Published in final edited form as:

Microvasc Res. 2009 December ; 78(3): 257–264. doi:10.1016/j.mvr.2009.08.006.

Hyperglycemia attenuates angiogenic capability of survivin in endothelial cells

Qinhui Song^{a,1}, Xiaojin An^{a,c,1}, Dongmei Li^{a,d}, Neel R. Sodha^b, Munir Boodhwani^b, Ye Tian^d, Frank W. Sellke^b, and Jian Li^{a,*}

^a Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Center for Life Science Building, 3 Blackfan Circle, Boston, MA 02115, USA

^b Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

^c Institute of Molecular Medicine, Peking University, Beijing, China

^d Department of Anesthesiology, The Second Affiliated Hospital of Harbin Medical University, Harbin, China

Abstract

Survivin, an anti-apoptotic protein, can be induced by hypoxia and contributes to angiogenic activity in endothelial cells. To determine the potential mechanism of survivin in endothelial dysfunction caused by hyperglycemia in diabetes, we evaluated the role of survivin in hyperglycemia and its effect on endothelial homeostasis. We demonstrated that an increase of D-glucose was sufficient to down-regulate survivin expression, impacting survivin's angiogenic role in endothelial cells. We additionally showed that survivin expression was increased in response to hypoxia yet this reaction was mitigated when the endothelial cells were in hyperglycemic conditions prior to hypoxia. Hyperglycemia also affected survivin-related proliferation and migration of endothelial cells and increased the number of apoptotic cells. In the ischemic porcine myocardium, the expression of survivin was induced. Moreover, survivin expression in the aorta, myocardium, and isolated endothelial cells was attenuated in a porcine model of diabetes in comparison to non-diabetes, which correlated negatively with the levels of fasting blood sugars and positively with territory perfusion. These results demonstrate that hyperglycemia critically alters survivin expression *in vitro* and *in vivo*, which leads to attenuation of angiogenic activity and impacts endothelial metabolism.

Keywords

Survivin; Angiogenesis; Apoptosis; Hyperglycemia

Introduction

Endothelial cell dysfunction is considered to be an initial event in the development of vascular complications in the setting of ischemic heart disease, peripheral vascular disease, and cerebrovascular disease, all of which are major contributors to the morbidity and mortality of diabetes. However, the mechanisms through which diabetes affects gene and protein regulation in the maintenance of the endothelial homeostasis still remain unclear.

*Corresponding author. jli@bidmc.harvard.edu (J. Li).

¹These two authors contribute equally to this paper.

Survivin is a protein which has both angiogenic and anti-apoptotic activities in endothelial cells (Ma et al., 2007; Yamagishi et al., 2002). It is strongly expressed in the G₂/M phases of the cell cycle in tumor cells (Deguchi et al., 2002) and under physiologic conditions, it has been reported that survivin enhances survival primarily through suppression of apoptosis-related cell death via direct inhibition of caspase-related proteins. Under pathologic conditions, survivin enhances survival of injured cells and angiogenic reactions in ischemia (Chandele et al., 2004; McKay et al., 2003).

Recent evidence suggests that survivin expression can be induced as an angiogenic factor in the ischemic brain to improve injury (Conway et al., 2003). In addition, the functional angiogenic factor, vascular endothelial growth factor (VEGF), has been reported to mediate survivin expression in neuroblastoma cells (Beierle et al., 2005). In angiogenesis studies, survivin has been shown to be involved in the growth of ovarian endometriotic cysts (Goteri et al., 2005), brain gliomas, and other tumors (Zhen et al., 2005). Inhibition of apoptosis by survivin suggested that survivin can be regulated by PI3-kinase/Akt (Ohashi et al., 2004; Zhang et al., 2007) and IL-11 (Kirkiles-Smith et al., 2004) to inhibit apoptotic reactions in endothelial cells. However, the effect of survivin in hyperglycemia has remained elusive, although there is mounting evidence suggesting that hyperglycemia has long been associated with impaired endothelial function (Sheetz and King, 2002). Studies have shown that hyperglycemia alters PI3 kinase/Akt signaling leading to endothelial cell proliferative dysfunction (Varma et al., 2005). Dohi et al. (2006) demonstrated that transplantation of survivin transgenic islets in diabetic recipient mice affords long-term engraftment and stable correction of hyperglycemia with intrinsic inhibition of beta-cell apoptosis, as well as promotes angiogenesis.

