An FGF8b-mimicking peptide with potent antiangiogenic activity

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Abstract. Fibroblast growth factor (FGF) 8b interacts with its receptors and promotes angiogenesis in hormone-dependent tumors. In the present study, we demonstrated that a short peptide, termed 8b-13, which mimics part of the FGF8b structure, significantly inhibited the proliferation and migration of human umbilical vein endothelial cells (HUVECs) triggered by FGF8b using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), flow cytometry and an *in vitro* scratch assay. In addition, the findings from western blotting and reverse transcription-quantitative polymerase chain reaction revealed that 8b-13 appeared to counteract the effects of FGF8b on the expression of cyclin D1, the activation of signaling cascades, and the expression of proangiogenic factors; these actions may be involved in the mechanism underlying the inhibitory effects of 8b-13 on FGF8b-induced HUVEC proliferation and migration. The present results suggested that 8b-13 may be considered a potent FGF8b antagonist with antiangiogenic activity, and may have potential as a novel therapeutic agent for the treatment of cancer characterized by abnormal FGF8b upregulation.

Introduction

Fibroblast growth factor (FGF) 8 is a member of the FGF family. Based on sequence homology and phylogeny, FGF8 can be classified in the same subfamily as FGF17 and FGF18. Four FGF8 isoforms have been reported in humans, including a, b, e and f, which are generated by alternative splicing of the FGF8 gene. Among them, FGF8b is the main isoform expressed in hormone-dependent tumors, where it displays the strongest mitogenic and angiogenic potential (1,2). Previous studies have reported that FGF8b may facilitate the progression of reproductive cancers, including prostate, breast and ovarian cancer, through the stimulation of cellular proliferation and angiogenesis (3-5). Therefore, FGF8b is considered a potential target for the treatment of hormone-dependent tumors. In addition, the antitumor activity of a monoclonal anti-FGF8 antibody has been reported in FGF8b-expressing human prostate cancer xenografts (6,7).

FGF8b binds to its receptor (FGFR) and its activation triggers intracellular signaling cascades that promote cellular proliferation and angiogenesis, which are involved in tumor progression (8). Based on structural data regarding the interaction of FGF8b with its receptor, the gN helix domain of FGF8b is responsible for the high affinity and specificity of the FGF8b-FGFR interaction. A short peptide 8b-13 mimicking the gN helix of FGF8b may inhibit the biological activity of FGF8b by disturbing the interaction between FGF8b and its receptors. Our previous study demonstrated that the 8b-13 peptide suppressed FGF8b-induced proliferation of human prostate cancer cells (9). FGF8b participates in autocrine cancer signaling by stimulating the proliferation of hormone-sensitive cancer cells that secrete it. In addition, it can also exert paracrine effects on vascular endothelial cells and promote angiogenesis. The present study aimed to evaluate the effect of the FGF8b-mimicking peptide 8b-13 on the angiogenic activity of FGF8b.

Materials and methods

Materials. Human recombinant FGF8b was purchased from PeproTech, Inc. (Rocky Hill, NJ, USA). Polyvinylidene difluoride membrane was purchased from EMD Millipore (Billerica, MA, USA). The enhanced chemiluminescence (ECL) detection kit was obtained from Thermo Fisher Scientific,

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Inc. (Waltham, MA, USA). Primary antibodies against the following proteins were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA): cyclin D1 mAb (cat. no. 2978), phosphorylated (p)-fibroblast growth factor receptor substrate (FRS)2α (Tyr196) antibody (cat. no. 3864), extracellular signal-regulated kinase (Erk) 1/2 mAb (cat. no. 4695), p-Erk1/2 (Thr202/Tyr204) mAb (cat. no. 4370), p38 MAPK antibody (cat. no. 9212), p-p38 (Thr180/Tyr182) mAb (cat. no. 4631), c-Jun N-terminal kinase (JNK) mAb (cat. no. 9258), p-JNK (T183/Y185) mAb (cat. no. 4671), Akt (pan) mAb (cat. no. 4691), p-Akt (Ser 473) mAb (cat. no. 4060), p-STAT5 (Tyr694) mAb (cat. no. 4322) and GAPDH mAb (cat. no. 2118). Horseradish peroxidase (HRP)-conjugated secondary antibodies (cat. no. 7074P2) were purchased from Cell Signaling Technology, Inc. The SYBR Green Q-PCR kit was obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Human umbilical vein endothelial cells (HUVECs) obtained from the Institute of Tissue Transplantation and Immunology, Jinan University (Guangzhou, China) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS) and maintained in a humidified atmosphere containing 5% CO_2 at 37˚C. The 8b-13 peptide with a purity >98% was synthesized by Beijing SBS Genetech, Co., Ltd. (Beijing, China).

