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Basic FGF Downregulates TSP50 Expression Via the ERK/Sp1 Pathway

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ABSTRACT

Previous studies demonstrated that the expression of testes-specific protease 50 (*TSP50*) was increased in breast cancer cells and that overexpression of TSP50 can promote tumorigenesis. Thus, it is important to identify the regulatory mechanisms of TSP50 for tumor therapy. In this study, we elucidated the mechanism underlying TSP50 downregulation by basic fibroblast growth factor (bFGF). We used MDA-MB-231 and HEK293T cell lines to address this issue. RT-PCR and promoter activity assays indicated that bFGF downregulates TSP50 expression via transcriptional activation. We next investigated the signaling pathway that mediated the effect of bFGF on TSP50 transcription, and identified that bFGF induced the phosphorylation of ERK and Sp1. An ERK inhibitor suppressed Sp1 phosphorylation and bFGF-reduced TSP50 expression at the mRNA level. In addition, the dominant negative (DN) mutants of ERK and Sp1 both suppressed the reduction of TSP50 by bFGF. Deletion and mutation analyses indicated that the Sp1 site, located within the +237/+239 region of the human TSP50 promoter, is the major responsive element for bFGF. Taken together, our results strongly suggest that bFGF mediates TSP50 downregulation by ERK activation, leading to the phosphorylation of Sp1 in this process. J. Cell. Biochem. 111: 75–81, 2010.

KEY WORDS: TSP50; bFGF; Sp1; ERK

Testes-specific protease 50 (TSP50) was discovered in a hypomethylated DNA fragment isolated from human breast cancer cells using the methylation sensitive representational difference analysis technique. TSP50 transcripts were detected predominantly in human testes but were not visible in other normal tissues. However, most patients with breast cancer showed abnormal TSP50 activation and expression [Yuan et al., 1999; Shan et al., 2002]. Our studies also revealed that overexpression of TSP50 can promote tumorigenesis [Wu et al., 2007]; therefore, determining the regulatory mechanisms of TSP50 may be important for tumor therapy. Previous studies that have characterized the promoter of TSP50 indicated that the putative Sp1 (+237 to +239) and CCAAT/

Abbreviations used: TSP50, testes-specific protease 50; bFGF, basic fibroblast growth factor; ERK, extracellular regulated protein kinases; MAPK, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; p38, p38 mitogenactivated protein kinase; PI3K, phosphoinositide 3-kinase; Sp1, Sp1 transcription factor; C/EBP, CCAAT/enhancer binding protein.

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enhancer binding protein (C/EBP) (−15 to −13) binding sites are more important for the basal transcription activity of the human TSP50 promoter. Additionally, these studies show that the tumor suppressor p53 inhibits TSP50 gene expression [Xu et al., 2007; Wang et al., 2008]. However, the regulatory mechanisms involved in TSP50 expression are still far from being fully understood.

Basic fibroblast growth factor (bFGF) is a pleiotropic growth factor, and bFGF-induced signal transduction involves the activation of multiple mitogen-activated protein kinases (MAPKs) such as ERK, p38, and c-Jun N-terminal kinase (JNK) [Tanaka et al., 1999; Im et al., 2007; Walsh et al., 2008]. bFGF can also activate the phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway [Abe et al., 2007; Hong et al., 2009]. These pathways are important for many fundamental cellular processes, including proliferation, differentiation, and survival. Sp1 and some other transcription factors are known to be phosphorylated and activated by ERK [Bouwman and Philipsen, 2002] and act as regulators of bFGF [Bonello and Khachigian, 2004]. The phosphorylation of Sp1 has been shown to regulate target genes in both positive and negative directions [Chu and Ferro, 2005].

Previous studies have proven that the putative Sp1 binding site in the TSP50 promoter is very important for TSP50 transcription [Xu et al., 2007; Wang et al., 2008] and that ERK can phosphorylate and activate Sp1 in response to bFGF activation. Therefore, we have investigated the regulation of TSP50 expression by bFGF and sought to identify the signal pathway involved in this process.

MATERIALS AND METHODS

CELL CULTURE AND EXPERIMENTAL REAGENTS

Human embryonic kidney 293T (HEK293T) cells and the human breast cancer cell line MDA-MB-231 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% newborn calf serum (NCS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C and under 5% CO_2. The generation of a human TSP50 promoter-luciferase reporter construct and its deletion and point mutant constructs were reported in the previous study [Wang et al., 2008]. The expression vector of a dominant negative (DN) mutant of ERK, in which the catalytic and putative activating phosphorylation sites were mutated [Sroka et al., 2007], was a gift from Dr. Bowden G Tim (Arizona Cancer Center, University of Arizona, Tucson, AZ). pEBGNLS and pEBGSp1 were obtained from Dr. Gerald Thiel (Department of Medical Biochemistry and Molecular Biology, University of Saarland Medical Center). U0126 was purchased from Promega (Madison, WI, USA). PD98059, SP600125, SB203580, and LY294002 were purchased from ALEXIS (San Diego, CA). Anti-ERK, anti-JNK, anti-p38, anti-AKT, anti-Sp1, and anti-Sp3 antibodies were obtained from Santa Cruz (Santa Cruz, CA). Anti-phospho-ERK, anti-phospho-JNK, anti-phospho-p38, and anti-phospho-AKT antibodies, and bFGF were obtained from Cell Signaling (Boston, MA). The anti-phospho-Sp1 antibody was obtained from Abcam (Cambridge, MA).