Considering that angiogenic therapy in ischemic vascular disorders failed to show efficacy in diabetic patients, we hypothesized that hyperglycemia-related endothelial dysfunction is one of the major factors leading to reduced angiogenesis. In the present study, we demonstrated that expression of survivin increases in hypoxia but decreases in hyperglycemia in endothelial cells. Moreover, exposing endothelial cells to high concentrations of glucose resulted in attenuation of survivin-induced angiogenesis. We found that myocardial survivin expression correlated negatively to levels of fasting blood sugar and positively to territory perfusion in a diabetic porcine model. Understanding the effect of survivin in hyperglycemia may provide beneficial knowledge in improving the angiogenic response in diabetes.

Materials and methods

Cell culture

Bovine aortic endothelial cells (BAECs) and porcine aortic endothelial cells were obtained using techniques previously described (Wu et al., 2004) under an Institutional Animal Care and Use Committee-approved protocol. The cells were cultured in DMEM with 10% FBS. Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza and cultured in endothelial basal medium-2 (EBM-2) supplemented by an endothelial growth kit (Lonza, MD).

Hypoxia and hyperglycemia

Endothelial cells were subjected to hypoxia (~1% O₂) for 6, 12, 24, 48, and 72 hours in an air-tight chamber (Billups-Rothenberg, CA) pre-gassed with 95% N₂ and 5% CO₂. Cells maintained under normoxic conditions (~21% O₂) served as controls. For hyperglycemic conditions, endothelial cells were cultured with varied concentrations of additional D-glucose (10, 20, 30, and 50 mM in addition to the 5 mM in baseline of DMEM). The glucose

concentration and duration is based on the cell viability assay and in a previous report that suggested that high glucose (30 mM) for 8 to 10 days did not affect its viability (Donnini et al., 2003).

Cell viability assay

Viable cell ratios were determined using the dye exclusion assay. This assay is based on the exclusion of Trypan blue dye from viable cells. Cells were treated with high D-glucose at a final concentration of approximately 35 mM and collected at 2, 4, 6, 8, and 10 days and then incubated with 0.4% Trypan blue (Sigma, MO) for 5 minutes. Cells were observed under a microscope and counted as stained or nonstained on a hemocytometer. From this, viable cell ratios were calculated according to the following formula: viable cell ratio (%) = nonstained cells vs. total cells $\times$ 100%.

Adenoviral constructs

The adenoviral constructs containing the full-length survivin cDNA were a kind gift from Dr. Dario Altieri, University of Massachusetts Medical School (Blanc-Brude et al., 2003). The adenoviral construct without survivin was used as the control. One milliliter of viral supernatant was used to infect 3×10^6 to 5×10^6 293T cells. Viruses were harvested at 3 day intervals reaching a total of 5×10^8 packaging cells, and viral particles were purified by cycle bandings and transformed to endothelial cells.

Western blot analysis

Lysates were generated from endothelial cells by placing in RIPA lysis buffer (Boston BioProducts, MA). Aortic or myocardial tissue specimens were homogenized in RIPA buffer and centrifuged at $12,000 \times g$ for 15 minutes at 4 °C to separate soluble from insoluble fractions. Bradford assays (Bio-Rad, Hercules, CA) were performed to determine total protein concentrations. Samples were run on 12–16% polyacrylamide gels. Wet transfer was performed using Immobilon-P Transfer membranes (Millipore, Bedford, MA) that were incubated with antibodies against survivin (Novus Biologicals, CO). Anti- β -actin antibody was used as an internal control (Sigma, MO).

