Angiogenesis plays an important role in a variety of physiological and pathological settings, including tumor growth and progression (1, 2). Tumor angiogenesis is regulated locally by the tight balance between proangiogenic and antiangiogenic factors (3). Vascular endothelial growth factor (VEGF)1 is not only an important angiogenic mediator but also a key survival factor for endothelial cells where it has been shown to protect them from the proapoptotic effects of ionizing radiation, antineoplastic drugs, and antiangiogenic factors (4–6). A number of human tumors express high levels of VEGF (7–9). Moreover, serum VEGF levels are often elevated in certain patients with malignant tumors after irradiation treatment, making the endothelial cells lining tumor blood vessels much less susceptible to apoptosis (10). Inhibition of VEGF-mediated angiogenic response with neutralizing antibodies or dominant negative soluble receptors has been shown to significantly reduce the growth of primary as well as metastatic tumors (4, 11).

VEGF, upon binding to its receptor, leads to the activation of various signaling molecules including phosphoinositide 3-kinase (PI3K)/Akt, the mitogen-activated protein kinases (MAPK), and stress-activated protein kinase-2 or p38 MAPK pathways (18–20). The p38 MAPK pathway is activated by a number of growth factors and has been implicated as a critical pathway for cell survival (15). Although the p38 pathway plays a critical role in apoptosis (15), its role in VEGF-mediated signaling has not been well characterized. Ionizing radiation is one of the most commonly used treatments for a wide variety of tumors. In addition to causing DNA damage, ionizing radiation can also activate the stress-activated protein kinases or c-Jun N-terminal kinase (SAPK/JNK) and p38 MAPK pathways (18–20). The SAPK/JNK pathway has been shown to activate caspases and may also target other factors that have been implicated in apoptosis regulation including p53, Bcl-2, and Bax (21–26). Wang et al. (20) have shown that p38γ (also known as ERK6 or SAPK3), but not the other p38 isoforms, is required for γ-irradiation-induced fibroblast cell arrest.

VEGF-mediated endothelial cell protection has been implicated as one of the key mechanisms responsible for tumor radioresistance (4). However, the signaling pathways by which VEGF protects endothelial cells against ionizing radiation are poorly understood. In the present study we explored strategies to attenuate the cytoprotective effects of VEGF in endothelial

1 The abbreviations used are: VEGF, vascular endothelial growth factor; HDMEC, human dermal microvascular endothelial cell; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling; MAPK, mitogen-activated protein kinases; PI3K, phosphoinositide 3-kinase; ERK, extracellular signal-related kinase; JNK, c-Jun N-terminal kinase; EBM, endothelial cell basal medium; EGM, endothelial growth medium; MAPRK, MAPK kinase; NS, non-stimulated; JNKI, JNK inhibitor; Irr, irradiation; SAPK, stress-activated protein kinase; Gy, gray.

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These results suggest that a combination of PI3K inhibitor was mediated predominantly via the PI3K-Akt-Bcl-2 pathway. This VEGF-mediated HDMEC protection for 3 days. On days 3 and 6, five random high-power fields ($\times 200$) of each sample group from three independent experiments were photographed by digital camera and then counted using Metamorph software. The percentage of HDMEC survival was calculated for each group by dividing day 6 cell counts by day 3 counts and multiplying by 100. A, photomicrograph of representative assays for: 1, NS control; 2, NS + irradiation (Irr); 3, NS + LY294002 + irradiation; 4, NS + PD98059 + irradiation; 5, NS + PD169316 + irradiation; 6, NS + JNK inhibitor; 7, NS + PD169316 + JNKi. B, percentage of HDMEC survival for each group. * represents a significant difference ($p < 0.05$) in irradiated groups and the control. ** represents a significant difference ($p < 0.005$) between PD169316 or PD169316 + JNKi-treated group and non-stimulated (NS + Irr) control.

