Expression of BDNF, TrkB, VEGF and CD105 is associated with pelvic lymph node metastasis and prognosis in IB2-stage squamous cell carcinoma

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Abstract. Brain-derived neurotrophic factor (BDNF), tropomyosin receptor kinase B (TrkB), vascular endothelial growth factor (VEGF) and CD105 are highly expressed in several types of cancer. The present study aimed to determine whether BDNF, TrkB, VEGF and CD105 are associated with the prognosis and metastasis of patients with cervical squamous cell carcinoma (SCC) at the IB2 stage. A total of 79 patients with IB2-stage SCC were enrolled in the present study. The expression levels of BDNF, TrkB, VEGF and CD105 in IB2-stage cervical cancer tissue were detected by immunohistochemistry and their association with clinicopathological indexes or prognostic factors was statistically analyzed. Reverse transcription quantitative PCR was used to detect whether the expression of VEGF was affected in SiHa cells co-cultured with BDNF. In addition, BDNF-induced SiHa cell migration and invasion were examined. BDNF expression in the cervical cancer samples was significantly associated with positive lymphovascular space invasion (P<0.001) and pelvic lymph node metastasis (P<0.05). In addition, microvessel density was verified as an independent prognostic factor for overall survival (P<0.05). *In vitro* analysis indicated that BDNF significantly induced cellular migration and invasion of SiHa cells in a dose-dependent manner (P<0.001). BDNF induced the expression of VEGF in SiHa cells, which was inhibited by BDNF antibodies or an inhibitor of TrkB receptor (P<0.05). BDNF may be considered a useful indicator of pelvic metastasis, which is involved in the aggressive spread of IB2-stage SCC. BDNF-induced upregulation of VEGF was revealed to act as a pro-angiogenic factor in SCC (Trial registration no. http://apps.who.int/trialsearch/; ChiCTR1800017778).

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Introduction

Cervical cancer is a common cancer type in females. Approximately 132,000 novel cases of cervical cancer are reported each year in China, accounting for 28% of the total number of cases worldwide. Furthermore, an increasing proportion of females <35 years old have developed cervical cancer in the past 5 years in China (1). The tumor microenvironment (comprising tumor cells, nerves and blood vessels), inflammatory cells and cytokines are closely linked to the origin and progression of tumors (2). Chen (3) revealed that nerve fibers and neurotrophins in the tumor microenvironment are associated with the progression of cervical cancer and the proportion of new nerves in cervical cancer was higher than that in the normal cervix (3). In addition, brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) were indicated to be widely expressed in cervical cancer. The TrkB expression level in cervical squamous cell carcinomas (SCC) was reported to be higher than that in the normal cervix. Furthermore, TrkB-positive vessels have been observed in foci of SCC (4). BDNF, a cytokine detected in a series of non-neurogenic tumors, belongs to the neurotrophin

family and has been indicated to be involved in tumor cell proliferation and invasion (2,5-7); it also induces perineural invasion (8-10).

The probability of recurrence and metastasis of SCC at the IB2 stage (also known as bulky-stage SCC) is much higher than at the early stage of cervical cancer. Furthermore, the prognosis of IB2-stage SCC is less favorable (11,12). However, to date, no biomarkers to accurately and effectively predict the recurrence and metastasis potential of cervical cancer at the bulky stage have been identified. Of note, the serum level of SSC antigen (SccA) reportedly is associated with the tumor grade, size and interstitial infiltration of cervical cancer; however, it only increased in 65% of patients with cervical cancers vs. normal volunteers, while serum level of cancer antigen 125 (CA125) was reported to increase in only 15% patients with cervical adenocarcinoma (13).

The role of the BDNF-TrkB regulatory system in SCC remains to be fully elucidated. In the present study, it was hypothesized that the potential for metastasis and recurrence of IB2-stage SCC is associated with the expression levels of BDNF and TrkB. Thus, data from clinical trials were retrospectively studied to investigate whether the expression of BDNF and TrkB, as well as other associated factors, is associated with the prognosis and metastasis of IB2-stage SCC. The SiHa cell line is a human papillomavirus 16-positive cervical cancer cell line, which has the characteristics of cervical SSC. In order to explore the role of BDNF in the progression of SCC, a Transwell assay was applied to evaluate the effect of BDNF on the migration and invasion in SiHa cells. The aim of the present study was to discover a biomarker that may serve as a predictor of progression of bulky-stage SCC and explore whether BDNF and its receptors are involved in the angiogenesis of SCC.