Quantitative PCR analysis

Reverse transcription was performed on 2 μ g of total RNA from endothelial cells in normal, hypoxic, and hyperglycemic conditions. The cDNA was used for quantitative real-time PCR amplification with SYBR Green I Chemistry (Applied Biosystems, CA) with the primers designed and selected as optimal primer pairs using Primer Premier 5 software (Premier Biosoft International, CA). Survivin was assessed by PCR with use of forward primer 5'-GTCGTCCGTACCATGGGTGCCCC-GACGTTG-3' and reverse primer 5'-CAGCAGGGATCCATCCATGGCA-GCCAGCTGCTC-3', corresponding to the human survivin sequence (GenBank NM_001012271.1). Primers for β -actin, 5'-GGACCTCACT-GACTACCTCATGAA-3' and 5'-GGTGAAGGTCAAACACCTAG-3' (GenBank NM_005159.4), were used for each sample to control for RNA loading. PCRs were run on an SDS 7000 System (ABI) in which the specificity of all individual amplification reactions was confirmed by melting curve analysis. All experiments were done in triplicate, and statistical analysis was based on the ratio of survivin transcripts and the amounts of β -actin.

Immunofluorescence assay

BAECs were cultured under hypoxic and/or hyperglycemic conditions. Cells were fixed with 4% formaldehyde and incubated with anti-survivin antibody (Novus Biologicals, CO) overnight at 4 °C followed by incubation with a Texas red-conjugated secondary antibody

(Santa Cruz, CA) for 1 hour. Slides were mounted by mounting medium with DAPI (Vector, CA) and imaged on a Leica DM IRB fluorescent microscope.

Apoptotic assays

Apoptosis was assayed using a FITC-labeled annexin V and propidium iodide (PI) apoptosis assay kit (Invitrogen, CA) based on the manufacturer's instruction. The stained cells were analyzed by flow cytometry using FACSscan (BD Biosciences, CA) with the fluorescence emission measuring 530 nm and >575 nm. For each treatment, 10,000 cells were counted and then analyzed using Cell Quest software. The flow cytometry results were confirmed by viewing the cells under a fluorescence microscope. Caspase-3 cellular activity was measured by using a caspase-3 assay kit (Calbiochem, CA) in endothelial cell extracts under hypoxic conditions 12 hours after incubation in D-glucose or mannitol for 8 days according to the manufacturer's directions. Caspase-3 activity was expressed as picomoles of *p*-nitroaniline released per minute per microgram of cellular protein.

Proliferation and migration assays

For measuring cell proliferation, endothelial cells were placed in six well dishes. ³H-Thymidine (Perkin Elmer, Boston, MA) was added at a concentration of 1 μ Ci/ μ l and incubated for 4 hours at 37 °C. After the trypsinization of cells, 200 μ l of medium and then 100 μ l of 0.8N NaOH (final concentration=0.2N) were added followed by incubation at room temperature for 20 minutes. Proliferating cells labeled with ³H-thymidine were measured using a multipurpose scintillation counter (Beckman LS 6500). Endothelial cell migration was measured using Transwell migration chambers with binding buffer (0.05% gelatin and 25 mM HEPES in DMEM). The migration assay was conducted at 37 °C for 48 hours. The polycarbonate membranes were removed and mounted onto glass slides, and the total number of cells was counted in each of six random fields (1.5 mm² per field). Only those cells with detectable nuclei were counted as positive.

Glucose oxidase assays

Glucose oxidase was measured by using an Amplex Red Glucose Oxidase Assay kit (Molecular Probe, CA) as per the manufacturer's protocol.

Porcine model of diabetes and acute myocardial ischemia

Yucatan miniswine (Sinclair Research Inc., Colombia, MO) were used (4 non-diabetic and 7 diabetic). Diabetes was induced with a single IV injection of alloxan (150 mg/kg). Animals with glycemia exceeding 400 mg/dl were treated with insulin to maintain glycemia between 250 and 400 mg/dl. After 8 weeks, diabetic animals underwent surgical procedures for acute ischemia–reperfusion (I/R) experiments. Anesthesia and surgical protocols have been previously described (Boodhwani et al., 2006). A median sternotomy was performed and the mid left anterior descending (LAD) artery was identified and occluded using an atraumatic Bulldog vascular clamp. Two cycles of I/R (10 minutes ischemia then 30 minutes reperfusion) were performed. All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee and the Harvard Medical Area Standing Committee on Animals. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85–23, revised 1996).

Statistical analysis

Data was obtained from at least three independent cell cultures or animals. For statistical analysis, a two-way analysis of variance (ANOVA) was followed by Scheffé's test. If differences were established, the values were compared using Student's *t*-test. The values

were expressed as mean $\pm$ SEM. Linear regression analyses were performed utilizing Pearson's correlation coefficient. The results were considered significant if $p<0.05$.