**Fig. 1.** γ-Irradiation mediates HDMEC growth arrest predominantly through p38 MAPK pathway. HDMECs were cultured in 60-nm dishes containing a thin layer of type I collagen gels. On day 3, HDMECs were treated with 10 μM LY294002 (LY), 20 μM PD98059 (PD), 10 μM PD169316 (PD16), or 2 μM JNK inhibitor for 1 h and then exposed to a single dose of γ-irradiation (10 Gy). The cells were further incubated at 37 °C for 3 days. On days 3 and 6, five random high-power fields ($\times 200$) of each sample group from three independent experiments were photographed by digital camera and then counted using Metamorph software. The percentage of HDMEC survival was calculated for each group by dividing day 6 cell counts by day 3 counts and multiplying by 100. A, photomicrograph of representative assays for: 1, NS control; 2, NS + irradiation (Irr); 3, NS + LY294002 + irradiation; 4, NS + PD98059 + irradiation; 5, NS + PD169316 + irradiation; 6, NS + JNKi + irradiation; 7, NS + PD169316 + JNKi. B, percentage of HDMEC survival for each group. * represents a significant difference ($p < 0.05$) in irradiated groups and the control. ** represents a significant difference ($p < 0.005$) between PD169316 or PD169316 + JNKi-treated group and non-stimulated (NS + Irr) control.

**MATERIALS AND METHODS**

**Reagents**—Recombinant human VEGF was purchased from Intergen (Purchase, NY). HDMECs, endothelial cell basal medium-2 (EBM-2), and growth supplements were purchased from BioWhittaker (Walkersville, MD). Inhibitors of PI3K (LY294002), MAPK (PD98059), p38 MAPK kinase (PD169316), and JNK (inhibitory peptide L-form), a DNA ladder kit, and mouse monoclonal anti-tubulin were obtained from Calbiochem. Rabbit polyclonal anti-phospho-Akt, rabbit polyclonal anti-phospho-ERK1/2, rabbit polyclonal anti-phospho-p38 MAPK, and mouse monoclonal anti-Bcl-2 antibodies were purchased from BIO-SOURCE International (Camarillo, CA). Rabbit polyclonal anti-phospho-JNK1/2 antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Apomorphine derivative terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay kit, RNase, proteinase K, and collagenase A were obtained from Sigma. A detergent-compatible protein assay kit was purchased from Bio-Rad. Enhanced chemiluminescence plus (ECL-plus) Western blotting detection reagents, anti-mouse, and anti-rabbit horseradish peroxidase-conjugated antibodies were purchased from Amersham Biosciences. Dominant negative mutant(s) plasmid propagation, purification, and transfection reagents were purchased from Invitrogen.

**Endothelial Cell Proliferation Assay**—HDMECs ($2 \times 10^5$) were plated in 60-nm dishes coated with a thin layer of type I collagen gel and incubated overnight at 37 °C in EGM2-MV. HDMECs were further incubated in growth factor-free EGM2-MV medium for 1 h prior to stimulation with 50 ng/ml VEGF. After 3 days of culture with VEGF, HDMECs were exposed to 10 Gy of γ-irradiation. One h prior to γ-irradiation, HDMECs were treated with the respective signaling inhibitors for PI3K (LY494002, 10 μM), MAPKK (PD98059, 20 μM), p38 MAPK (PD169316, 10 μM), or JNK inhibitor (2 μM). On days 0, 3, and 6, five random high-power fields ($\times 200$) from each sample group were photographed with a digital camera (Nikon Inc., Melville, NY) and then counted using Metamorph (Universal Imaging Corporation, Downingtown, PA). The percentage of HDMEC survival was calculated for each group by dividing day 6 cell counts by day 3 cell counts and multiplying by 100.