ANALYSIS OF LUCIFERASE ACTIVITY

The TSP50 promoter deletion constructs pGL3 − 170/+454, pGL3 + 6/+454, pGL3 + 176/+454, and pGL3 + 176/+239, and the following point mutation constructs that disrupt specific transcription factor binding sites were previously described in detail: Sp1, pGL3 − 170/+454(pm + 237/+239); NF-κB, pGL3 − 170/+454(pm − 11/−9); and C/EBP, pGL3 − 170/+454(pm − 15/−13) [Wang et al., 2008]. Cells were plated onto 6-well plates and grown overnight, and were then co-transfected with 4 μg of full-length, deletion or mutant TSP50 promoter-luciferase constructs and 1 μg of β-galactosidase plasmid. Twenty-four hours after transfection, cells were treated with various drugs in 1% NCS medium for 48 h. The promoter activity was determined by a luciferase assay system. For some experiments, expression vectors of the constitutive DN-ERK mutant were co-transfected with TSP50 promoter-luciferase constructs and promoter activity was assayed at 48 h after transfection. To evaluate transfection efficiencies, we co-transfected the cells with the β-galactosidase plasmid and measured β-galactosidase activity by a chemiluminescent assay [Wang et al., 2008]. The luciferase activity was measured using FLUOstar OPTIMA (BMG Labtech, Germany).

RNA EXTRACT AND RT-PCR

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was quantified by measuring the absorbance (A260) and stored at −80°C until use. One microgram of total RNA was reverse transcribed by oligo (dT) primers using the Reverse Transcription System (TAKARA, Dalian, China). The single-stranded cDNA was amplified by PCR using TSP50-specific primer and β-actin primer pairs (Table I). PCR was performed for 30 cycles (each cycle consisting of 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s). The PCR products were analyzed by electrophoresis on a 1% agarose gel.

WESTERN BLOT ANALYSIS

Subconfluent cell monolayers were washed 3 times with PBS and then lysed in HEPES containing 0.5% NP-40 and a mixture of protease inhibitors. An aliquot of the cell lysates was used to evaluate the protein content by colorimetric assay. Equal protein concentrations from each of the cell lysates were analyzed by 10% SDS–PAGE. After electroblotting the gels onto polyvinylidene difluoride (PVDF) membranes, the membranes were probed with different primary antibodies. Enhanced ChemiLuminescence (ECL) (Beyotime, Shanghai, China) reagents were used to visualize the protein bands on the blots.

STATISTICAL ANALYSIS

The data were analyzed by the analysis of variance using Student's *t*-test. Values of $P < 0.05$ were considered statistical significant.

TABLE I. Primer for RT-PCR

Gene name	Primer sequence
TSP50	Forward: 5′ cggatccATGCAGGGGAAGCC3′ Reverse: 5′gctctagaAGTCAGAGGGCAG3′
β-Actin	Forward: 5′TCGTGCGTGACATTAAGGAG 3′ Reverse: 5′ATGCCAGGGTACATGGTGGT3′

RESULTS

bFGF DECREASES TSP50 VIA TRANSCRIPTIONAL ACTIVATION

Members of the FGF family play critical roles during organogenesis and carcinogenesis [Maher, 1999; Dvorak et al., 2006; Abate-Shen and Shen, 2007]. Thus, we speculated that there might be an association between FGF and TSP50. To investigate the effects of bFGF on TSP50 expression, we treated MDA-MB-231 and HEK293T cells with or without bFGF (10 ng/ml), and then compared the TSP50 mRNA levels by semiquantitative RT-PCR analysis. The results indicated that the TSP50 mRNA level decreased by an average of 50–30% in bFGF-treated MDA-MB-231 and HEK293T cells when compare with that in control cells, which were not exposed to bFGF (Fig. 1A,B). This result indicates a similar mechanism of bFGF-dependent transcriptional regulation of TSP50 in the two cell lines. A promoter activity assay also revealed that bFGF downregulated TSP50 expression via transcriptional activation in HEK293T cells (Fig. 1C).

ERK INVOLVED IN THE REDUCTION OF TSP50 BY bFGF

We next addressed the signal transduction pathway by which bFGF downregulates TSP50 expression. Since bFGF activates both the PI3K/AKT pathway [Abe et al., 2007; Hong et al., 2009] and MAPK pathway, including ERK, p38, and JNK [Im et al., 2007; Tanaka et al., 1999], we investigated the effect of bFGF on the phosphorylation of the transcription factors in the MAPK and AKT pathways. bFGF enhanced ERK and JNK phosphorylation, but had no effect on p38 and AKT. To evaluate the importance of each signaling pathway in this process, we used chemical inhibitors targeting the ERK, JNK, p38, and AKT kinase pathways. After 24 h of transfection with the TSP50 promoter construct, the cells were incubated with PD98059 (10 mM), U0126 (10 mM), SP600125 (20 mM), SB203580 (10 mM), or LY294002 (10 mM) for 12 h in HEK293T cells (Fig. 2A). Promoter activity was assayed and normalized using β-gal activity. Our results demonstrated that the ERK pathway inhibitor PD98059 induced a 30% increase of TSP50 activity (Fig. 2B), and another specific inhibitor of ERK1/2, namely, U0126, had the same effect. Chemical inhibitors of JNK (SP600125), p38 kinase (SB203580), and PI3K (LY294002) had little effect.