Results

Reduction of survivin expression by a high concentration of glucose

Survivin mRNA expression was decreased around 50% after 8 days of incubation with 35 mM D-glucose in comparison to control cells with 5 mM D-glucose. Thirty-five millimolars of mannitol did not affect the expression of survivin, which excludes the possibility of an osmotic effect of glucose on survivin in endothelial cells (Fig. 1A). High glucose-decreased survivin expression was shown in a dose-dependent manner in mRNA levels (Fig. 1B) as well as in protein levels (Fig. 1C). Furthermore, a cell survival count assay demonstrated that there were no significant differences in response to high D-glucose (35 mM) up to 10 days, indicating that long-term hyperglycemia did not cause endothelial cell damage (Fig. 1D).

Hyperglycemia attenuates hypoxia-induced survivin expression

The expression of survivin mRNA was induced and peaked at approximately 12 hours during a time course of hypoxic exposure (1% O₂) (Fig. 2A). The effect of hypoxia on survivin protein level (Fig. 2B) was also time-dependent. To determine whether endothelial response to hypoxia was negatively affected by hyperglycemia, BAECs were pre-incubated with 35 mM D-glucose or mannitol for 8 days prior to 12 hours of hypoxia. The expressions of survivin mRNA (Fig. 2C) and protein (Fig. 2D) were no longer induced by hypoxia in hyperglycemic endothelial cells, while the BAEC exposed to mannitol maintained its expression. To avoid limitation of BAEC, we duplicated the experiment in primary cultured human umbilical vein endothelial cells (HUVECs) (Fig. 2C, right), in which the effect of survivin expression in hypoxia and hyperglycemia was similar to BAEC (Fig. 2C, left). These results suggested that hyperglycemia is responsible for the reduction of endothelial ability in response to hypoxic stimuli. Immunofluorescence analysis indicated that survivin localizes in both the nucleus and the cytoplasm in normal conditions. Under hypoxia conditions, more nuclear-localized survivin was detected compared with that in normoxia, but hyperglycemia has no influence on its localization (Fig. 2E).

Hyperglycemia attenuates survivin-induced proliferation and migration

The decrease in survivin expression following exposure to hyperglycemia suggests that the endothelial cells may reduce their sensitivity and activity in response to stimulation. To confirm this possibility, an adenoviral construct carrying survivin was transduced to infect the cultured BAEC. Western blot analysis indicated that survivin overexpression (survivin o/e) was significantly higher compared with the BAEC with adenoviral vector only (Fig. 3A). The proliferating rate of endothelial cells was measured in BAEC under hyperglycemic conditions. ³H-Thymidine uptake assay demonstrated a significantly greater proliferative response in survivin o/e cells. However, the survivin-induced proliferation was attenuated in BAEC under hyperglycemic conditions (Fig. 3B). In addition, hyperglycemia attenuated the survivin-related migration rate of BAEC, with survivin o/e BAEC being significantly reduced (Fig. 3C).

Hyperglycemia attenuated the survivin-related anti-apoptosis

Considering that angiogenesis and apoptosis are reciprocal processes, we further examined whether survivin-induced endothelial cell proliferation is also related to its anti-apoptotic ability. Using an annexin V assay to measure early-stage apoptotic cells, the number of apoptotic BAEC with survivin o/e was significantly decreased in comparison to the control

BAEC (4.23% vs. 0.98%, $*p < 0.05$). Hyperglycemia significantly increased the number of apoptotic cells from 4.23% to 6.69% ($*p < 0.05$) in the control BAEC, and from 0.98% to 4.71% in survivin o/e BAEC (Fig. 4A). These results suggested that survivin may have the ability to protect endothelial cells from being affected by hyperglycemia. To further confirm that survivin can protect endothelial cells from undergoing apoptosis in hyperglycemic conditions, the caspase-3 activity was measured in BAEC under 12 hours of hypoxia after pre-incubation in either 35 mM D-glucose or 35 mM mannitol for 8 days. As shown in Fig. 4B, hyperglycemia can increase caspase-3 activity (1.7-fold, $*p < 0.05$) in comparison to the control (5 mM D-glucose and mannitol). Caspase-3 cellular activity in survivin o/e BAEC under hyperglycemic conditions showed a significantly reduced cell apoptotic rate.