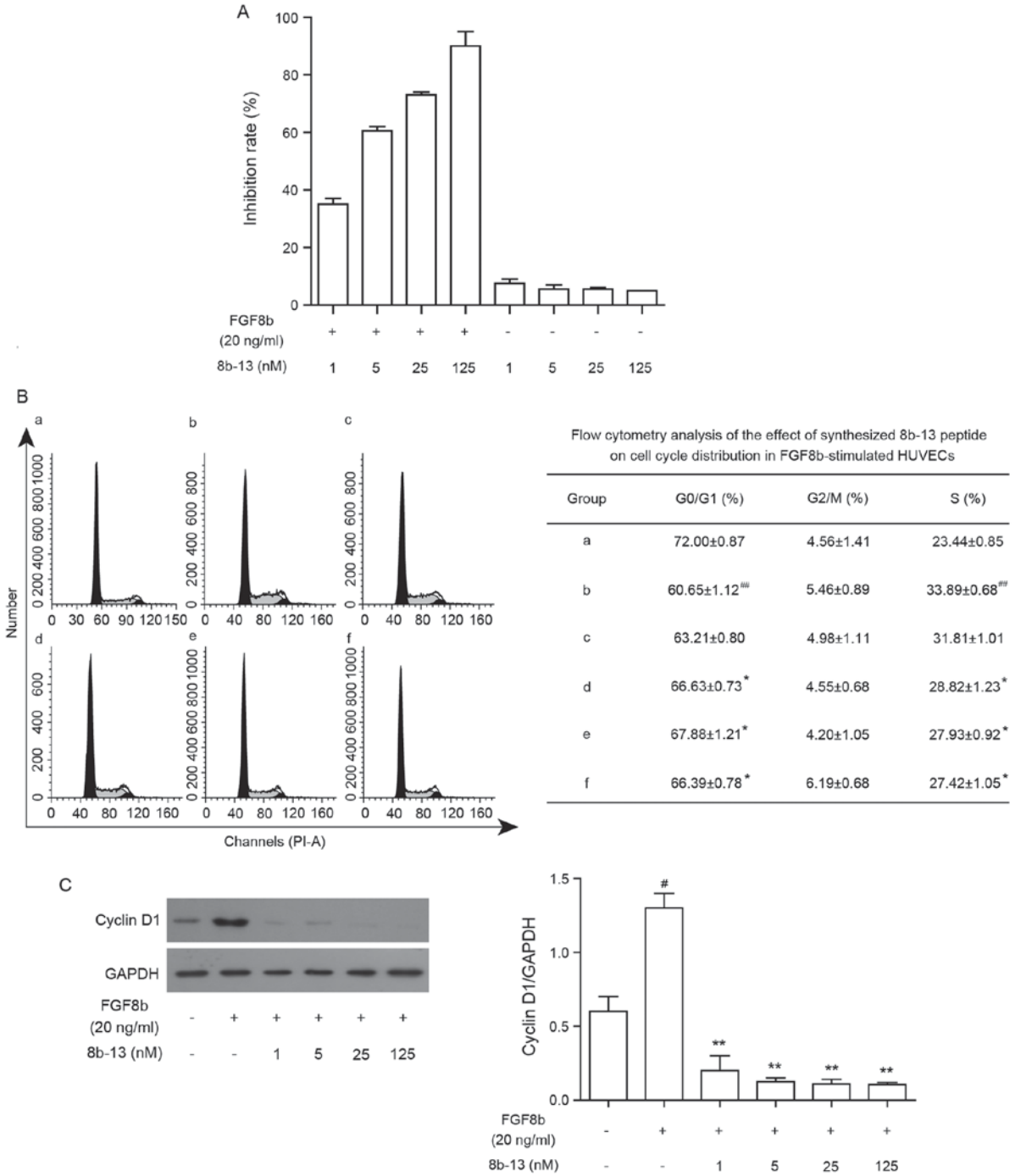
Cell viability assay. HUVECs were seeded in 96-well plates at a density of $1x10^4$ per well, and allowed to adhere overnight. Cells were cultured in DMEM supplemented with 0.4% FBS for 24 h, and then treated with the following: 1, 5, 25, 125 nM 8b-13 alone, 20 ng/ml FGF8b alone, or 20 ng/ml FGF8b preceded by treatment with 1, 5, 25, 125 nM 8b-13 for 5 min in a humidified atmosphere containing 5% CO_2 at 37˚C. After 48 h, the number of viable cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 20 µl of MTT (5 mg/ml) was added to each well, medium was discarded 4 h later and 100 µl of dimethyl sulfoxide (DMSO) was added to each well. The plate was maintained at room temperature for 30 min and the absorbance was immediately measured at 570 nm. The inhibition rate was calculated according to the following formula: Inhibition rate (%)=[($OD_{(FGF8b)}$-$OD_{(FGF8b+8b-13)}$)/($OD_{(FGF8b)}$-$OD_{(control)}$)]x100%. The control group cells did not receive FGF8b or 8b-13.