Next, we examined the role of ERK in the transcriptional regulation of TSP50 mRNA in MDA-MB-231 cells. We found that the ERK inhibitor U0126 blocked FGF-stimulated TSP50 mRNA suppression (Fig. 2C).

To further confirm the effect of bFGF-induced ERK signaling on TSP50 expression, we transfected DN-ERK into HEK293T cells (Fig. 3A), and then analyzed the TSP50 promoter activity. We observed that by reducing cellular ERK activity, TSP50 promoter activity was enhanced in response to bFGF (Fig. 3B). These results suggest that bFGF downregulated TSP50 expression via the ERK signaling pathway.

Sp1 IS INVOLVED IN THE REDUCTION OF TSP50 BY bFGF

Previous studies indicated the Sp1 and C/EBP binding sites are important for the basal transcriptional activity of the human TSP50 promoter. Some reports also revealed that Sp1 can be phosphorylated and activated by bFGF [Bachmeier and Löffler, 1997; Geller

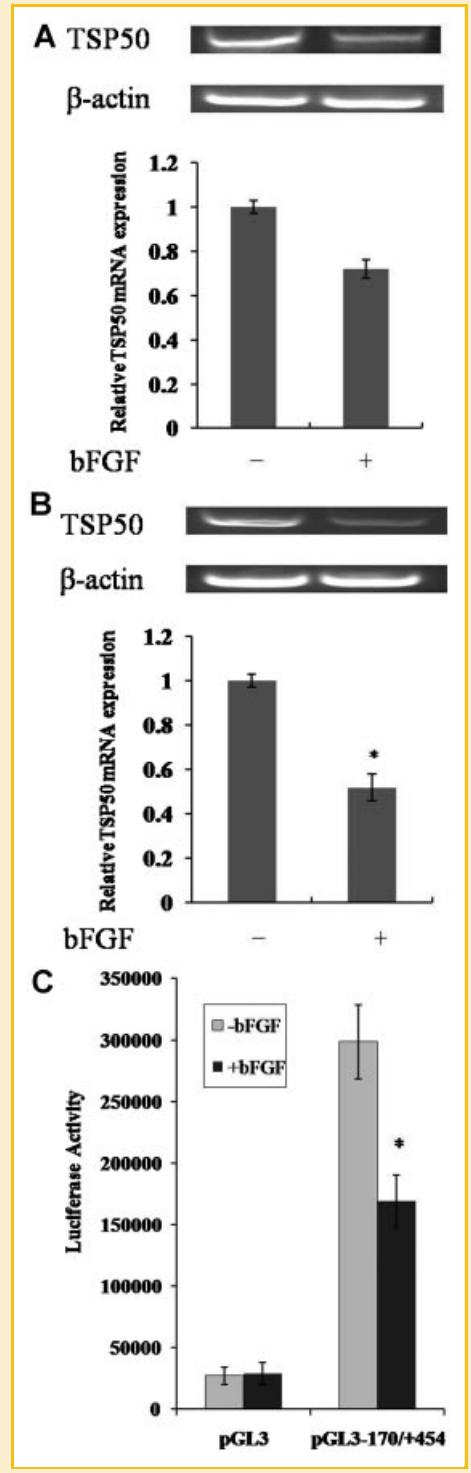

Fig. 1. Decrease of TSP50 expression by bFGF is mediated by transcriptional activation. TSP50 mRNA levels in HEK293T (A) and MDA-MB-231 (B) cells were analyzed by RT-PCR. β-Actin was used as an internal control to check the efficiency of cDNA synthesis and PCR amplification. C: TSP50 promoter luciferase construct was transfected into HEK293T cells and promoter activity was assayed and normalized to β-gal activity. Data are presented as the mean luciferase activity of three independent transfections. Statistically significant differences ($P < 0.05$) in paired Student's *t*-test are marked with an asterisk.