Metabolic changes in response to survivin expression in endothelial cells

To further explore the role of survivin in glucose metabolism in endothelial cells, glucose oxidase levels were measured in survivin transfected BAEC. Hyperglycemia alone did not significantly affect glucose oxidase level but did cause decreased glucose oxidase levels in survivin o/e BAEC (Fig. 5). These findings suggest that the protective effects of survivin in endothelial cells may be mediated through changes in cell metabolism, which hyperglycemia could impact.

Down-regulation of survivin expression in diabetic heart and aorta

To determine whether the hypoxia-induced survivin expression observed in cultured cells also occurs in response to ischemia in the *in vivo* setting, we used a porcine model of acute myocardial ischemia. Survivin expression was significantly increased in ischemic myocardium compared to non-ischemic myocardium (1.5-fold, $*p < 0.05$, Fig. 6A). Furthermore, ischemic myocardial tissue from diabetic and non-diabetic controls was examined to assess the expression pattern of survivin *in vivo* in the setting of diabetes. Diabetic myocardium and aorta demonstrated a reduction in survivin expression relative to non-diabetic myocardium and aorta (Fig. 6B). To evaluate the survivin level in diabetic porcine endothelial cells, aortic endothelial cells were obtained using techniques previously described (Wu et al., 2004) from diabetic pigs. Survivin expression was significantly decreased in the diabetic aortic endothelial cells versus non-diabetic aortic endothelial cells (Fig. 6C).

Myocardial survivin expression correlates to both fasting blood sugar and territory perfusion in a diabetic porcine model

In the porcine diabetic model, linear regression analysis utilizing Pearson's correlation coefficient demonstrated a negative association between myocardial survivin expression and mean fasting blood sugars, with $R = -0.841$ (Fig. 6D). Linear regression analysis demonstrated a positive association between the degree of tissue perfusion in ischemic myocardium and survivin expression ($R = 0.702$), consistent with prior evidence that hypoxia up-regulated survivin expression (Fig. 6E).

Discussion

Diabetes mellitus is a major risk factor for the development of cardiovascular complications. However, the mechanism of vascular injury in the setting of diabetes with hyperglycemia remains unclear. The major finding of this study indicates that hyperglycemia, as a metabolic stressor, decreases expression and function of survivin, causes endothelial dysfunction, and impacts hypoxia-induced endothelial cell ability *in vitro* and *in vivo*. Multiple lines of results support this finding. First, survivin expression was induced by hypoxia in endothelial cells, which could be attenuated with exposure to hyperglycemia prior to hypoxia. Second, survivin-induced endothelial cell proliferation and migration were

significantly inhibited by hyperglycemia. Third, high glucose decreased endothelial metabolism, which could be enhanced by survivin. Finally, survivin expression was markedly diminished in the diabetic porcine heart and aorta, which correlated with blood glucose concentrations and tissue perfusion. Our studies are complementary to previous reports demonstrating the role of survivin in angiogenesis in ischemic brain (Conway et al., 2003). In addition to the role of survivin in hypoxic/ischemic endothelial cells, hyperglycemia-related endothelial dysfunction may play a role in the inhibition of angiogenic responses in terms of hypoxia through attenuation of survivin expression, suggesting that diabetes may affect homeostatic balance in post-ischemic microvascular function.

Survivin has recently been described as an important regulator of cellular angiogenesis and apoptosis and has also been implicated in ischemic cardiovascular disease (Conway et al., 2003; Lu et al., 2004; Santini et al., 2004). In endothelial cells, we demonstrated that exposure to hypoxia led to a time-dependent increase in survivin expression. This was supported by the reports detailing survivin-related angiogenesis in ischemic brain (Conway et al., 2003) and survivin expression up-regulation in hypoxic HUVECs (Conway et al., 2003). Thus, these results together reinforce the crucial role played by survivin in mediating hypoxia-induced neovascularization. Normally, survivin is localized in both the cytoplasm and the nucleus of endothelial cells, but hypoxia only or combined with hyperglycemia induced its predominant nuclear localization, which might increase sensitivity to pro-apoptotic stimuli (Temme et al., 2007). Consequently, subcellular localization of survivin may be important for its anti-apoptotic capacity.