**Plasmid Constructions and Transient Transfections**—HDMECs were transiently co-transfected with pIRES-enhanced green fluorescent protein and pcDNA3 plasmid containing the dominant negative mutants of ERK1/2, Akt, p38 MAPK or c-Jun (a gift from Dr. Cun-Yu Wang, University of Michigan, Ann Arbor) using Lipofectin (Invitrogen) as described previously (27). Four μg of each plasmid and 20 μl of Lipofectin reagent were separately diluted in 200 μl of EBM-2 and incubated for 45 min at room temperature. The two solutions were gently mixed and further incubated for 15 min at room temperature to prepare the lipid-DNA complexes. HDMECs were washed twice with EBM, and then the lipid-DNA complexes were overlaid onto the cells. The cells were incubated for 6 h at 37 °C in a CO₂ incubator. At the end of the incubation, DNA containing medium was removed and replaced with EGM2-MV. Forty-eight h after transfection HDMECs were used for cell survival assays. HDMECs transfected with
pcDNA3 plasmids with no insert were used as a negative control. In cell survival assays, the percentage of HDMEC survival was calculated for each group (co-transfected with respective dominant negative mutant and enhanced green fluorescent protein) by counting the green fluorescent protein-positive cells using fluorescence microscope (Leica DM I RB).

Retroviral Vector Construction and HDMEC Transduction—The generation of HDMEC-Bcl-2 and HDMEC-LXSN was performed as previously described (28). The Bcl-2 construct or the vector alone was introduced into PA317 amphotropic packaging cells with Lipofectin. Viral supernatants were collected after 24 h, centrifuged, filtered, and stored at −70 °C. HDMECs were transduced with either Bcl-2 or control vector by overnight incubation with a one-tenth dilution of the viral supernatant in the presence of 4 µg/ml Polybrene. Endothelial cell growth medium supplemented with 400 ng/ml G418 was used to select the resistant clones. Bcl-2 expression was confirmed by Northern and Western blot analysis.

Immunoblotting—HDMECs (2 × 10^5) were plated in 60-mm dishes containing a thin layer of type I collagen gel and incubated as previously described. HDMECs were further incubated in serum-free EGM2-MV medium. The medium was changed, and the HDMECs were treated with 50 ng/ml VEGF in growth factor-free EGM2-MV medium. The medium was changed, and the HDMECs were exposed to 10 Gy of γ-irradiation and incubated for an additional 3 days. In signaling inhibition experiments, HDMECs were pretreated with the respective inhibitors for 1 h prior to irradiation. On day 6 HDMECs were harvested from the collagen with a solution of 2.5 mg/ml collagenase A. Both fragmented and high molecular weight DNA were extracted from each group using a DNA ladder isolation kit (Suicide-Track, Calbiochem, San Diego, CA) according to the manufacturer’s instructions. DNA was resolved in a 1.5%-agarose gel and stained with ethidium bromide.

TUNEL Assay and Flow Cytometry—HDMECs were cultured on collagen and treated with γ-irradiation as described previously for DNA ladder analysis. Cells were retrieved from collagen with a solution of 2.5 mg/ml collagenase A, washed, fixed in 1% paraformaldehyde for 15 min at 4 °C, and then stored overnight in 70% ethanol at −20 °C. The percentage of apoptotic cells was evaluated using the apocardodeoxyuridine TUNEL assay according to the manufacturer’s instructions (Sigma). Apoptotic cells were quantitated by flow cytometry using an argon laser excited at 488 nm (BD Biosciences).

Statistical Analysis—Data from all the experiments are expressed as mean ± S.E. Statistical differences were determined by two-way analysis of variance and Student’s t test. A P value of <0.05 was considered significant.

RESULTS

γ-Irradiation Induces Endothelial Cell Apoptosis Predominantly through p38 MAPK—HDMECs were cultured in 60-mm dishes on a thin layer of collagen in growth factor-free EGM2-MV medium. HDMECs exposed to γ-irradiation showed significantly lower survival rates as compared with non-treated cells (Fig. 1A, compare panel 2 with 1). HDMEC survival was further decreased when cells were pretreated with the PI3K