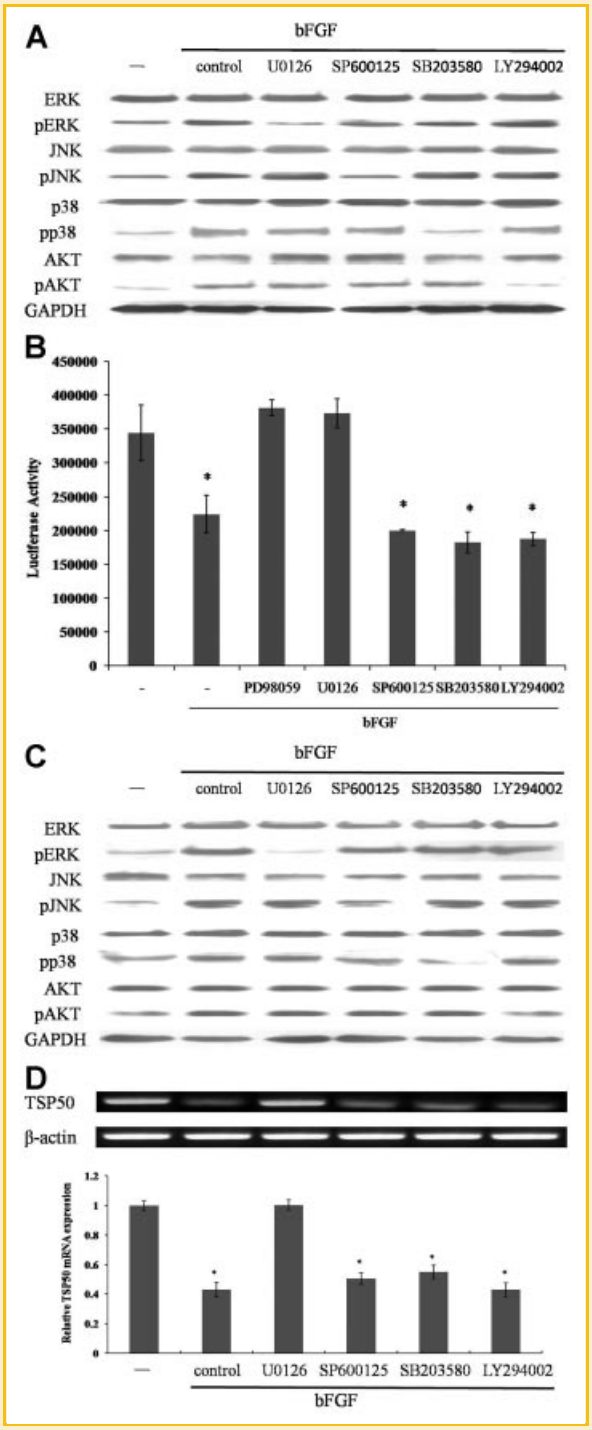

Fig. 2. Effects of signaling pathway inhibitors on the bFGF-induced down-regulation of TSP50 expression. HEK293T cells were transfected with the TSP50 gene promoter. The phosphorylation of ERK, JNK, p38, and AKT (A) and TSP50 Luciferase activity (B) were measured in HEK293T cells. The phosphorylation of ERK, JNK, p38, and AKT (C) and TSP50 mRNA expression (D) were measured in MDA-MB-231 cells. Cells were cultured with or without 10 ng/ml of bFGF in the presence or absence of PD98059, U0126 (ERK inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor), or LY294002 (PI3K inhibitor). Statistically significant differences ($P < 0.05$) in paired Student's *t*-test are marked with an asterisk.

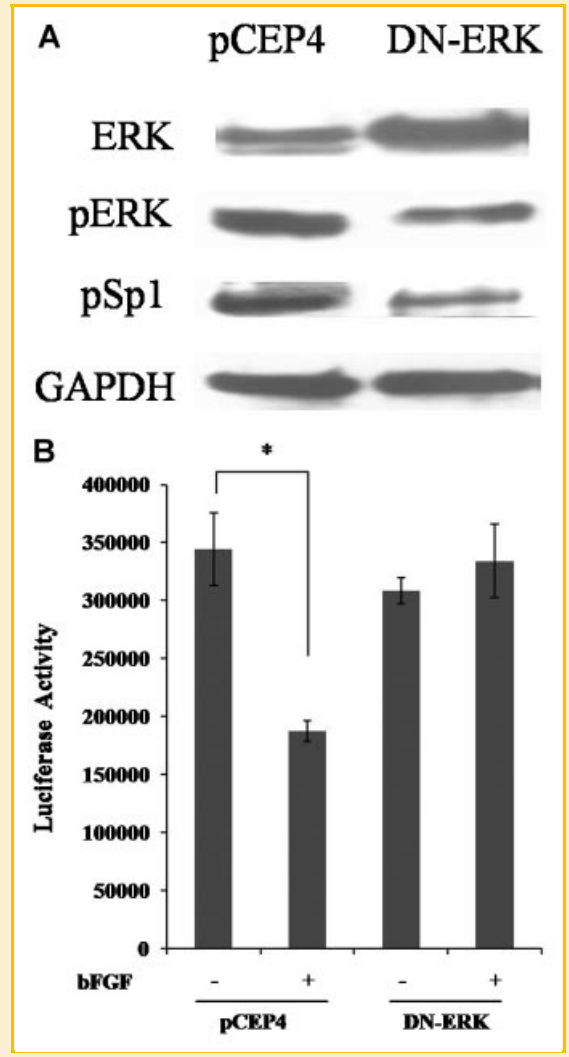

Fig. 3. The phosphorylation of ERK promotes bFGF-reduced transcriptional activity of TSP50. A: Effect of ERK phosphorylation and Sp1 phosphorylation induced by bFGF in HEK293T cells. Cells were treated with bFGF after 48 h transfection with control (pCEP4) or DN-ERK expression vector. Cells were then lysed and the phosphorylation of ERK and Sp1 were analyzed by Western blotting. B: Effect of ERK phosphorylation on TSP50 promoter activity stimulated by bFGF. The pGL3 − 170/+454 reporter construct was co-transfected with control or DN-ERK expression vector into HEK293T for 48 h and promoter activity was assayed and normalized by β-gal activity. Statistically significant differences ($P < 0.05$) in paired Student's *t*-test are marked with an asterisk.