Multiple clinical trials have failed to reproduce the excellent results of pro-angiogenic therapy published in pre-clinical large animal studies, and patient co-morbidities such as diabetes are hypothesized to account for this discordance, secondary to the presence of endothelial dysfunction in these conditions. Our data provide evidence that hyperglycemia reduces expression of survivin based on the following: hypoxia-induced survivin expression is attenuated when endothelial cells are exposed to hyperglycemic conditions, and survivin expression is diminished in hyperglycemic endothelial cells as well as in diabetic porcine hearts and aortas. To the best of our knowledge, this is the first report that survivin is down-regulated in hyperglycemic endothelial cells. We also found that survivin overexpression led to enhanced cellular metabolism, as evidenced by increased cellular glucose oxidase levels, which may be important for its angiogenic and anti-apoptotic effects.

The effects of survivin's activity in terms of angiogenesis and apoptosis have previously been evaluated in tumor cells and in models of vascular injury, but less so in the context of hypoxia- and hyperglycemia-mediated regulation of angiogenesis in endothelial cells. We demonstrated that overexpression of survivin enhanced endothelial cell proliferation and migration. Furthermore, endothelial cells in hyperglycemic conditions showed significant down-regulation of survivin's activities. Our *in vivo* porcine model of short-term myocardial ischemia led to an increase in survivin expression in the myocardium. This *in vivo* finding is consistent with prior *in vitro* reports of hypoxia-induced up-regulation of survivin expression. In the porcine diabetic model, survivin expression was markedly diminished in both the myocardium and aorta and demonstrated a strong negative linear correlation with fasting blood glucose levels. These results supported our *in vitro* findings of diminished expression and function of survivin in hyperglycemic conditions. The development of coronary collateralization was markedly impaired in diabetic swine, leaving the myocardium significantly more ischemic in this group, consistent with the survivin expression observed in their myocardium. Taken together, the results of our study demonstrate a critical role for survivin in hypoxia-mediated angiogenesis and suggest potential mechanisms for its induction and downstream effects.

In conclusion, our findings confirm that survivin plays an important role in modulating key events required for angiogenesis and metabolism in endothelial cells, namely, cellular proliferation, migration, and reduction of apoptosis. These activities of survivin can be significantly blocked in endothelial cells by incubating under hyperglycemic conditions. These findings may provide new areas for therapeutic intervention in ameliorating diabetic cardiovascular complications.

Acknowledgments

We thank Dr. Dario Altieri (University of Massachusetts Medical School, MA) for providing the adenoviral constructs of survivin and Ms Brittany L. Cully for editing the manuscript. This study was supported by NIH R21HL088219 (J.L.), NIH R01HL69024 (F.W.S.), and by NIH Training Grant HL07374-22 (Q.S.).