Cell cycle analysis. HUVECs were seeded in 12-well plates at a density of $1x10^5$ cells per well, and allowed to adhere overnight. Cells were incubated for 24 h in DMEM supplemented with 0.4% FBS in a humidified atmosphere containing 5% CO_2 at 37˚C. Subsequently, cells were treated with 20 ng/ml FGF8b alone, or 20 ng/ml FGF8b plus 1, 5, 25, 125 nM 8b-13 at 37˚C for 48 h. The cells were then washed three times with cold PBS, trypsinized and centrifuged at 250 x g at 4˚C for 10 min. The pellets were resuspended in PBS containing 10% FBS. Ice-cold 70% ethanol was added dropwise, the samples were maintained at 4˚C for 30 min, and were stained with propidium iodide (PI; Sigma-Aldrich; Merck Millipore, Darmstadt, Germany). Then cell cycle was analyzed by FACSCalibur-sort 4 (Becton Dickinson, Franklin Lakes, NJ, USA). The ModFit DNA analysis version 3.1 (Verity Software House, Topsham, ME, USA) was used to analyze the distribution of cells at various phases of the cell cycle.

Western blot analysis. HUVECs were seeded in 6-well plates at a density of $2x10^5$ cells/well and allowed to adhere overnight. Cells were incubated for 24 h in DMEM supplemented with 0.4% FBS in a humidified atmosphere containing 5% CO_2 at 37˚C. Subsequently, cells were treated with 20 ng/ml FGF8b alone, 20 ng/ml FGF8b preceded by treatment with 1, 5, 25, 125 nM 8b-13. HUVECs were collected and lysed in 1x SDS-PAGE loading buffer. The protein samples were then separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% non-fat dry milk at room temperature for 1 h, followed by incubation with the primary antibodies, including anti-cyclin D1 (1:2,000), anti-p-FRS2α (1:4,000), anti-Erk1/2 (1:4,000), anti-p-Erk1/2 (1:4,000), anti-p38 (1:4,000), anti-p-p38 (1:4,000), anti-JNK (1:4,000), anti-p-JNK (1:4,000), anti-Akt (1:4,000), anti-p-Akt (1:4,000) and anti-p-STAT5 (1:4,000) at 4˚C overnight. The membrane was then probed with goat anti-rabbit HRP-conjugated immunoglobulin G (1:5,000) for 1 h at room temperature. The bands were visualized with ECL detection kit according to the manufacturer's protocol. GAPDH (1:4,000 dilution for anti-GAPDH antibody) was used as a loading control. The bands were analyzed by QuantityOne version 4.6.2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Analysis of cell migration by in vitro scratch assay. HUVEC cells ($1x10^5$) were incubated on 12-well plates overnight, allowing cells to adhere and spread completely. A 'scratch' was created by scraping the cell monolayer in a straight line with a 10 µl pipette tip. After three washes with PBS to remove debris, cells were maintained for 24 h in DMEM supplemented with 0.4% FBS, and subsequently treated with 20 ng/ml FGF8b alone, or 20 ng/ml FGF8b plus 25 nM 8b-13. Photomicrographs were acquired using a phase-contrast microscope at the defined time points (0, 12, 24 and 48 h). The Image Pro-Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) was used for the quantitative analysis of the scratch distance. The migration distance was calculated according to the formula: Migration distance=scratch distance (0 h)-scratch distance (n h).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). HUVECs cultured in DMEM supplemented with 0.4% FBS were treated with 20 ng/ml FGF8b alone or 20 ng/ml FGF8b together with 25 nM 8b-13 for 48 h. Total RNA was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA (1 µg) was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). Briefly, 12 µl mixture containing 1 µg total RNA and 1 µl oligo$(dT)_{18}$ primer was incubated at 65˚C for 5 min prior to the addition of 4 µl (5x reaction buffer), 1 µl RiboLock RNase Inhibitor (20 u/µl), 2 µl (10 mM) dNTP mix and 1 µl RevertAid M-MuLV reverse transcriptase and incubation at 42°C for 1 h. qPCR analysis was performed on cDNA using the SYBR Green Q-PCR kit (Bio-Rad Laboratories, Hercules, CA, USA). The qPCR reaction mixture contained 10 µl 2x real-time PCR mix, 1 µl forward primer, 1 µl reverse primer, 1 µl cDNA