Fig. 2. γ-Irradiation induces time-dependent increase in p38 MAPK and JNK phosphorylation, which is further increased in cells pretreated with PI3K inhibitor (LY294002) and MAPKK inhibitor (PD98059). HDMECs were cultured in 60-mm dishes containing a thin layer of type I collagen gels until they were around 80–90% confluent. A and B, serum-starved HDMECs were treated with a single dose of γ-irradiation (10 Gy), and cell lysates were prepared at different time points. Phospho-p38 (p-p38), phospho-JNK (p-JNK), and tubulin content were analyzed by Western blot of total cell lysates using fluorescence microscope (Leica DM I RB).
enhanced in cells pretreated with LY294002 and PD98059. To further understand the cross-talk between the cell growth signal pathways (PI3K/Akt and ERK1/2) and cell death signal pathways (p38 MAPK and JNK), we pretreated the HDMECs with the PI3K inhibitor (PD169316), the MAPKK inhibitor (PD98059), the p38 MAPK inhibitor (PD169316, or the JNK inhibitor (inhibitory peptide LY294002), the MAPK inhibitor (PD98059), the p38 MAPK and JNK1/2), we pretreated the HDMECs with the PI3K inhibitor (PD169316) or the MAPK inhibitor (PD98059) significantly enhanced p38 MAPK phosphorylation in HDMECs (Fig. 2D). VEGF treatment of HDMECs prior to irradiation showed a significant decrease in JNK1/2 phosphorylation as compared with non-stimulated and irradiated cells (Fig. 2D).

γ-Irradiation-induced apoptosis of HDMECs was evaluated using both DNA ladder and TUNEL analysis of DNA fragmentation. HDMECs exposed to γ-irradiation showed a dose- and time-dependent increase in TUNEL-positive cells. HDMECs exposed to 5 and 10 Gy showed 16 and 26% TUNEL-positive cells, respectively. However, at 20 Gy a significantly higher number of TUNEL-positive cells (65%) was observed. In a time course study, the 72-h post-irradiation time point showed the maximal number of TUNEL-positive cells. Accordingly, all future studies were performed at 72 h time points and with a 10-Gy dose. HDMECs exposed to γ-irradiation showed a moderate level of DNA fragmentation (Fig. 3A, 1), which was further enhanced in cells pretreated with LY294002 and PD98059 (Fig. 3A, 7 and 8). In contrast, cells pretreated with the p38 MAPK inhibitor (PD169316) or the JNK inhibitor (Fig. 3A, 9 and 10) were resistant to γ-irradiation-mediated DNA fragmentation. VEGF-treated HDMECs were protected from γ-irradiation-induced DNA fragmentation as compared with non-stimulated cells. Similarly, VEGF-treated HDMECs showed significantly fewer TUNEL-positive cells (9%) when exposed to γ-irradiation as compared with non-stimulated and irradiated cells (25%, Fig. 3B). Pretreatment of HDMECs with the PI3K inhibitor (LY294002) or the MAPK inhibitor (PD98059) significantly enhanced γ-irradiation-induced HDMEC apoptosis as the number of apoptotic cells further increased to 35 and 30%, respectively. As observed in earlier experiments, blocking of p38 MAPK with PD169316 significantly protected HDMECs from γ-irradiation-mediated apoptosis (Fig. 3B). These results demonstrate the role of p38 MAPK in γ-irradiation-induced HDMEC apoptosis.