Materials and methods

Patients. A total of 79 IB2-stage SCC patients treated between January 2006 and December 2012 at the Department of Obstetrics and Gynecology of Nanfang Hospital (affiliated to Southern Medical University, Guangzhou, China) were enrolled in the present study. The diagnoses of all SCC patients enrolled were performed based on the post-operative pathological results. The general and clinical information of the SCC patients was collected, including age, sex, gestation, parity, Fédération Internationale de Gynécologie et d'Obstétrique stage, pre-operative hemoglobin level, duration of operation, type of surgery, complications, lymphovascular space involvement (LVSI) status, depth of cervical infiltration, uterine body invasion, parametrical infiltration, pelvic lymph node status and post-operative therapy. The clinical and pathological data of the patients were entered into Epidata software 3.1 version (The EpiData Association) separately by two gynecologists, who revised the date together, to ensure input errors were minimized. In order to compare the expression levels of BDNF, TrkB, vascular endothelial growth factor (VEGF) and CD105 between cervical cancer tissue and normal cervix tissue, samples of normal comparable cervix tissue (n=10) were obtained from uterine leiomyoma patients who underwent a hysterectomy in the same time period as patients in the cervical cancer cohort at the Department of Obstetrics and Gynecology of Nanfang Hospital.

Ethics approval and informed consent. This study was part of a clinical research project based on a multicenter study of cervical cancer in China. It was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University (Guangzhou, China; code: NEEC-2017-135). Written informed consent for the use of specimens for scientific research was provided by all patients included in the current study.

Cell culture. SiHa cells purchased from American Type Culture Collection were provided by the laboratory at Southern Medical University and incubated with complete Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Inc.) in an incubator at 37˚C with 5% CO_2. For stimulation with BDNF (recombinant human BDNF; cat. no. 450-1; PeproTech EC, Ltd.), the SiHa cells starved for 12 h and incubated with serum-free DMEM supplemented with 20, 50 or 100 ng/ml BNDF for 18 h. In another test to neutralize the effect of BDNF, the SiHa cells were divided into three groups: The 100 ng/ml BDNF group, the BDNF (100 ng/ml) plus BDNF antibody (1:500; cat. no. 500-P84; PeproTech) group, and BDNF (100 ng/ml) plus TrkB inhibitor ANA12 (1 mg/ml; BioVision, Inc.) group. SiHa cells cultured in DMEM were used as the control group.

Immunohistochemical staining. The cervical tissue specimens were taken from surgically cut tissue from the SCC patients and the uterine leiomyoma patients, embedded in paraffin, cut into 4-μm thick sections, placed on glass slides and incubated for 10 min in 3% H_2O_2 at room temperature to inhibit endogenous peroxidases. The slides were then incubated with 3,3-diaminobenzidine (DAB) for 10 min, washed with PBS, stained with 10% hematoxylin, dehydration with gradient ethanol. After washing with PBS, the samples were blocked by incubation with 5% goat serum (cat. no. ab 138478; Abcam) at room temperature for 10 min. The primary antibody reaction was performed at 4˚C overnight in 1% goat serum in PBS containing the corresponding antibody [BDNF (1:500; cat. no. ab203573; Abcam), TrkB (1:500; cat. no. SC-8316; Santa Cruz Biotechnology, Inc.), VEGF (1:250; cat. no. ab32152; Abcam), CD105 (1:500; cat. no. ab135528; Abcam) and PBS (as the negative control)]. After triple washing with PBS, the slides were incubated with the secondary antibody (Goat Anti-Mouse Anti-Rabbit IgG/IgM H&L; 1:100; cat. no. ab2891; Abcam) for 30 min at room temperature.

Average optical density (IOD) and microvessel density (MVD) evaluation. The images were captured on a Nikon Bx51 camera (Nikon Corporation). DAB-stained regions of interest were defined based on their immunohistochemical staining profiles in in a constant manner. To analyze the immunohistochemically stained slides, 10 fields of the target slide at a magnification of x400 were captured and the average integrated optical density (IOD) was analyzed with Image Pro Plus v6.0 software (Media Cybernetics, Inc.). Microvessel density (MVD) was determined by immunostaining for CD105 according to Weidner's method (14). In brief, single brown-stained endothelial cell or clusters of brown-stained endothelium (with or without lumen) were counted as individual vessels. The slides were screened at a magnification of x100-200 under an upright microscope to