Flow cytometry analysis of the effect of synthesized 8b-13 peptide on cell cycle distribution in FGF8b-stimulated HUVECs

Group	G0/G1 (%)	G2/M (%)	S (%)
a	72.00±0.87	4.56±1.41	23.44±0.85
b	60.65±1.12##	5.46±0.89	33.89±0.68##
c	63.21±0.80	4.98±1.11	31.81±1.01
d	66.63±0.73*	4.55±0.68	28.82±1.23*
e	67.88±1.21*	4.20±1.05	27.93±0.92*
f	66.39±0.78*	6.19±0.68	27.42±1.05*

Figure 1. Effects of 8b-13 on the FGF8b-induced proliferation of HUVECs. (A) HUVECs were treated with 8b-13 alone, or 20 ng/ml FGF8b plus 8b-13 at the indicated concentrations. Cell viability was measured after 48 h using MTT assay. (B) Effects of 8b-13 on the cell cycle progression of FGF8b-stimulated HUVECs were assessed by PI staining combined with flow cytometry. HUVECs were starved in DMEM supplemented with 0.4% FBS for 24 h and treated with 20 ng/ml FGF8b (Group b), or 20 ng/ml FGF8b plus increasing concentrations (1, 5, 25 and 125 nM) of 8b-13 (Groups c-f) for 48 h. Control cells did not receive FGF8b or 8b-13 (Group a). (C) 8b-13 downregulated the expression of cyclin D1 in HUVECs. Starved cells were pretreated with 8b-13 at the indicated concentrations for 40 min prior to stimulation with 20 ng/ml FGF8b for 12 h. Western blot analysis was performed using cell lysates and anti-cyclin D1 primary antibody. Data are expressed as the mean ± standard deviation. Experiments were performed in triplicate. #P<0.05, ##P<0.01 vs. control group; *P<0.05, **P<0.01 vs. FGF8b alone group. FGF, fibroblast growth factor; HUVEC, human umbilical vein endothelial cells; PI, propidium iodide.

and 7 μl ddH_2O. The following primers were used for RT-qPCR: GAPDH, forward 5'-CCCACT CCTCCACCTTTGAC-3', reverse 5'-TGTGCTGTAGGAAGCTCA-3'; urokinase-type plasminogen activator (uPA), forward 5'-CGCAG TCACACCAAGGAAGAGAATG-3', reverse 5'-TCTGTGC AGAGCCTATCTTCCCAGT-3'; vascular endothelial growth factor (VEGF), forward 5'-GGCAGAATCATCACGAAG-3', reverse 5'-TGTGCTGTAGGAAGCTCA-3'; and matrix metalloproteinase-9 (MMP9), forward 5'-CAGAGATGCG TGGAGAGT-3' and reverse 5'-TCTTCCGAGTAGTTTTGG-3'. Amplification was performed using the CFX96 Touch Deep Well Real-Time PCR Detection system (Bio-Rad Laboratories, Inc.) under the following conditions: 95˚C for 5 min, 95˚C for 10 sec, 59˚C for 5 sec for 40 cycles, 95˚C for 10 sec, melt

