Central Role of p53 on Regulation of Vascular Permeability Factor/ Vascular Endothelial Growth Factor (VPF/VEGF) Expression in Mammary Carcinoma

Soumitro Pal, Kaustubbh Datta, and Debabrata Mukhopadhyay

Departments of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215

ABSTRACT

The process of angiogenic switching is one of the most important factors in the growth and development of breast tumors. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is considered to be one of the most important direct angiogenic proteins that has been shown to be up-regulated in breast cancer cells. Hypoxia seems to be an important stimulant for inducing VPF/VEGF mRNA expression in human mammary tumors. Here, we have studied the roles of the tumor suppressor gene p53 and the proto-oncogene c-Src in regulating the transcription of VPF/VEGF in breast cancer cell lines MCF-7 and MDA-MB 435 under both normoxic and hypoxic conditions. p53 significantly inhibited the transcription of VPF/VEGF involving the transcription factor Sp1. Increased binding of Sp1 to the VPF/VEGF promoter has been observed when the cells were exposed to hypoxia. It has been shown that p53 makes a complex with Sp1 and inhibits its binding to the VPF/VEGF promoter to prevent the transcriptional activation. Furthermore, c-Src kinase activity was found to be increased in the hypoxic condition, and in the presence of antisense of Src, there was down-regulation of the total mRNA level and also the promoter activity of VPF/VEGF. The present study indicates that p53 can also inhibit the hypoxic induction of Src kinase activity and thereby may prevent VPF/VEGF transcription. Taken together, our data suggest a central role of p53, through which it can inhibit VPF/VEGF expression by regulating the transcriptional activity of Sp1 and also by down-regulating the Src kinase activity, under both normoxic and hypoxic conditions.

INTRODUCTION

The importance of tumor angiogenesis has been widely accepted for its role in the growth and development of solid tumors (1, 2). In most of the tumors, angiogenesis is mediated by several angiogenesis-promoting growth factors, such as VPF/VEGF, platelet derived growth factor, transforming growth factor-α, and basic fibroblast growth factor (3–7). Importantly, breast tumors are not an exception (8, 9). VPF/VEGF is a secreted protein that plays a critical role in tumor-associated microvascular hyperpermeability and angiogenesis (3, 6). Some recent studies have demonstrated the importance of microvesSEL density for malignant progression in breast cancer, underscoring the importance of angiogenesis in this type of tumor (7, 10, 11). It has been shown that, in response to hypoxic conditions, human mammary fibroblasts dramatically up-regulate VPF/VEGF mRNA and increase VPF/VEGF protein levels in accordance with the degree of oxygen deprivation (12). Oncogenes and antioncogenes also play an important role in regulating VPF/VEGF expression and angiogenesis (13–16).

The genetic alterations responsible for oncogenesis and tumor progression may underlie the ability of breast tumors to switch to an angiogenic phenotype (17). The p53 tumor suppressor gene is one of the most frequently mutated genes in human cancers (18). The loss of p53 function, via somatic mutations or the expression of viral oncoproteins, contributes to the activation of the angiogenic switch during tumorigenesis (13, 15, 17, 19). We have shown that wt p53 can significantly inhibit the VPF/VEGF transcriptional activation, although the exact mechanism of this inhibition was not explored (13). Human p53 encodes a multifunctional transcription factor that mediates cellular responses to diverse stimuli, including DNA damage and hypoxia (20). In addition to being an integral component of the surveillance mechanisms that arrest cell cycle progression under adverse conditions, p53 is also involved in mediating hypoxia-induced apoptosis (21) and inducing inhibitors of angiogenesis such as thrombospondin-1 (22, 23). It has been reported that microvesSEL density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinomas (24).