**VEGF Protects HDMECs against γ-Irradiation through PI3K-Akt and ERK1/2 Pathways**—VEGF-treated HDMECs exposed to γ-irradiation showed significantly higher survival rates as compared with non-treated controls (Fig. 4A, compare panel 2 with 1). This cytoprotective effect was significantly reversed by pretreating the cells with the PI3K inhibitor (LY294002) or the MAPKK inhibitor (PD98059). VEGF-treated HDMECs when treated with the p38 MAPK inhibitor (PD169316) prior to irradiation showed significantly greater protection (Fig. 4A, 5) as compared with the VEGF-treated and irradiated group (Fig. 4A, 2). This protection was marginally but not significantly increased when HDMECs were treated with a combination of the p38 MAPK and JNK inhibitors (Fig. 4A, 7). As assessed by DNA ladder analysis and TUNEL assay, VEGF-treated HDMECs showed significantly less apoptosis following γ-irradiation as compared with non-treated cells (Fig. 3A, 2 and Fig. 3B, 2). Pretreatment of these VEGF-stimulated cells with LY294002 or PD98059 prior to γ-irradiation reversed the protective effect of VEGF (Fig. 3A, 3 and 4 and Fig. 3B, 3 and 4). These results suggest that VEGF protects HDMECs through the PI3K and ERK1/2 pathways. VEGF-activated HDMECs showed a time-dependent increase in Akt and ERK1/2 phosphorylation (data not shown).
Furthermore, we investigated the cross-talk between pro-growth pathways (PI3K/Akt and ERK1/2) and prodeath pathways (p38 MAPK and JNK) in VEGF-treated and γ-irradiation-exposed HDMECs. When exposed to γ-irradiation, non-stimulated HDMECs showed a high level of phosphorylation of cell death-signaling mediators (p38 MAPK and JNK1/2, Fig. 5B) and a very low level of phosphorylation of cell growth pathway mediators (ERK1/2 and Akt, Fig. 5A). In contrast, VEGF-stimulated and γ-irradiated cells showed high levels of phosphorylation of cell growth pathway mediators (ERK1/2 and Akt) and similar or lower levels of phosphorylation of cell death pathway mediators (Fig. 5B).

The role of different signaling pathways in γ-irradiation-mediated cell death and VEGF-mediated protection was further examined using dominant negative mutants for ERK1/2, Akt, p38, and c-Jun. HDMECs were co-transfected with enhanced green fluorescent protein and the respective mutant plasmids using the Lipofectin kit. HDMECs transfected with p38 MAPK dominant negative mutants showed significantly higher survival levels when treated with γ-irradiation as compared with vector controls (Fig. 6A). HDMECs transfected with c-Jun dominant negative plasmids also showed higher survival levels when exposed to irradiation. However, this increased survival was not statistically significant. As observed with pharmacological inhibitors, HDMECs transfected with Akt mutants showed significant inhibition of VEGF-mediated cell survival when exposed to irradiation (Fig. 6B). HDMECs transfected with ERK1/2 mutants were significantly less protected against γ-irradiation as compared with HDMECs transfected with Akt mutants. These results confirm our findings that γ-irradiation mediates endothelial cell death predominantly via the p38 MAPK pathway, and VEGF protects these endothelial cells from γ-irradiation-mediated apoptosis through the PI3K-Akt pathway.

**Bcl-2 Up-regulation by VEGF Is Predominantly Mediated via the PI3K Pathway**—VEGF treatment of HDMECs showed significant up-regulation of Bcl-2 expression as reported previously (28). This was predominantly mediated via the PI3K/Akt pathway as pretreatment with the PI3K inhibitor (LY294002) completely inhibited VEGF-mediated Bcl-2 expression (Fig. 7A). The MAPKK inhibitor (PD98059) was only partially effective in inhibiting VEGF-mediated Bcl-2 expression. However, the p38 MAPK and JNK inhibitors did not show a significant effect on VEGF-induced Bcl-2 expression (Fig. 7A). Next, we
examined the effect of irradiation on Bcl-2 expression in HDMECs by immunostaining analysis. HDMECs exposed to irradiation showed a significant decrease in Bcl-2 expression as compared with non-irradiated HDMECs (compare Fig. 7B, 3 with Fig. 7C, 3). In contrast, VEGF-stimulated cells showed significantly higher Bcl-2 expression and maintained higher Bcl-2 levels even after irradiation (Fig. 7B, 2 and 4). Pretreatment with the PI3K inhibitor (LY294002) completely blocked Bcl-2 expression in VEGF-stimulated HDMECs, and the MAPKK inhibitor (PD98059) was only partially effective in inhibiting Bcl-2 expression (Fig. 7B, 5 and 6).