References

- Beierle EA. VEGF-mediated survivin expression in neuroblastoma cells. *J Surg Res* 2005;127:21–28. [PubMed: 15882878]
- Blanc-Brude OP. Therapeutic targeting of the survivin pathway in cancer: initiation of mitochondrial apoptosis and suppression of tumor-associated angiogenesis. *Clin Cancer Res* 2003;9:2683–2692. [PubMed: 12855648]
- Boodhwani M. Mortality and myocardial infarction following surgical versus percutaneous revascularization of isolated left anterior descending artery disease: a meta-analysis. *Eur J Cardiothorac Surg* 2006;29:65–70. [PubMed: 16337800]
- Chandele A. Upregulation of survivin in G₂/M cells and inhibition of caspase 9 activity enhances resistance in staurosporine-induced apoptosis. *Neoplasia* 2004;6:29–40. [PubMed: 15068669]
- Conway EM. Survivin-dependent angiogenesis in ischemic brain: molecular mechanisms of hypoxia-induced up-regulation. *Am J Pathol* 2003;163:935–946. [PubMed: 12937134]
- Deguchi M. Expression of survivin during liver regeneration. *Biochem Biophys Res Commun* 2002;297:59–64. [PubMed: 12220508]
- Dohi T. Inhibition of apoptosis by survivin improves transplantation of pancreatic islets for treatment of diabetes in mice. *EMBO Rep* 2006;7:438–443. [PubMed: 16470228]
- Donnini D. New *in vitro* model to study high glucose-dependent endothelial dysfunctions. *Biochimie* 2003;85:701–705. [PubMed: 14505826]
- Goteri G. Endothelial cell survivin is involved in the growth of ovarian endometriotic cysts. *Anticancer Res* 2005;25:4313–4318. [PubMed: 16309234]
- Kirkiles-Smith NC. IL-11 protects human microvascular endothelium from alloinjury *in vivo* by induction of survivin expression. *J Immunol* 2004;172:1391–1396. [PubMed: 14734714]
- Lu QP. Multiple gene differential expression patterns in human ischemic liver: safe limit of warm ischemic time. *World J Gastroenterol* 2004;10:2130–2133. [PubMed: 15237451]
- Ma A, et al. The role of survivin in angiogenesis during ebrafish embryonic development. *BMC Dev Biol* 2007;7:50. [PubMed: 17511868]
- McKay TR. Procaspase 3 expression in ovarian carcinoma cells increases survivin transcription which can be countered with a dominant-negative mutant, survivin T34A; a combination gene therapy strategy. *Oncogene* 2003;22:3539–3547. [PubMed: 12789262]
- Ohashi H. Phosphatidylinositol 3-kinase/Akt regulates angiotensin II-induced inhibition of apoptosis in microvascular endothelial cells by governing survivin expression and suppression of caspase-3 activity. *Circ Res* 2004;94:785–793. [PubMed: 14963002]
- Santini D. Surviving acute myocardial infarction: survivin expression in viable cardiomyocytes after infarction. *J Clin Pathol* 2004;57:1321–1324. [PubMed: 15563676]
- Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 2002;288:2579–2588. [PubMed: 12444865]
- Temme A. Nuclear localization of survivin renders HeLa tumor cells more sensitive to apoptosis by induction of p53 and Bax. *Cancer Lett* 2007;250:177–193. [PubMed: 17084966]

- Varma S. Hyperglycemia alters PI3k and Akt signaling and leads to endothelial cell proliferative dysfunction. *Am J Physiol Heart Circ Physiol* 2005;289:H1744–H1751. [PubMed: 15964918]
- Wu J. PR39 inhibits apoptosis in hypoxic endothelial cells: role of inhibitor apoptosis protein-2. *Circulation* 2004;109:1660–1667. [PubMed: 15023888]
- Yamagishi S. Advanced glycation end products-induced apoptosis and over-expression of vascular endothelial growth factor in bovine retinal pericytes. *Biochem Biophys Res Commun* 2002;290:973–978. [PubMed: 11798169]
- Zhang Y. Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. *Am J Physiol Heart Circ Physiol* 2007;292:H2573–H2581. [PubMed: 17337592]
- Zhen HN. Survivin expression and its relation with proliferation, apoptosis, and angiogenesis in brain gliomas. *Cancer* 2005;104:2775–2783. [PubMed: 16284993]

Fig. 1.

Hyperglycemia decreases expression of survivin in endothelial cells. (A) Data from real-time Q-PCR show that survivin mRNA expression was decreased from days 3 to 8 in response to high glucose. (B) Different concentrations of D-glucose decreased survivin mRNA expression 20%–40% as a dose-dependent effect. (C) Western blotting demonstrated that the expression of survivin was decreased when the BAECs were cultured in a high concentration of D-glucose (5, 10, 15, 35, and 55 mM) for 8 days. Incubation of BAECs with mannitol (35 mM) for 8 days served as an osmotic control. (D) There was no significant difference in response to high D-glucose (35 mM) at 2, 4, 6, 8, and 10 days ($p>0.05$) by the cell survival count assay.

Fig. 2.