Amplification and overexpression of the neu (c-erbB2) proto-oncogene has been implicated in the pathogenesis of 20–30% of human breast cancers (25–27). Although the activation of Neu receptor tyrosine kinase appears to be a pivotal step during mammary tumorigenesis, the mechanism by which Neu signals cell proliferation is unclear. Molecules bearing a domain shared by the c-Src proto-oncogene (Src homology 2) are thought to be involved in signal transduction from activated receptor tyrosine kinases such as Neu (28). The polyomavirus middle T oncogene in the mammary epithelium develop multifocal mammary tumors that metastasize with high frequency (29). It has been reported that the potent transformational activity of polyomavirus middle T antigen is also attributed to its ability to associate with and to activate a number of c-Src family tyrosine kinases (c-Src, c-Yes, and Fyn; Refs. 29 and 30). Recent reports have indicated that human breast carcinomas contain functionally active p60c-Src (31, 32). In previous studies, we have shown that c-Src activation by hypoxia up-regulates VPF/VEGF expression, and constitutive v-Src increased VPF/VEGF mRNA (33). Thus, Src family kinases seem to play a significant role in the advancement of human breast tumors (31, 34). We have also reported that wt p53 and v-Src exert an opposing influence on VPF/VEGF gene expression (13).

In the present study, we examine in more detail the mechanistic role of p53 and c-Src in regulating the transcriptional expression of VPF/VEGF in breast cancer cell lines MCF-7 and MDA-MB 435 under both normoxic and hypoxic conditions. We demonstrate that p53 makes a complex with Sp1 and thereby inhibits Sp1-mediated VPF/VEGF transcriptional activation. p53 also plays a significant role in down-regulating hypoxia-induced Src kinase activity.

MATERIALS AND METHODS

Cell Culture. Human breast carcinoma cell lines, MCF-7 and MDA-MB 435 were maintained in DMEM with 10% fetal bovine serum (HyClone Laboratories).
Plasmids. The VPF/VEGF reporter constructs used in transient transfection assays contain sequences derived from human VPF/VEGF promoter driving expression of firefly luciferase. The 0.35- and 0.07-kb deletion mutant constructs were made by PCR from the 2.6-kb promoter fragment and subcloned into pGL-2 basic vector (Promega), as described earlier (14). Human cytomegalovirus immediate-early promoter-driven wt and mutant p53 expression plasmids (generously provided by Prof. Arnold J. Levine, Princeton University, Princeton, NJ) were used in this study. wt p53 contains wt p53 cDNA, whereas p53-SCX3 is a single-nucleotide mutation that results in a valine to alanine alteration at codon 143 in p53-wt, and p53-42N3 is a mutant human p53 cDNA derived from an epidermoid carcinoma cell line A431 (13). This p53 gene encodes an arginine to histidine change at codon 273. Src sense and antisense expression vectors were generous gifts from G. E. Gallick (The University of Texas, M.D. Anderson Cancer Center, Houston, TX; Ref. 35).

Transfection Assays. Cells were plated at 2 × 10^5 cells/60-mm dish 1 day before transfection with VPF/VEGF promoter-luciferase construct and expression plasmids using calcium-phosphate precipitation (36). The expression was normalized with a control empty-expression vector. Cells were harvested for luciferase assay 40 h after transfection. In all cotransfection experiments, transfection efficiency was normalized by assaying β-galactosidase activity using the β-galactosidase gene under control of the cytomegalovirus immediate-early promoter as internal control.

Immunoprecipitations and Western Blot Analyses. Cells were washed twice with cold PBS, lysed with ice cold lysis buffer [50 mM Tris (pH 7.5), 1% NP40, 150 mM NaCl, 1 mM NaVO₄, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 0.5% aprotinin, and 2 mM pepstatin A] and centrifuged for 10 min on ice. Cell extracts were incubated for 15 min on ice. Cells were then lysed with 0.5% NP40 and the pellets were collected and stored at -70°C. Immunoprecipitations were carried out at antibody excess using 0.5 mg of total protein with rabbit polyclonal antibodies (1 μg) directed against p53 (Santa Cruz Biotechnology) and Sp1 (Santa Cruz Biotechnology). Immunocomplexes were captured with protein A agarose beads (Pharmacia). After incubation with lysis buffer, washes, and bead-bound proteins were separated by SDS-PAGE. Western blot analysis was carried out as described earlier (14).