**HDMECs Overexpressing Bcl-2 Are Resistant to γ-Irradiation-induced Cell Death**—Previously, we have shown that HDMECs overexpressing Bcl-2 are resistant to the proapoptotic effect of antiangiogenic factors such as thrombospondin-1 (6). In this study, we further investigated if HDMECs overexpressing Bcl-2 were also resistant to γ-irradiation-mediated apoptosis. Similar to VEGF treatment, HDMECs overexpressing Bcl-2 showed significantly higher survival rates (160%) when exposed to γ-irradiation as compared with HDMECs transduced with vector control (Fig. 8).

**DISCUSSION**

Radiotherapy is one of the most widely used cancer treatments, but it is often unsuccessful because of the acquisition of radioresistance by tumor cells and vascular endothelial cells (29, 30). The mechanisms by which tumor cells and endothelial cells lining the tumor blood vessels acquire resistance to radiotherapy are poorly understood. One of the reasons might be the abundance of growth factors at the tumor site, including VEGF, which enhances endothelial cell survival and protects them from the apoptotic effects of irradiation (31). In this study we have examined the effect of γ-irradiation on endothelial cell apoptosis and the mechanisms by which VEGF protects endothelial cells from γ-irradiation-induced cell death. HDMECs exposed to γ-irradiation showed significantly higher levels of apoptosis. This endothelial cell death was predominantly mediated via p38 MAPK as HDMECs treated with the p38 MAPK inhibitor (PD169316), or HDMECs transfectected with dominant negative p38 MAPK mutants were significantly protected against γ-irradiation-induced apoptosis. In fibroblasts the p38 MAPK isotype is predominantly activated by γ-irradiation to induce cell growth arrest at G1 (20). A selective increase in c-Jun expression has been observed in brain cells undergoing apoptosis after irradiation (32). Ionizing radiation also utilizes the JNK pathway to induce apoptosis in U937 human monoblastic leukemia and bovine aortic endothelial cells (33). However, we found that the JNK inhibitor or c-Jun dominant negative mutants were only partially effective in blocking γ-irradiation-induced apoptosis in endothelial cells.

To further understand the cross-regulation of cell growth (PI3K/Akt and ERK1/2) cell death signal pathways (p38 MAPK and JNK1/2), we pretreated the HDMECs with the PI3K inhibitor (LY294002), the MAPKK inhibitor (PD98059), the p38 MAPK inhibitor (PD169316), or the JNK inhibitor, respectively, for 1 h prior to exposure to γ-irradiation. Inhibition of the PI3K and MAPK pathways by signaling inhibitors significantly up-regulated p38 MAPK activation in HDMECs. These findings could explain the enhanced cell death observed in irradiated cells where up-regulation of the proapoptotic signal via p38 MAPK occurred in cells treated with the PI3K inhibitor (LY294002) and the MAPKK inhibitor (PD98059). Similarly, Gratton et al. (16) have shown that infection with
adenovirus expressing constitutively active Akt in bovine aortic endothelial cells induces MEKK3 kinase phosphorylation, which is associated with decreased p38 MAPK activation. Conversely, activation-deficient Akt up-regulated the p38 phosphorylation.