et al., 2001; Chu and Ferro, 2005; Ulbrich et al., 2008] and that bFGF can induce Sp1 activation via ERK [Merchant et al., 1999; Milanini-Mongiat et al., 2002]. Therefore, we analyzed the induction of Sp1 phosphorylation after bFGF stimulation in MDA-MB-231 and HEK293T cells. Our results indicated that bFGF increased the amount of Sp1 phosphorylation. Furthermore, the ERK inhibitor U0126 blocked Sp1 phosphorylation, but did not affect Sp3 translocation to the nucleus in both MDA-MB-231 and HEK293T cells treated with bFGF (Fig. 4A,B). Additionally, DN-ERK also reduced Sp1 phosphorylation (Fig. 3A).

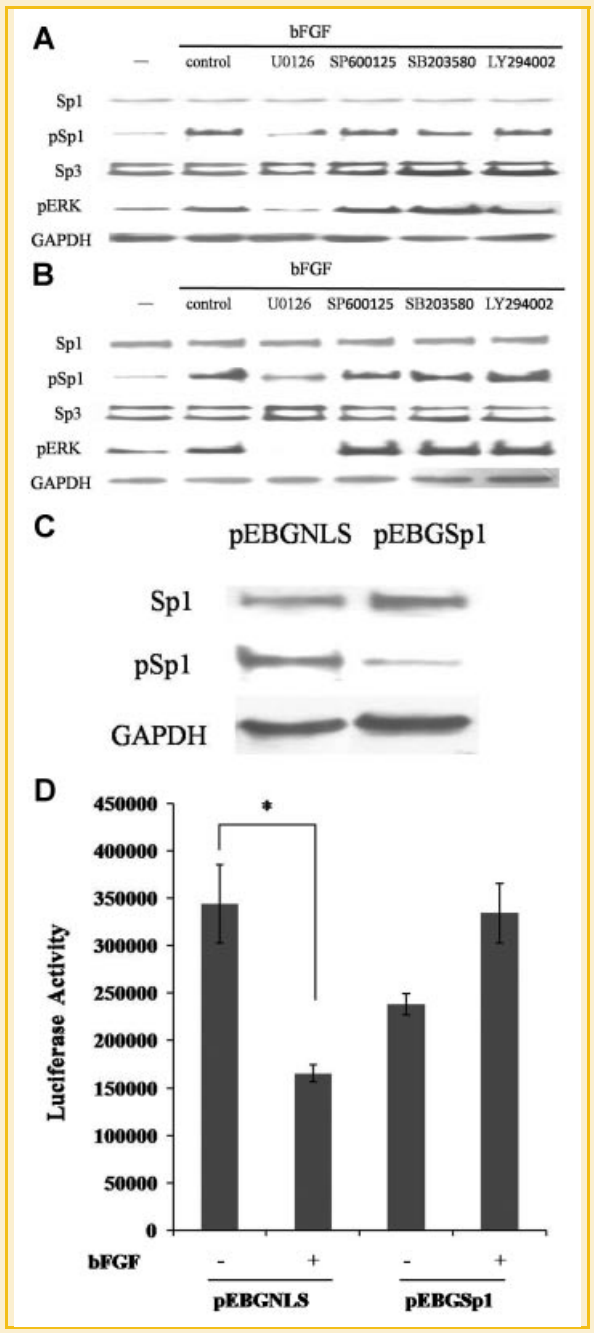

Fig. 4. Phosphorylation of Sp1 is induced by bFGF via the ERK pathway. Sp1, phosphorylation of Sp1, Sp3, and phosphorylation of ERK was measured by Western blotting in MDA-MB-231 cells (A) and HEK293T cells (B) cultured with or without 10 ng/ml of bFGF in the presence or absence of U0126 (ERK inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor), or LY294002 (PI3K inhibitor). C: Effect of DN-Sp1 on Sp1 phosphorylation in HEK293T cells. Cells were treated with bFGF after 48 h transfection with control (pEBGNLS) or DN-Sp1 (pEBGSp1) expression vector. Cells were then lysed and the phosphorylation of Sp1 was analyzed by Western blotting. D: DN-Sp1 suppresses bFGF-induced TSP50 transcriptional activity. The pGL3 − 170/+454 reporter construct was co-transfected with control (pEBGNLS) or DN-Sp1 (pEBGSp1) expression vector into HEK293T for 48 h and the promoter activity was assayed and normalized against β-gal activity. Reproducibility of these results was confirmed in three experiments. Statistically significant differences ($P < 0.05$) in paired Student's *t*-test are marked with an asterisk.