Kinase Assay. Src kinase activity was assayed by measuring the incorporation of [γ-32P]ATP into a Src-specific peptide (33). The cellular extracts were immunoprecipitated with Src-specific antibody (Santa Cruz Biotechnology) and the immunoprecipitates were incubated in a 25 μl-reaction mixture consisting of 30 mM Tris-HCl (pH 7.5), 0.01% Triton X-100, 10 mM MgCl₂, 0.4 mM Src-specific peptide (Upstate Biotechnology, Inc.), and 50 μM [γ-32P]ATP for 30 min at 30°C. The reaction was stopped by the addition of ice-cold 25% trichloroacetic acid. Precipitates were collected on phosphocellulose filter paper. The filters were washed with 0.75% phosphoric acid and counted for 32P using liquid scintillation spectroscopy.

Nuclear Extract Preparation and EMSAs. Nuclear extracts were prepared using MCF-7 cells following a standard protocol, with modifications (14, 16). Cells were washed in cold PBS, suspended in buffer A (10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM MgCl₂, 0.1 mM EDTA, 10 μg/ml aprotinin, 3 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride) and incubated for 15 min on ice. The nuclei were lysed with 0.5% NP40 and the pellets were resuspended in buffer C [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 3 mM DTT and 0.1 mM phenylmethylsulfonyl fluoride]. After incubation on a rotator for 25 min, samples were centrifuged at 14,000 RPM for 10 min. Clear supernatants containing the nuclear proteins were collected and stored at -70°C.

EMSAs were performed as described previously (14, 16). Briefly, EMSA binding reaction mixtures (25 μl) contained 20 mM HEPES (pH 8.4), 100 mM KCl, 20% glycerol, 0.1 mM EDTA, 0.2 mM ZnSO₄, 0.05% NP40, and 1 μg BSA. Extract protein and 200 ng of poly(dA-dT).poly(dA-dT) were added at room temperature 10 min before the addition of ~0.1 ng of radiolabeled oligonucleotide probe. After 20 min incubation at 4°C, samples were run on 7% acrylamide gel in 1X TAE (40 mM Tris-acetate and 1 mM EDTA) buffer. The radiolabeled oligonucleotide used in EMSA studies was a 188-bp PCR-generated fragment (bp -195 to -7, relative to the transcription start site) of the VPF/VEGF promoter containing the four putative Sp1 binding sites (14, 16).

RNA Analysis by Northern Blot Hybridization. Total RNA, isolated by the single-step acid-phenol extraction method (37), was separated on a formaldehyde-agarose gel, transferred to a GeneScreen (DuPont) membrane by using 10× SSC, and probed with random-primer-labeled cDNAs in a solution containing 0.5 m sodium phosphate (pH 7.2), 7% SDS, 1% BSA, 1 mM EDTA, and sonicated herring sperm DNA (50 μg/ml) at 68°C. Blots were washed three times with a solution containing 40 mM sodium phosphate (pH 7.2), 0.5% SDS, 0.5% BSA, and 1 mM EDTA at 68°C and autoradiographed.