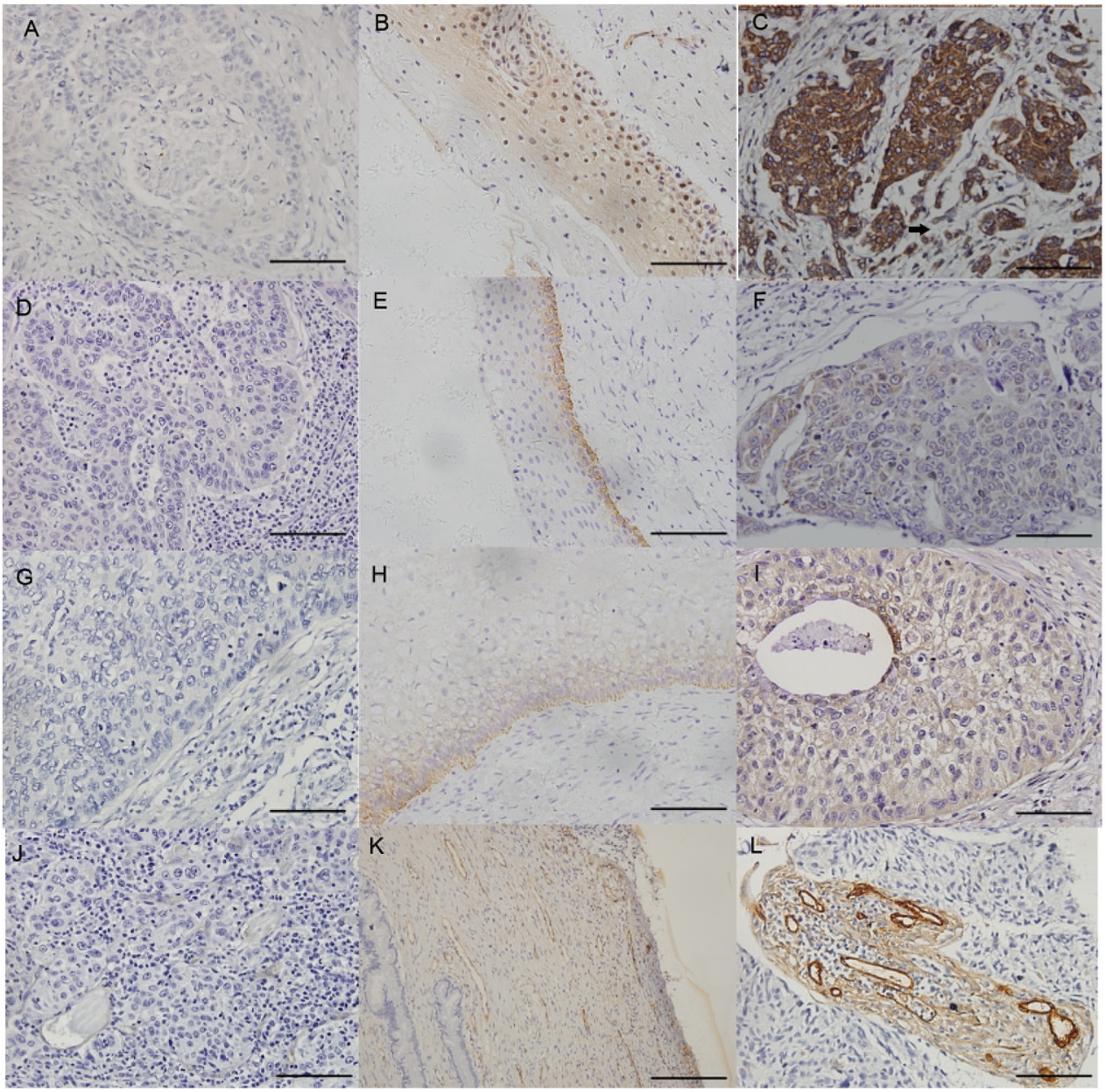

Figure 1. Immunohistochemical staining for BDNF, TrkB, VEGF and CD105 in cervical cancer specimens. The expression of (A-C) BDNF, (D-F) TrkB, (G-I) VEGF and (J-L) CD105 was detected by immunohistochemical staining in negative controls, normal cervical epithelium and cervical cancer specimens, respectively, from left to right. Negative controls were incubated with PBS only instead of primary antibody. The scale bar was 50 μm in A-I and 100 μm in J-L. The arrow in C indicates negative staining for BDNF in the cervical cancer stroma. BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; VEGF, vascular endothelial growth factor.

determine the field of the target area containing large number of positive vessels. Three fields at the magnification of x400 that had the highest concentrations of microvessels were selected and the number of microvessels was counted. The mean value of the three fields was taken as the mean MVD.