To confirm the effect of Sp1 on bFGF-reduced TSP50 expression, we transfected the DN-Sp1 into HEK293T cells (Fig. 4C). Transient transfection of the DN-Sp1 mutant [Al-Sarraj et al., 2005] revealed that bFGF could not suppress TSP50 transcription (Fig. 4D). These results suggest that bFGF downregulated TSP50 expression in an ERK- and Sp1-dependent manner.

bFGF REGULATES TSP50 PROMOTER ACTIVITY VIA THE Sp1 TRANSCRIPTION FACTOR BINDING SITE

Next, we investigated the critical elements that mediate the regulatory effect of bFGF on TSP50 promoter activity using various deletion mutants. Our results showed that pGL3 − 170/+454, pGL3 + 6/+454, pGL3 + 176/+454, and pGL3 + 176/+239 deletion mutants, containing the region of human TSP50 promoter, were fully responsive to bFGF (Fig. 5A). Conversely, reduction of bFGF-induced promoter activity was found in the pGL3 + 240/+454 deletion, which only contained a 217 bp region of the TSP50 promoter. These data indicate that the TSP50 promoter region from +176 to +239 encodes information important for conveying full bFGF responsiveness. We have reported that putative Sp1 and C/EBP transcription factor binding sites located within the +237 to +239 and −15 to −13 regions of the TSP50 promoter play important roles in the control of TSP50 expression [Wang et al., 2008]. Therefore, it is possible that bFGF downregulates TSP50 expression via these Sp1 and/or C/EBP sites. To confirm our hypothesis, we mutated the Sp1, C/EBP, and other transcription factor binding sites. We found that mutation of the Sp1 site, similar to that in the pGL3 − 170/+454(pm + 237/+239) construct, completely abrogated bFGF-induced responsiveness (Fig. 5C). These results suggest that this Sp1-response element mediates bFGF repression of the TSP50 promoter activity.

Our findings show that the ERK signaling pathway regulates TSP50 transcription, and that this involves the activation of the Sp1 transcription factor by bFGF.

DISCUSSION

TSP50 is a testis-specific gene that was determined to be abnormally activated in many breast cancer biopsies [Shan et al., 2002; Xu et al., 2004]. Our previous result revealed that overexpression of TSP50 in CHO cells that did not express TSP50 promoted tumorigenesis, while knockdown of TSP50 in the mouse breast cancer cells EMT6 inhibited tumor cell proliferation, migration, and invasion [Wu et al., 2007]. These results suggested that the downregulation of TSP50 may be a powerful approach for breast cancer therapy.

Previously, we have characterized the TSP50 gene promoter and identified the *cis*- and *trans*-acting elements influencing TSP50 transcription [Wang et al., 2008]. The minimal promoter region of the TSP50 gene was located between nucleotides −170 and +454 relative to the transcription initiation site (+1). Sp1 and C/EBP transcription factor binding sites are important for basal transcription activity.

In this study, we provide the evidence of bFGF acting through the ERK/Sp1 signaling pathway to decrease TSP50 expression. We now expand on these findings and identify the signaling pathway that