RESULTS

p53 Inhibits Sp1-mediated Transcription of VPF/VEGF in Breast Cancer Cells. It has been shown previously that overexpression of the tumor suppressor wt p53 can inhibit transcription of many cellular and viral promoters (38). Interestingly, wt p53 specifically repressed the activity of TATA promoters that do not contain a wt p53 DNA binding sequence (39). The VPF/VEGF promoter does not possess a TATA box or wt p53 DNA binding sequence, but it does contain five Sp1 binding sites (40). Although we have shown previously that wt p53 can inhibit the VPF/VEGF promoter activity, the mechanism of this inhibition is still unclear (13). Here, we sought to determine whether wt p53 is involved in Sp1-mediated transcriptional regulation of VPF/VEGF in human breast cancer cells. To this end, MCF-7 cells were cotransfected with a 2.6-kb VPF/VEGF promoter-luciferase construct and plasmid containing wt p53 cDNA. VPF/VEGF reporter activity was decreased by 60% in comparison with the cells transfected with expression vector alone (Fig. 1A). To define the region of the VPF/VEGF promoter that is responsive to wt p53, we used two different 5’ deletions of the 2.6-kb promoter-reporter vector and cotransfected these deletions with the plasmid expressing wt p53. It has been found that wt p53 decreased the reporter activity by 70% in the 0.35-kb segment of the VPF/VEGF promoter that contains multiple Sp1 binding sites, although there was no change of reporter activity in the 0.07-kb VPF/VEGF promoter having the deleted Sp1 binding site (Fig. 1A). We also observed a similar result in MDA-MB 435 cells (data not shown). These observations reveal that in breast cancer cells, wt p53 may inhibit VPF/VEGF transcription by interfering with Sp1-mediated promoter activation. Interestingly, when we used two different mutants of wt p53, they could not down-regulate the Sp1-mediated VPF/VEGF promoter activity in MCF-7 cells (Fig. 1B).

p53 Associates with Sp1 and Prevents Its Binding to the VPF/VEGF Promoter. Because we have observed that wt p53 inhibits Sp1-mediated VPF/VEGF transcription, here we set out to determine whether, in breast cancer cells, wt p53 might form a complex with Sp1. To test this possibility, we transfected both MCF-7 and MDA-MB 435 cells with the expression vector containing wt p53. Lysates of transfected and nontransfected cells were immunoprecipitated with antibody purified antibodies to wt p53, and then Western blotting with antibody directed against Sp1 was performed. We found a strong band corresponding to Sp1 in the cases of the transfected samples of both cell lines (Fig.1C).

To examine the consequences of the association between p53 and Sp1 on VPF/VEGF transcription, we performed EMSA using a 188-bp VPF/VEGF promoter fragment containing all four Sp1 binding sites, with the nuclear extracts of MCF-7 cells transfected with or without wt p53. As shown in Fig. 1D, wt p53 inhibited the binding of Sp1 to the VPF/VEGF promoter. This specific protein-DNA complex formation was competed away with wt p53, and thereby, may prevent the VPF/VEGF transcriptional activation by blocking Sp1 and promoter interaction.

p53 Can Also Down-Regulate Hypoxia-induced Activation of VPF/VEGF Transcription. Hypoxia appears to be an important stimulus in promoting the overexpression of VPF/VEGF. Previous studies...
have indicated that p53 can down-regulate hypoxia-mediated VPF/VEGF transcription through degradation of HIF-1α (17). Here, we attempted to explore whether wt p53 can also inhibit the hypoxic induction of VPF/VEGF transcriptional activation involving Sp1. To this end, MCF-7 cells were transfected with 2.6-kb promoter-reporter construct (containing both HIF-1α and Sp1 binding sites) and wt p53 (0.5 μg) expression vectors. Cells were harvested for luciferase assays 40 h after transfection, and fold activation was calculated as relative to the activity of same reporter construct cotransfected with an empty expression vector. A, empty expression vector; B, wt p53 expression. MCF-7 cells were cotransfected with 0.35-kb VPF/VEGF promoter-luciferase construct (1.0 μg) and mutant p53 (p53-SCX3 and p53–4.2N3) expression vectors. After harvesting the cells, luciferase activity was measured as described above. C, extracts were prepared from MCF-7 and MDA-MB 435 cells and transfected with wt p53 expression vector. Both the transfected and untransfected cell lysates were immunoprecipitated with a polyclonal antibody directed against wt p53. All immunoprecipitates were then captured by protein A-Sepharose beads. After thorough washings, the Sepharose beads were boiled in SDS buffer and separated by SDS-PAGE. Western blotting (Blot) were performed by using Sp1 polyclonal antibody. Lane 1, transfected with wt p53 expression vector; Lane 2, transfected with empty expression vector; Lanes 3 and 4: control cell lysates, immunoprecipitated with polyclonal antibodies, directed against Sp1 and IGF-1 respectively. D, by using a 188-bp VPF/VEGF promoter fragment (having all of the four Sp1 binding sites) as the probe, EMSA was performed with partially purified nuclear extracts of MCF-7 cells, transfected with wt p53 (0.5 μg), and subjected to both normoxic (I) and hypoxic conditions (II). In hypoxic conditions, the cells were exposed to hypoxia overnight. Nuclear extracts were prepared from the transfected cells 40 h after transfection. Lane 1, probe control, without any nuclear extract. In Lane 2 of the normoxic condition, the unlabeled Sp1 consensus oligonucleotide (oligo; 10-fold molar excess) was added to the binding reaction mixture of the control sample (without any transfection) to show that it can compete away the specific protein-DNA complexes. In Lane 5 of the hypoxic condition, Sp1 polyclonal antibody (2 μg) was added to the reaction mixture of the hypoxic sample to show the supershift of the Sp1 band.