Cell death induced by ionizing radiation varies according to cell type. Thymocytes, lymphocytes, and cells from the hematopoietic and germinal lineage are usually apoptosis-sensitive, whereas endothelial cells lining the tumor blood vessels are relatively resistant to apoptosis (34). Analysis of signaling mechanisms that may be involved in VEGF-mediated endothelial cell protection could provide valuable insight into how this protection can be reversed, which in turn would enhance the antitumor effect of γ-irradiation. Pretreatment of HDMECs with the PI3K inhibitor (LY294002) or transfection with dominant negative Akt mutants significantly reversed VEGF-mediated protective effects against γ-irradiation. Akt has been shown to phosphorylate the Bcl-2 family member Bad and Caspase-9, thus inhibiting their proapoptotic functions (35, 36). Inhibition of MAPK pharmacologically with PD98059 or by dominant negative ERK1/2 mutants was also able to reverse VEGF-mediated protection against γ-irradiation. However, it was not as effective as inhibition of the PI3K pathway. Non-stimulated HDMECs, when exposed to γ-irradiation, exhibited high levels of phosphorylation of cell death-signaling mediators (p38 MAPK and JNK) and low levels of phosphorylation of cell survival pathway mediators (Akt and ERK1/2). In contrast, VEGF-stimulated and γ-irradiated cells showed high phosphorylation of cell growth pathway mediators (Akt and ERK1/2) and similar or lower phosphorylation of cell death pathway mediators. These results demonstrate that in non-stimulated HDMECs, when exposed to γ-irradiation, the net balance is in favor of the proapoptotic pathways leading to cell death. However, in VEGF-stimulated cells, when exposed to γ-irradiation, the balance is in favor of antiapoptotic pathways. Therefore, VEGF may protect the endothelial cells from γ-irradiation by maintaining the net balance in favor of antiapoptotic pathways along with increasing the expression of antiapoptotic factors such as Bcl-2, survivin, and interleukin-8 (3, 37). Similarly, Choi et al. (38) have shown that differential activation of PI3K/Akt and p38 MAPK signaling by Ha-Ras and Ki-Ras in Rat2 cells lead to either increased resistance or increased sensitivity, respectively, to the ionizing radiation.

We have shown earlier that VEGF up-regulates the expression of the antiapoptotic protein Bcl-2 (28). In this study, we further investigated whether VEGF-mediated Bcl-2 expression is also mediated through the PI3K/Akt pathways and whether γ-irradiation has any effect on Bcl-2 expression. VEGF-induced Bcl-2 expression in HDMECs was predominantly mediated via the PI3K pathway. γ-Irradiation treatment significantly decreased Bcl-2 expression (50%) in HDMECs. These results suggest that for endothelial cells, γ-irradiation, in addition to activating the proapoptotic p38 MAPK pathway, also decreases the expression of the antiapoptotic protein Bcl-2 in HDMECs. We had shown earlier that HDMECs overexpressing Bcl-2 are resistant to thrombospondin-1 treatment (6). In the present
study, we demonstrate that Bcl-2-overexpressing HDMECs are also resistant to γ-irradiation-induced cell death.

Taken together, our data show that γ-irradiation-induced HDMEC apoptosis is predominantly mediated through the p38 MAPK pathway. VEGF protects endothelial cells from γ-irradiation, predominantly through the PI3K/Akt-Bcl-2 pathway (Fig. 9). Recent reports (39–41) have suggested that combination therapy approaches may be more effective than using a single agent, as tumor and endothelial cells lining the tumor blood vessels become resistant to individual antitumor therapies because of the abundance of growth factors at the tumor site (42). Therefore, treatment with a selective PI3K inhibitor prior to irradiation treatment may be a potentially novel strategy to overcome resistance in endothelial cells lining the tumor blood vessels, thereby enhancing the effectiveness of irradiation therapy.
FIG. 9. A schematic representation of signaling pathways involved in VEGF-mediated protection of endothelial cells against γ-irradiation-induced cell death. VEGF upon binding to its receptor on endothelial cells activates two different and independent pathways, ERK1/2 and PI3K/Akt. Activation of the PI3K/Akt pathway led to increased expression of Bcl-2 protein. Exposure of endothelial cells to γ-irradiation induced increased endothelial cell apoptosis via the activation of the p38 MAPK and JNK1/2 pathways. VEGF protected endothelial cells against γ-irradiation via the enhanced expression of Bcl-2. VEGF induced activation of the PI3K/Akt and ERK1/2 pathways and also negatively regulated the activation of p38 MAPK and JNK1/2 pathways. Solid arrows represent the known pathways, and dashed arrows represent pathways that remain to be elucidated. Solid lines represent intact DNA and dashed lines represent fragmented DNA.

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