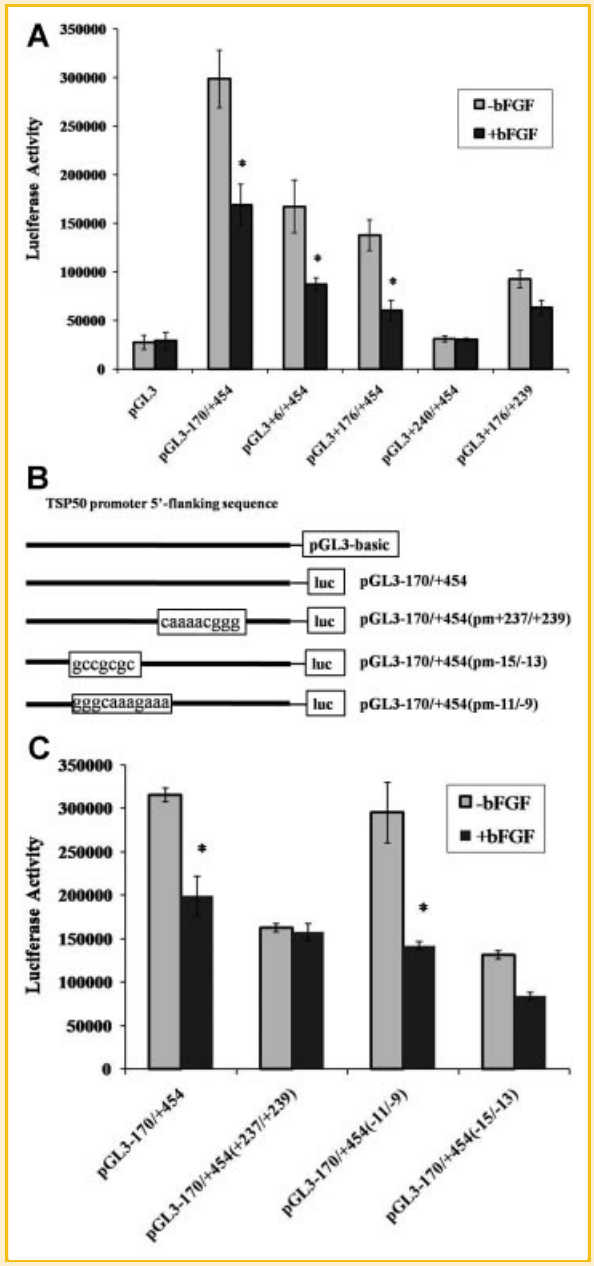

Fig. 5. The TSP50 promoter region encodes a functional bFGF responsive element. A: Serial deletion TSP50 promoter constructs analyzed for their ability to respond to bFGF. Serial deletion constructs of TSP50 promoter pGL3 − 170/+454, pGL3 + 6/+454, pGL3 + 176/+454, pGL3 + 176/+239, and pGL3 + 240/+454 were transfected into HEK293T cells, and 24 h after transfection, the cells were treated with bFGF for an additional 12 h. Luciferase activity was then assayed and normalized against β-gal activity. B: Schematic representation of the point mutation constructs of the TSP50 promoter. C: Analysis of the point mutation constructs activities of the TSP50 gene promoter stimulated by bFGF. A serial mutant of the TSP50 promoter was transfected into HEK293T cells, and 24 h after transfection, the cells were treated with bFGF for another 12 h. Luciferase activity was then assayed and normalized by β-gal activity. The reproducibility of these results was confirmed in three experiments. Statistically significant differences ($P < 0.05$) in paired Student's *t*-test are marked with an asterisk.

leads to bFGF-mediated decrease of TSP50 transcription. Using an inhibitor of PI3K, a downstream molecule in the AKT pathway, we found no role for the PI3K/AKT pathway in bFGF-mediated reduction of TSP50 promoter activation. In addition, inhibition of p38 or JNK did not affect bFGF-mediated reduction of TSP50 promoter activity. We identified that ERK1/2-inducible Sp1 phosphorylation mediates the repression of TSP50 gene expression.

Previous studies demonstrated Sp1 as a master regulator of TSP50 [Wang et al., 2008] and showed Sp1 binding to the TSP50 promoter by EMSA [Xu et al., 2007]. However, when we mutated the Sp1 binding site in the TSP50 promoter, we did not observe a reduction in the levels of TSP50 promoter activity in response to bFGF. Our study suggests that bFGF enhances the binding of Sp1 transcription factor to the +237 to +243 GC-rich region of the TSP50 promoter to decrease gene transcription.

bFGF is a pleiotropic growth factor, synergizing with many cytokines to stimulate proliferation; however, its activity inhibits cell differentiation [Xu et al., 1999; Nakazawa et al., 2006] and induces cell growth inhibition that occurs in the G2 phase of the cell cycle [Smits et al., 2000]. In addition, bFGF-activated ERK1/2-phosphorylation of Sp1 increases the interaction of Sp1 with the platelet-derived growth factor receptor (PDGFR) gene promoter resulting in a decrease in PDGFR expression [Bonello and Khachigian, 2004]. In this study, we demonstrate that TSP50 transcription and mRMA expression are active by native Sp1 phosphorylation via ERK1/2 by bFGF, which is similar with PDGFR gene. However, we do not rule out a possible co-operative effect with other transcription factors for TSP50 promoter regulation; however, in our model of bFGF-stimulated human breast cancer cells, we found Sp1 to be the main transcription factor responsible for TSP50 transcription. Moreover, bFGF-dependent downregulation of TSP50 expression implies a relationship between TSP50 and anti-apoptosis pathways.

This study provides the basis for improved understanding of the role of the TSP50 gene in signal-dependent transcription regulation and for the consideration of these regulatory mechanisms when developing treatments for the therapeutic downregulation of TSP50 in human breast cancer. We propose a pathway involving bFGF-induced ERK activation followed by ERK-induced phosphorylation of the transcription factor Sp1, which then translocates to the nucleus and binds to its specific sequence within the TSP50 promoter where it acts to decrease the transcription of the TSP50 gene.

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REFERENCES

Abate-Shen C, Shen MM. 2007. FGF signaling in prostate tumorigenesis-new insights into epithelial-stromal interactions. Cancer Cell 12:495–497.

Abe M, Sogabe Y, Syuto T, Yokoyama Y, Ishikawa O. 2007. Evidence that PI3K, Rac, Rho, and Rho kinase are involved in basic fibroblast growth factor-stimulated fibroblast-Collagen matrix contraction. J Cell Biochem 102:1290–1299.

Al-Sarraj A, Day RM, Thiel G. 2005. Specificity of transcriptional regulation by the zinc finger transcription factors Sp1, Sp3, and Egr-1. J Cell Biochem 94:153–167.

Bachmeier M, Löffler G. 1997. The effect of platelet-derived growth factor and adipogenic hormones on the expression of CCAAT/enhancer-binding proteins in 3T3-L1 cells in serum-free conditions. Eur J Biochem 243:128–133.