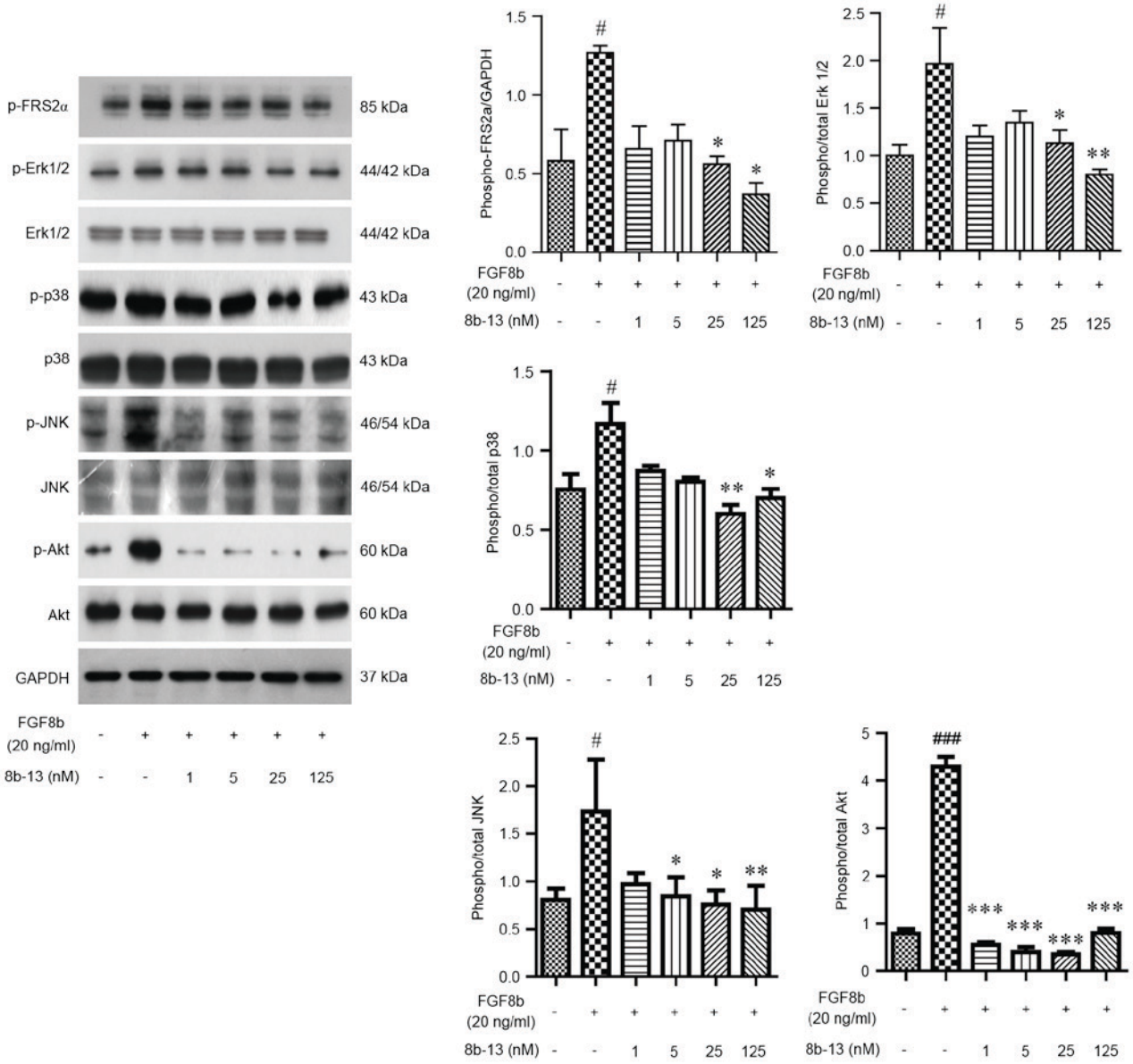


Figure 2. Effects of 8b-13 on FGF receptor-mediated activation of Akt and mitogen-activated protein kinases. Starved human umbilical vein endothelial cells were pretreated with increasing concentrations of 8b-13 peptide (1, 5, 25 and 125 nM) for 5 min prior to stimulation with 20 ng/ml FGF8b for 10 min. Control cells did not receive FGF8b or 8b-13. The phosphorylated levels of FRS2a, Erk1/2, p38, JNK, and Akt were assessed using western blot analysis. Data are expressed as the mean ± standard deviation. Experiments were performed in triplicate. $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 vs. control group; *P<0.05, **P<0.01, ***P<0.001 vs. FGF8b alone group. FGF, fibroblast growth factor; FRS, fibroblast growth factor receptor substrate; Erk, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; p-, phosphorylated.

curve 65 to 95˚C (increment 0.5˚C) for 5 sec. Finally, the relative changes in gene expression determined from real-time quantitative PCR experiments were calculated using the $2^{-\Delta\Delta Cq}$ method (10).

Statistical analysis. The statistical significance of differences between groups was assessed by one-way analysis of variance, followed by a post hoc Tukey test for multiple comparisons. The Student's t-test was used to assess the difference between two groups. Data are expressed as the mean ± standard deviation, and P<0.05 was considered to indicate a statistically significant difference. The analysis was performed using GraphPad Prism software version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

8b-13 peptide suppresses FGF8b-induced HUVEC proliferation. The effects of the synthetic 8b-13 peptide on the FGF8b-induced proliferation of HUVECs were examined using the MTT method. 8b-13 inhibited FGF8b-triggered cellular proliferation in a dose-dependent manner, with the inhibition rate rising to 90% at a concentration of 125 nM (Fig. 1A). Notably, 8b-13 alone had little effect on HUVEC proliferation.