Fig. 1. Effect of p53 on Sp1-mediated transcriptional activation of VPF/VEGF. A, MCF-7 cells were cotransfected with 2.6-, 0.35-, or 0.07-kb VPF/VEGF promoter-luciferase constructs (1.0 μg) and wt p53 (0.5 μg) expression vectors. Cells were harvested for luciferase assays 40 h after transfection, and fold activation was calculated as relative to the activity of same reporter construct cotransfected with an empty expression vector. A, empty expression vector; B, wt p53 expression. MCF-7 cells were cotransfected with 0.35-kb VPF/VEGF promoter-luciferase construct (1.0 μg) and mutant p53 (p53-SCX3 and p53–4.2N3) expression vectors. After harvesting the cells, luciferase activity was measured as described above. C, extracts were prepared from MCF-7 and MDA-MB 435 cells and transfected with wt p53 expression vector. Both the transfected and untransfected cell lysates were immunoprecipitated with a polyclonal antibody directed against wt p53. All immunoprecipitates were then captured by protein A-Sepharose beads. After thorough washings, the Sepharose beads were boiled in SDS buffer and separated by SDS-PAGE. Western blottings (Blot) were performed by using Sp1 polyclonal antibody. Lane 1, transfected with wt p53 expression vector; Lane 2, transfected with empty expression vector; Lanes 3 and 4: control cell lysates, immunoprecipitated with polyclonal antibodies, directed against Sp1 and IGF-1 respectively. D, by using a 188-bp VPF/VEGF promoter fragment (having all of the four Sp1 binding sites) as the probe, EMSA was performed with partially purified nuclear extracts of MCF-7 cells, transfected with wt p53 (0.5 μg), and subjected to both normoxic (I) and hypoxic conditions (II). In hypoxic conditions, the cells were exposed to hypoxia overnight. Nuclear extracts were prepared from the transfected cells 40 h after transfection. Lane 1, probe control, without any nuclear extract. In Lane 2 of the normoxic condition, the unlabeled Sp1 consensus oligonucleotide (oligo; 10-fold molar excess) was added to the binding reaction mixture of the control sample (without any transfection) to show that it can compete away the specific protein-DNA complexes. In Lane 5 of the hypoxic condition, Sp1 polyclonal antibody (2 μg) was added to the reaction mixture of the hypoxic sample to show the supershift of the Sp1 band.