Bonello MR, Khachigian LM. 2004. Fibroblast growth factor-2 represses platelet-derived growth factor receptor-alpha (PDGFR-alpha) transcription via ERK1/2-dependent Sp1 phosphorylation and an atypical cis-acting element in the proximal PDGFR-alpha promoter. J Biol Chem 279:2377–2382.

Bouwman P, Philipsen S. 2002. Regulation of the activity of Sp1-related transcription factors. Mol Cell Endocrinol 195:27–38.

Chu S, Ferro TJ. 2005. Sp1: Regulation of gene expression by phosphorylation. Gene 348:1–11.

Dvorak P, Dvorakova D, Hampl A. 2006. Fibroblast growth factor signaling in embryonic and cancer stem cells. FEBS Lett 580:2869–2874.

Geller SF, Lewis GP, Fisher SK. 2001. FGFR1, signaling, and AP-1 expression after retinal detachment: Reactive Müller and RPE cells. Invest Ophthalmol Vis Sci 42:1363–1369.

Hong BZ, Park SA, Kim HN, Ma TZ, Kim HG, Kang HS, Kim HG, Kwak YG. 2009. Basic fibroblast growth factor increases intracellular magnesium concentration through the specific signaling pathways. Mol Cells 28:13–17. Epub 2009 July 8.

Im HJ, Muddasani P, Natarajan V, Schmid TM, Block JA, Davis F, van Wijnen AJ, Loeser RF. 2007. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes. J Biol Chem 282:11110–11121. Epub 2007 February 20.

Maher P. 1999. p38 mitogen-activated protein kinase activation is required for fibroblast growth factor-2-stimulated cell proliferation but not differentiation. J. Biol. Chem 274:17491–17498.

Merchant JL, Du M, Todisco A. 1999. Sp1 phosphorylation by Erk 2 stimulates DNA binding. Biochem Biophys Res Commun 254:454–461.

Milanini-Mongiat J, Pouyssegur J, Pages G. 2002. Identification of two Sp1 phosphorylation sites for p42/p44 mitogen-activated protein kinases: Their implication in vascular endothelial growth factor gene transcription. J Biol Chem 277:20631–20639.

Nakazawa F, Nagai H, Shin M, Sheng G. 2006. Negative regulation of primitive hematopoiesis by the FGF signaling pathway. Blood 108:3335–3343.

Shan J, Yuan L, Xiao Q, Chiorazzi N, Budman D, Teichberg S, Xu HP. 2002. TSP50, a possible protease in human testes, is activated in breast cancer epithelial cells. Cancer Res 62:290–294.

Smits VA, van Peer MA, Essers MA, Klompmaker R, Rijksen G, Medema RH. 2000. Negative growth regulation of SK-N-MC cells by bFGF defines a growth factor-sensitive point in G2. J Biol Chem 275:19375–19381.

Sroka IC, Nagle RB, Bowden GT. 2007. Membrane-type 1 matrix metalloproteinase is regulated by sp1 through the differential activation of AKT, JNK, and ERK pathways in human prostate tumor cells. Neoplasia 9:406–417.

Tanaka K, Abe M, Sato Y. 1999. Roles of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase in the signal transduction of basic fibroblast growth factor in endothelial cells during angiogenesis. Jpn J Cancer Res 90:647–654.

Ulbrich C, Westphal K, Baatout S, Wehland M, Bauer J, Flick B, Infanger M, Kreutz R, Vadrucci S, Egli M, Cogoli A, Derradji H, Pietsch J, Paul M, Grimm D. 2008. Effects of basic fibroblast growth factor on endothelial cells under conditions of simulated microgravity. J Cell Biochem 104:1324–1341.

Walsh CT, Wei Y, Wientjes MG, Au JL. 2008. Quantitative image analysis of intra-tumoral bFGF level as a molecular marker of paclitaxel resistance. J Transl Med 18:4.

Wang M, Bao YL, Wu Y, Yu CL, Meng XY, Xu HP, Li YX. 2008. Identification and characterization of the human testes-specific protease 50 gene promoter. DNA Cell Biol 27:307–14.

Wu P, Bao YL, Wu Y, Yu CL, Meng XY, Li YX. 2007. Testes-specific protease 50 promotes tumorigenesis. 9th Chin Conf Cell Biol 11:60.

Xu RH, Ault KT, Kim J, Park MJ, Hwang YS, Peng Y, Sredni D, Kung H. 1999. Opposite effects of FGF and BMP-4 on embryonic blood formation: Roles of PV.1 and GATA-2. Dev Biol 208:352–361.

Xu HP, Yuan L, Shan J, Feng H. 2004. Localization and expression of TSP50 protein in human and rodent testes. Urology 64:826–832.

Xu H, Shan J, Jurukovski V, Yuan L, Li J, Tian K. 2007. TSP50 encodes a testis-specific protease and is negatively regulated by p53. Cancer Res 67:1239–1245.

Yuan L, Shan J, De Risi D, Broome J, Lovecchio J, Gal D, Vinciguerra V, Xu HP. 1999. Isolation of a novel gene, TSP50, by a hypomethylated DNA fragment in human breast cancer. Cancer Res 59:3215–3221.