Hyperglycemia attenuates hypoxia-induced survivin expression in endothelial cells. (A) Induced survivin mRNA levels in response to hypoxia occurs in a time-dependent manner from 6 hours to 48 hours ($p < 0.05$). The peak was at 12 hours (2.5-fold compared to the control). (B) Survivin protein expression was induced by hypoxia and peaked also at 12 hours in response to hypoxia ($\sim 4.66 \pm 0.35$ -fold) and returned to baseline at 72 hours. β -Actin expression in response to hypoxia did not show a significant difference and represent for equal protein loading. Data were from triplet experiments and presented as means $\pm$ SD. $*p < 0.05$. (C and D) BAECs were pre-incubated with 35 mM D-glucose or mannitol for 8 days and then exposed to hypoxia for 12 hours. Expressions of survivin mRNA (C, left) and protein (D) were no longer induced by hypoxia in hyperglycemic endothelial cells. BAECs exposed to mannitol did not change hypoxia-driven survivin expression. Similar treatment performed on HUVEC resulted in the same negative effect of hyperglycemia in the hypoxia-induced level of survivin mRNA (C, right). The results were quantified based on three experiments by ImageQuant and presented as mean $\pm$ SD. $*p < 0.05$. (E) Immunofluorescence staining for survivin localization showed that survivin was detected in both the cytoplasm and the nucleus. Hypoxia promoted its nuclear translocation, while hyperglycemia did not show any change. Arrows indicate nuclear localization, and stars (*) indicate cytoplasm.

Fig. 3.

Hyperglycemia attenuates survivin-stimulated endothelial cell proliferation and migration. (A) Western blot indicates the overexpression of survivin (survivin o/e) in BAEC that were infected with adenoviral constructs of survivin and empty adenoviral vectors as a control. (B) ^3H -Thymidine incorporation uptake in BAEC indicated that overexpression of survivin increased proliferation about 1.4-fold compared to controls (black bars). The increase in BAEC proliferation was significantly attenuated after BAECs were pre-treated with 35 mM glucose (gray bars). (C) Migration of BAEC was measured using Transwell migration chambers. The figure illustrates the quantitative analysis of the number of cells that migrated to the upper chambers. Migration rate was enhanced 1.2-fold in BAECs over-expressing survivin compared with wild-type BAECs. However, survivin-enhanced endothelial cell migration was significantly attenuated by the BAECs in hyperglycemic conditions.

Fig. 4.

Hyperglycemia increases survivin-reduced apoptotic endothelial cells. (A) Quantitative analysis of apoptotic cells with annexin V is shown as percentage changes in apoptotic-positive BAECs of total cells with survivin overexpression and in the presence of 35 mM D-glucose (gray bars). The number of apoptotic cells was 4.23% in control and declined to 0.98% in BAECs with survivin o/e. Hyperglycemia significantly increased the number of apoptotic cells in both control (from 4.23% to 6.69%) and survivin o/e BAECs (from 0.98% to 4.71%) compared to that in normal glucose condition. The results were quantified based on three experiments and are presented as mean $\pm$ SD. * p <0.05. (B) Hyperglycemia increased BAEC's caspase-3 activity (1.7-fold, * p <0.05) in comparison to the control (5 mM D-glucose and 35 mM mannitol). Caspase-3 activity in survivin o/e BAECs under hyperglycemic and normal glucose conditions showed no significant difference in comparison to the rate in the control BAECs.

Fig. 5. Metabolic effects in response to hypoxia-induced survivin expression. Glucose oxidase levels were increased in the BAEC survivin o/e in comparison to the controls but decreased in high glucose conditions. Data are expressed as the average of three independent experiments and presented as mean $\pm$ SD, * p <0.05.

Fig. 6.

Hyperglycemia reduces expression of survivin in the porcine diabetic model. (A) Survivin expression was significantly increased in the ischemic tissue (1.5-fold, $*p < 0.05$). (B) Survivin expression was significantly decreased in the myocardium (46% of control) and aorta (50% of the control, $*p < 0.05$) of the porcine models of diabetes versus non-diabetics. (C) Survivin expression was significantly decreased in the diabetic porcine aortic endothelial cells versus non-diabetic porcine aortic endothelial cells. (D) The fasting blood sugar and perfusion rate were examined in the diabetic model ($n=7$) and normal porcine ($n=4$). Linear regression analysis utilizing Pearson's correlation coefficient demonstrated a negative association between myocardial survivin expression and mean fasting blood sugars with $R = -0.841$, $p < 0.05$. (E) Linear regression analysis demonstrated a positive association between the degree of tissue perfusion in chronically ischemic myocardium and survivin expression, $R = 0.702$, $p < 0.05$, consistent with prior evidence that hyperglycemia and hypoxia regulate survivin expression.