Fig. 2. Effect of p53 on hypoxia-induced activation of VPF/VEGF transcription. MCF-7 cells were cotransfected with 2.6-kb VPF/VEGF promoter-luciferase construct (1.0 μg) and wt p53 expression vector (0.5 μg). One set of cells was subjected to the hypoxic condition for overnight. Cells were harvested for luciferase assays 40 h after transfection, and fold activation was calculated as relative to the activity of the same reporter construct cotransfected with an empty expression vector.
Activation of Src Kinase Activity in Breast Cancer Cells Under Hypoxia. Hypoxia is a potent inducer of tyrosine kinase cascade, and the activation of tyrosine kinases has been found to be critical in signaling triggered by growth factors (33). Here, we measured the Src kinase activity in MDA-MB 435 cells under hypoxic conditions. The breast cancer cells were exposed to hypoxia for 15, 30, and 60 min, respectively. The cell extracts were used to perform Src kinase assays using an Src-specific peptide as a substrate. As shown in Fig. 3A, the substrate was strongly phosphorylated when the cells were exposed to hypoxic condition for 30 min, although the Src protein levels were the same in all of the conditions (Fig. 3B). This result suggests that Src, activated by hypoxia, is a key signaling molecule in human breast cancer cells.

c-Src Promotes Increased VPF/VEGF mRNA Levels in Breast Cancer Cells Subjected to Hypoxia. To analyze the role of c-Src on hypoxia-induced VPF/VEGF gene expression in human breast carcinoma, MCF-7 cells were transfected with Src antisense and sense cDNA containing expression vectors and afterward subjected to hypoxia. Interestingly, in Northern blot analysis, it has been observed that the hypoxic induction of VPF/VEGF mRNA expression was effectively blocked in Src antisense-transfected MCF-7 cells as compared with the Src sense-transfected ones (Fig. 4). We also observed a similar type of inhibition of hypoxia-induced VPF/VEGF expression in MDA-MB 435 cells using Src antisense cDNA (data not shown). This result clearly suggests the importance of c-Src in increasing the VPF/VEGF mRNA level in human breast cancer cells under hypoxic conditions.

c-Src Induces Hypoxia-mediated Transcriptional Activation of VPF/VEGF. As the activation of c-Src has been found to be an important factor in the regulation of hypoxia-induced up-regulation of VPF/VEGF, we set out to determine whether the antisense of Src could block the hypoxia-induced VPF/VEGF transcriptional activation. Indeed, Fig. 5 shows that in MCF-7 cells cotransfected with 2.6-kb promoter-luciferase construct and Src antisense cDNA, the hypoxia-induced VPF/VEGF transcriptional activation was significantly reduced in comparison to the cells transfected with Src sense cDNA. This result indicates that in breast cancer cells, c-Src activation is critical for hypoxia-mediated up-regulation of VPF/VEGF transcription.

p53 Can Down-Regulate Hypoxia-induced Src Kinase Activity in Breast Cancer. Inasmuch as p53 plays an important role in regulating tumor angiogenesis and because c-Src activity seems to be crucial for transcriptional expression of VPF/VEGF in breast cancer cells under hypoxia, we tried to examine whether p53 could inhibit Src kinase activity in the MDA-MB 435 cell line. Src kinase activity was measured in the p53-transfected cells, subjected to both normoxic and hypoxic conditions. Interestingly, p53 significantly decreased the normoxic as well as hypoxic induction of Src kinase activity (Fig. 6).
The mutant p53 could not decrease either normoxic or hypoxic induction of Src kinase activity (Fig. 6). This result clearly suggests that p53 may play an important role in regulating tumor angiogenesis by inhibiting Src kinase activity under both normoxic and hypoxic conditions.

**DISCUSSION**

The genetic alterations involved in tumorigenesis are responsible for the phenotypic characteristics of cancer cells (17, 18, 41). The process of tumor angiogenesis is tightly controlled by the balance of positive and negative regulatory pathways (13, 42). The molecular mechanism of the angiogenic switch is a fundamental determinant of breast tumor growth and progression (7, 10, 11). Although several factors have been described as the inducer of tumor angiogenesis, VPF/VEGF is considered as the most important directly acting angiogenic cytokine (3, 6). Recent clinical studies have demonstrated quantification of intratumoral VPF/VEGF levels may be useful to quantify of intratumoral VPF/VEGF levels may be useful to assess the angiogenic phenotype of breast tumors (7, 10, 11). It plays an important role in vascuogenesis in the presence of wt p53 and other factors have been associated with VPF/VEGF expression in breast cancer cell lines (7, 10, 11). It has been shown that p53 interacts with the transcription factor Sp1 and thereby prevents its binding to the VPF/VEGF promoter for its transcriptional activation (17, 46). In this study, we report that, like HIF-1α, Sp1 also plays a significant role in promoting VPF/VEGF transcription in breast cancer cells under hypoxic conditions (Fig. 1D). We have shown that, in this condition, there is increased binding of Sp1 to the VPF/VEGF promoter (Fig. 1D). We have also demonstrated that, as under normoxic conditions, p53 can also down-regulate the hypoxic induction of VPF/VEGF transcriptional activation in breast cancer cells that prevents the binding of Sp1 to its promoter (Figs. 1D and 2). The exact role of HIF-1α in this situation needs to be explored.