In order to explore the effects of 8b-13 on cell cycle progression in FGF8b-stimulated HUVECs, PI staining combined with flow cytometry was used. FGF8b increased the percentage of cells at S phase from 23.44±0.85% to

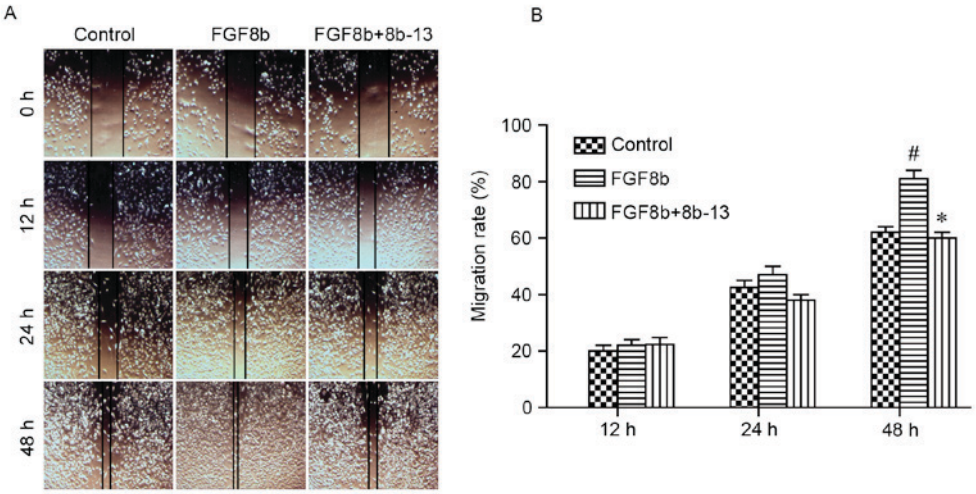


Figure 3. Effects of 8b-13 on FGF8b-stimulated human umbilical vein endothelial cell migration. Cells starved in DMEM containing 0.4% FBS were treated with FGF8b or FGF8b plus 8b-13 (25 nM) for 12, 24 or 48 h. Control cells did not receive FGF8b or 8b-13. Cellular migration was examined by scratch wound healing assay under a microscope (magnification, x100). (A) Graphic representation of the wound closure showing changes in the wound distance. (B) Migration rate was evaluated by measuring the remaining scratch distance, and is expressed as a percentage of the initial scratch distance. Data are expressed as the mean ± standard deviation. Experiments were performed in triplicate. $^{\#}$P<0.05 vs. control group; *P<0.05 vs. FGF8b alone group. FGF, fibroblast growth factor.

33.89±0.68%, and reduced the percentage of cells at G_0/G_1 phase from 72.00±0.87% to 60.65±1.12%. The addition of 8b-13 to FGF8b-stimulated cells appeared to counteract the effects of FGF8b on the cell cycle, as it decreased the percentage of cells at S-phase and increased the percentage of cells at G_0/G_1 phase (Fig. 1B).

To explore the mechanism underlying the inhibitory actions of 8b-13 on the FGF8b-induced progression from G_0/G_1 to S phase, the effects of 8b-13 on the expression of cyclin D1 were analyzed. Cyclin D1 has been demonstrated to promote the G_1/S cell cycle transition (11). As presented in Fig. 1C, 8b-13 downregulated the expression of cyclin D1, which was enhanced by FGF8b stimulation. These results suggested that the decreased cyclin D1 expression may partly contribute to the G_0/G_1 phase arrest triggered by 8b-13 administration.

8b-13 peptide blocks FGFR signaling cascades. FGF8b exerts autocrine and paracrine effects by acting through its receptor, FGFR. It has previously been reported that the docking protein FRS2α can directly interact with the activated FGFR and mediate the downstream Ras-mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-Akt cascades, which are essential for cellular proliferation and survival (12,13). The present study investigated the effects of 8b-13 on the FGF8b-induced activation of FRS2α and its downstream cascades. Pretreatment with 8b-13 significantly reduced the phosphorylation levels of FRS2α, Erk1/2, p38, JNK and Akt, which were enhanced by FGF8b (Fig. 2). These results suggested that 8b-13 may inhibit the proliferation of HUVECs via preventing the activation of FRS2α and its downstream pathways; the PI3K-Akt cascade appeared to be markedly affected by 8b-13, as suggested by the considerable decrease in FGF8b-stimulated Akt phosphorylation levels (Fig. 2).