Transwell migration and invasion assay. The migration and invasion capacity of SiHa cells were determined using Transwell plates for 24-well plates (6.5 mm; pore size 8 μm; Corning, Inc.) according to the manufacturer's protocol. For the cellular migration assay, the pre-treated SiHa cells were suspended in 600 μl serum-free DMEM at a density of 10^5 cells/ml and seeded into the upper chambers of Transwell plates; 100 μl of serum-free DMEM was supplied into the lower chamber. After 24 h of incubation at 37˚C, the migrated tumor cells on the lower side of the filter were fixed with 4% paraformaldehyde and stained with crystal violet (Ubio).

For the cellular invasion assay, the procedure was similar to that described above, except that the inserted filter was pre-coated with Matrigel (BD Biosciences; diluted at 1:6) and the plates were incubated for 24 h at 37˚C.

The migrated and invaded cells were counted in triplicate fields of view three times under an Olympus CKX41 inverted microscope (Olympus Corporation) at a magnification of x100.

Western blot analysis. The treated cells were lysed with radioimmunoprecipitation assay protein lysis buffer (containing protease inhibitors) and centrifuged at 12,000 x g or 4 min at

Table I. Analysis of the association between BDNF and TrkB with clinicopathological indexes of IB2-stage cervical differentiated squamous cell carcinoma.

	BDNF			TrkB		
Characteristic	N	Mean ± SD	P-value	N	Mean ± SD	P-value
Age (year)						
≤35	5	0.06±0.03	0.978	5	0.01±0.01	0.068
>35 or ≤50	48	0.06±0.04		44	0.03±0.03	
>50	26	0.07±0.03		26	0.02±0.02	
Tumor diameter (cm)			0.209			0.425
>4, <5	40	0.06±0.04		37	0.02±0.02	
≥5	39	0.07±0.04		38	0.03±0.03	
Gross type			0.046			0.249
Exogenous	63	0.07±0.04		59	0.02±0.02	
Ulcerative	11	0.05±0.02		11	0.03±0.02	
DSI			0.188			0.441
No	33	0.06±0.03		31	0.03±0.02	
Yes	44	0.07±0.04		42	0.02±0.02	
LVSI			0.001			0.256
Yes	13	0.09±0.04		11	0.04±0.04	
No	66	0.06±0.03		64	0.02±0.02	
Pelvic lymph node metastasis			0.010			0.202
No	56	0.06±0.03		54	0.02±0.02	
Yes	23	0.08±0.04		21	0.03±0.03	

DSI, deep stromal invasion; LVSI, lymphovascular space invasion; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; SD, standard deviation.

4˚C. The protein concentration of the supernatant was detected with a bicinchoninic acid assay kit (BioVision). A total of 40 mg of the total protein samples were loaded onto 12% SDS-PAGE gel, separated by electrophoresis and transferred onto a polyvinylidene difluoride membrane (Sigma-Aldrich; Merck KGaA). The membranes were blocked with Tris-buffered saline containing 5% skimmed milk for 1 h, incubated with VEGF antibody (1:500; cat. no. 251622; ZEN-Biotech) overnight at 4˚C, triple-washed with Tris-buffered saline containing Tween-20, reacted with secondary antibody (1:500; cat. no. 220173; ZEN-Biotech Pvt. Ltd.) at room temperature for 1 h, triple washed again, and visualized using an enhanced chemiluminescence detection kit (GE Healthcare). The protein bands were then exposed to X-ray film (Fujifilm).

Reverse transcription quantitative PCR (qPCR). Total RNA was extracted with an RNA Extraction kit (Promega Corp.), The reaction mix contained 175 μl of RNA polymerase, 300 nM of primer and 100 ng of RNA. The RNA was amplified with a starting denaturation for 10 min at 95˚C, 95˚C for 30 sec, 56˚C for 60 sec, and 72˚C for 60 sec, and was repeated for 40 cycles. The PCR mixture was prepared with SYBR Green PCR Master Mix (Toyobo), according to the manufacturer's protocol. 18srRNA (15) was used as the endogenous reference gene for qPCR analysis. Each reaction was performed in triplicate. The results were analyzed using the $2^{-\Delta\Delta Cq}$ method (16). The primers used for qPCR were as follows: VEGF forward, 5'-GCAGATTATGCGGATCAAACC-3' and reverse, 5'-TTTCGTTTTTGCCCCTTTCC-3'; endogenous reference forward, 5'-CCTGGATACCGCAGCTAGGA-3'; and reverse, 5'-GCGGCGCAATACGAATGCCCC-3'.