It has been reported that higher activity of the proto-oncogene c-Src is necessary for the increased expression of VPF/VEGF in different tumor cell lines, including glioma (47), colon carcinoma (35), ovarian carcinoma (48), lung carcinoma (49), and also in primary culture like mouse brown adipocytes (50) or in retinal vascular disorder (51). This phenomenon was observed not only in hypoxia but also in growth factor-regulated or X-ray- or C-ion-induced VPF/VEGF expression (49, 50, 52). In the present paper, we report increased c-Src kinase activity in MDA-MB 435 cells under hypoxia (Fig. 3). In breast cancer cell lines, c-Src is an important modulator of VPF/VEGF transcription under hypoxia because, in the presence of the antisense of Src, there was a down-regulation of the total mRNA level and also of the promoter activity (Figs. 4 and 5). Previously, we have reported that v-Src in presence of wt p53 was unable to activate the transcription of the VPF/VEGF promoter (13). The present study suggests that wt p53, apart from its inhibitory role on the transcription factor Sp1, may also regulate c-Src activity and thereby down-regulate VPF/VEGF transcription, either directly or indirectly (Fig. 6). To understand the function of p53 and c-Src in breast cancer cells, we transiently overexpressed wt p53 as well as its dominant-negative mutant in MCF-7 and MDA-MB 435 cell lines, and we determined their Src kinase activity in both normoxic and hypoxic conditions (Fig. 6). The observation of down-regulation of c-Src kinase activity, only through wt p53, has led us to conclude that p53 has a more direct effect on the inhibition of VPF/VEGF transcription by c-Src and that this inhibitory function is probably different from its effect on Sp1.

In conclusion, here we define a central inhibitory role of p53 on Src kinase activity of breast cancer cells, subjected to both normoxic and hypoxic conditions. A, MDA-MB 435 cells were transfected with wt- p53 or mutant p53 (4.2N3) expression vector (0.5 μg). After 24 h of transfection, one set of cells from both transfected and untransfected groups were subjected to hypoxic conditions overnight. Afterward, a kinase assay of c-Src was performed using specific substrate peptide for Src kinase. The percentage of activation of each experiment was determined in comparison with [γ-32P]ATP incorporation under normoxic conditions, which was considered as 100%. Results were the average of three independent experiments. B, expression of c-Src has been shown in all of the transfected samples of MDA-MB 435 cells.
region of the VPF/VEGF gene, under both normoxic and hypoxic conditions. p53 also regulates c-Src kinase activity and thereby inhibits c-Src-induced VPF/VEGF expression under the above conditions.

ACKNOWLEDGMENTS

We thank Dr. Arthur Mercurio for helpful comments on the manuscript.

REFERENCES

Central Role of p53 on Regulation of Vascular Permeability Factor/Vascular Endothelial Growth Factor (VPF/VEGF) Expression in Mammary Carcinoma

Soumitro Pal, Kaustubh Datta and Debrabrata Mukhopadhyay


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/18/6952

Cited articles
This article cites 51 articles, 26 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/18/6952.full.html#ref-list-1

Citing articles
This article has been cited by 39 HighWire-hosted articles. Access the articles at:
/content/61/18/6952.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.