8b-13 peptide inhibits HUVEC migration. The effects of 8b-13 on the migratory ability of HUVECs were evaluated using an *in vitro* scratch assay. Images taken at various post-scratch time points revealed complete wound closure within 48 h in FGF8b-treated cells; however, only partial closure was achieved within the same time in cells treated with 8b-13 (Fig. 3A and B). Analysis of the migration distance demonstrated that the increase in migration rate triggered by FGF8b was significantly reduced by treatment with 8b-13 (Fig. 3B).

Previous studies have revealed that the activation of STAT5 is essential for the FGF8b-induced migration of vascular endothelial cells (14,15). The present study assessed the effect of 8b-13 on STAT5 activation using western blot analysis. The results revealed that FGF8b-induced phosphorylation of STAT5 was reduced by 8b-13 in a dose-dependent manner. Therefore, it may be hypothesized that 8b-13 exhibits anti-angiogenic potential, executed via inhibiting FGF8b-induced STAT5 activation (Fig. 4).

8b-13 peptide decreases the expression of uPA, VEGF and MMP9. Proangiogenic factors, including uPA, VEGF and MMPs, have critical roles in various angiogenic stages. RT-qPCR was used to investigate the influence of 8b-13 on the expression levels of uPA, VEGF and MMP9. FGF8b increased the mRNA expression levels of uPA, VEGF and MMP9, whereas treatment with 8b-13 significantly decreased the mRNA expression levels of these genes (Fig. 5). These results suggested that 8b-13 downregulation of angiogenic factors may contribute to the antiangiogenic potential of 8b-13.

Discussion

Angiogenesis, which refers to the formation of new blood vessels, is critical for the continued growth and metastatic spread of malignant tumor cells (16). Therefore, therapeutic approaches aiming at inhibiting angiogenesis are promising in cancer treatment. The roles of FGF8b, alongside another

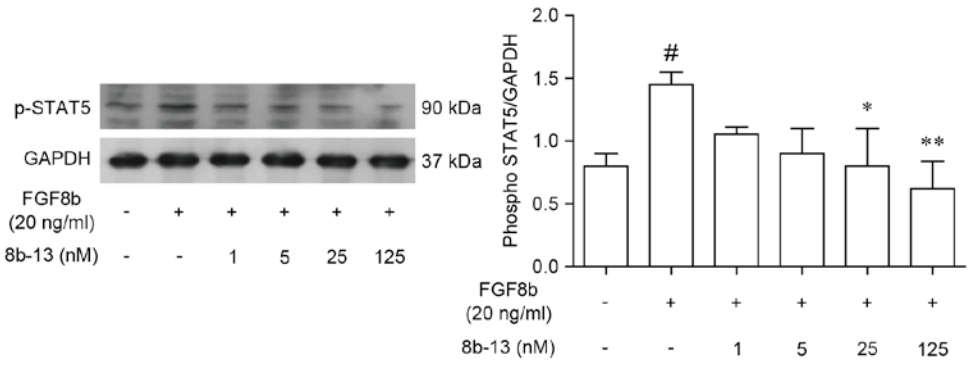


Figure 4. 8b-13 inhibits FGF8b-induced phosphorylation of STAT5. Human umbilical vein endothelial cells starved in DMEM containing 0.4% FBS were pretreated with increasing concentrations of 8b-13 peptide (1, 5, 25 and 125 nM) for 5 min prior to stimulation with 20 ng/ml FGF8b for 10 min. Control cells did not receive FGF8b or 8b-13. The phosphorylated STAT5 and GAPDH were assessed by western blot analysis. Data are expressed as the mean ± standard deviation. Experiments were performed in triplicate. $^{\#}$P<0.05 vs. control group; *P<0.05, **P<0.01 vs. FGF8b alone group. FGF, fibroblast growth factor; STAT, signal transducer and activator of transcription.