Statistical analysis. SPSS v21.0 software (IBM Corp.) was employed for statistical analysis. Values are expressed as the mean ± the standard deviation or standard error. A Student's t-test was applied to evaluate the differences between two groups. Statistical analysis of multiple groups was performed using one-way analysis of variance, followed by Dunnett's test. The χ^2-square test was used to analyze categorical variables. The univariate analysis of the association of factors with survival were performed using one-way ANOVA and Student's t-test, for categorical variables and continuous variables respectively. The Cox proportional hazards model was employed to assess the association of a variety of factors with survival. Survival curves were plotted using the Kaplan-Meier method. $P<0.05$ was considered to indicate statistical significance.

Results

SCC patients. The average age of the 79 SCC patients was 47.84±8.46 years (range, 24-68 years). The follow-up period

Table II. Analysis of the association of VEGF and MVD with clinicopathological characteristics of IB2-stage cervical squamous cell carcinoma.

	MVD			VEGF		
Characteristic	N	Mean ± SD	P-value	N	Mean ± SD	P-value
Age (year)						
≤35	7	11.76±2.81	0.878	11	0.78±0.05	0.567
>35 or ≤50	43	12.11±2.71		34	0.08±0.03	
>50	25	10.90±2.71		30	0.08±0.01	
Tumor diameter (cm)			0.463			0.006
>4, <5	37	11.84±2.89		40	0.06±0.05	
≥5	38	12.31±2.60		39	0.09±0.06	
Gross type			0.381			0.412
Exogenous	59	11.79±2.67		63	0.07±0.06	
Ulcerative	11	12.57±2.90		11	0.08±0.05	
DSI			0.862			0.852
No	31	11.99±2.86		33	0.07±0.05	
Yes	42	12.10±2.60		44	0.07±0.06	
LVSI			0.695			0.005
Yes	12	12.36±1.83		13	0.11±0.04	
No	63	12.02±2.88		66	0.06±0.05	
Pelvic lymph node metastasis			0.403			0.222
No	54	12.24±2.94		56	0.07±0.06	
Yes	21	11.65±2.10		23	0.08±0.05	

DSI, deep stromal invasion; LVSI, lymphovascular space invasion; SD, standard deviation; MVD, microvessel density; VEGF, vascular endothelial growth factor.

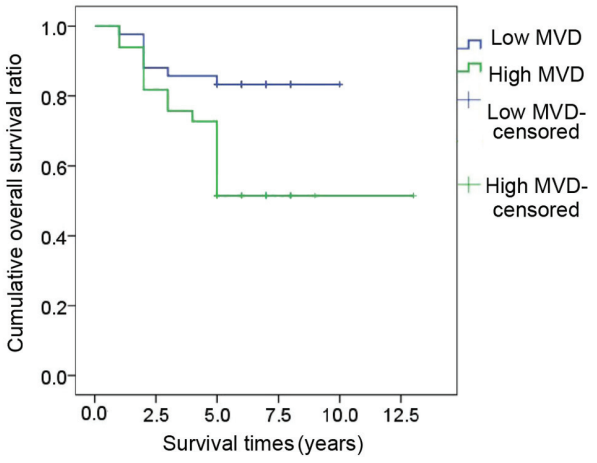

Figure 2. Kaplan-Meier analysis of the association between MVD and survival. An analysis of the associative link between the MVD and survival (MVD-) in patients with low CD105 (blue) or higher CD105 (green) levels is provided (cutoff value: 12.2). High MVD is an independent prognostic factor for OS. MVD, micro-vessel density.

after surgical resection ranged from 1 to 13 years (average, 5.9±2.0 years). During the follow-up, 23 patients died. Additional information on the cohort is listed in Supplemental Table SI.