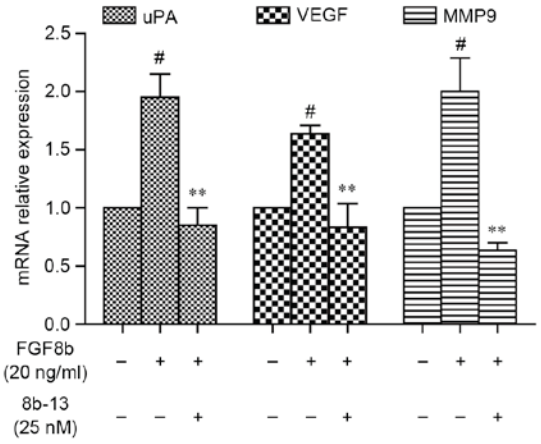


Figure 5. 8b-13 inhibits the expression of uPA, VEGF, and MMP-9. Human umbilical vein endothelial cells starved in DMEM containing 0.4% FBS were treated with 20 ng/ml FGF8b alone or 20 ng/ml FGF8b together with 25 nM 8b-13 for 48 h. Control cells did not receive FGF8b or 8b-13. Total RNA was extracted and the relative mRNA expression levels of uPA, VEGF and MMP-9 were measured by reverse transcription-quantitative polymerase chain reaction. Data are expressed as the mean ± standard deviation of four independent experiments. $^{\#}$P<0.05 vs. control group; **P<0.01 vs. FGF8b alone group. uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; FGF, fibroblast growth factor.

member of the FGF family, FGF2, in tumor angiogenesis have been well documented (14,15). An FGF8b-mimicking peptide, 8b-13, which was designed based on the structure of the FGF8b-FGFR complex, appeared to markedly suppress the proliferation of human prostate cancer cells stimulated by endogenous and exogenous FGF8b (9). The results of the present study demonstrated that the synthetic peptide can exert inhibitory effects on the angiogenic action of FGF8b.

Since proliferation and migration of endothelial cells are critical events in angiogenic processes (17,18), the present study evaluated the effects of 8b-13 on FGF8b-induced endothelial cell proliferation and migration. The results revealed that 8b-13 significantly inhibited the proliferation and migration of HUVECs, which were stimulated by FGF8b. It has previously been reported that 8b-13 may suppress prostate cancer cell proliferation induced by endogenous FGF8b (9); conversely, in the present study, the inhibitory effect of 8b-13 on the growth of HUVECs without exogenous FGF8b stimulation was insignificant. Since it has been demonstrated that almost no endogenous FGF8b is present in HUVECs (19), the absence of an 8b-13-mediated effect on HUVEC proliferation without exogenous FGF8b stimulation further confirmed the specificity of 8b-13 in targeting FGF8b.

The mechanism of the 8b-13-induced inhibition of HUVEC proliferation and migration was further explored. The present results revealed that 8b-13 counteracted the effects of FGF8b on G_1/S-specific cyclin D1 expression, on FGFR-mediated signaling cascades involving the kinases Erk1/2, p38, JNK, Akt and STAT5, and on the expression of the proangiogenic factors uPA, VEGF and MMP9. Cyclin D1 functions as a regulatory subunit of cyclin-dependent kinases, and regulates the transition from G_1 phase to S phase, which is essential for cell cycle progression. It has previously been reported that FGF8b can induce cyclin D1 expression via the activation of PI3K-Akt and p38 MAPK pathways (20). In addition, the activation of STAT5 is involved in the regulation of cyclin D1 expression, via transactivation of the cyclin D1 promoter (21). Therefore, it may be hypothesized that 8b-13 can inhibit the activation of p38, Akt and STAT5 signaling cascades induced by FGF8b, and cause a downregulation in the G_1/S-specific protein cyclin D1, leading to cell cycle arrest at the G_0/G_1 phase and contributing to the suppression of FGF8b-induced cellular proliferation.

STAT5 activation is implicated in cellular proliferation, and appears to occur downstream of the activation of the proangiogenic factor VEGF, which is involved in FGF8b-induced cellular migration (7,14,22). The present results revealed that 8b-13 inhibited the expression of VEGF and the phosphorylation of STAT5, which were stimulated by FGF8b. STAT5 activation can also upregulate cyclin D1 expression (21). Therefore, it may be hypothesized that the VEGF/STAT5 pathway partially mediates the inhibitory effects of 8b-13 on HUVEC proliferation and migration. Erk1/2 activation has been reported to enhance cellular migration, via enhancing the activity of myosin light chain kinase, causing phosphorylation

of myosin light chains (23). Therefore, the suppression of Erk1/2 signaling cascades may be involved in the mechanism of 8b-13-mediated inhibition of cellular migration.

In conclusion, the present study demonstrated that the synthetic peptide 8b-13 antagonized the proangiogenic action of FGF8b by inhibiting the FGF8b-induced proliferation and migration of HUVECs. Our previous study demonstrated that 8b-13 may exhibit significant inhibitory effects on prostate cancer cell proliferation (9). These results suggested that 8b-13 may exercise antiangiogenic and antitumor effects via targeting FGF8b-mediated pathways, and may have therapeutic potential in various cancer types, which are characterized by aberrant FGF8b levels.

Acknowledgements

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