Expression and location of BDNF, TrkB, VEGF and CD105 in IB2-stage cervical SCC tissue according to immunochemical staining. The protein expression of BDNF, TrkB, VEGF and CD105 in the cervical cancer tissue specimens and the normal cervix samples was evaluated by immunochemical staining with the corresponding antibodies. BDNF, TrkB and VEGF were identified to be widely expressed in the tumor cells with positive rates of 97.6, 88.2 and 93.0%, respectively. CD105 was located in the capillary wall with a positive rate of 92.4%. TrkB and VEGF were also detected in the capillary walls of the cancer specimens (Fig. 1). Furthermore, the expression levels of BDNF, VEGF and CD105 in SCC tissues were higher than those in normal cervical tissues ($P<0.001$, $P<0.05$ and $P<0.05$, respectively). However, the expression levels of TrkB in SCC tissues and normal tissues were not significantly different ($P>0.05$; Table SII).

Association of the expression of BDNF, TrkB and VEGF, as well as MVD, with clinicopathological parameters of the IB2-stage SCC patients. The correlation of the expression levels of BDNF, TrkB and VEGF, as well as the MVD (CD105 positivity), with various clinical indexes was statistically analyzed. The expression of BDNF was significantly correlated with positive LVSI ($P<0.001$), pelvic lymph node metastasis ($P<0.05$) and exogenous gross type ($P<0.05$). Furthermore, the expression of VEGF was associated with larger tumor

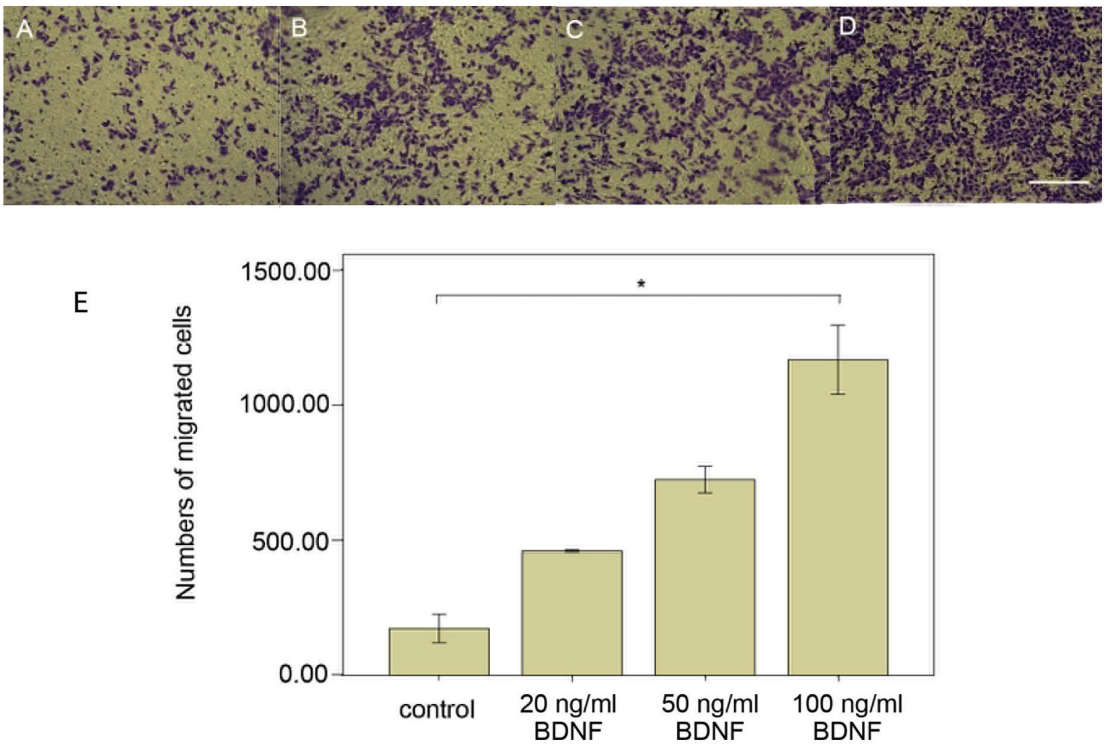

Figure 3. Transwell cellular migration assay using SiHa cells. Starved SiHa cells were (A) treated with PBS or stimulated with BDNF at (B) 20, (C) 50 or (D) 100 μg/ml for 18 h. Subsequently, the cells were subjected to a Transwell cellular migrative assay, the migrated cells on the filters were stained and representative images are provided in the left-hand panel (scale bar, 1 mm). (E) The average numbers of migrated cells in each group (three assays) are provided in a graph. BDNF significantly promoted cellular migration of SiHa cells in a dose-dependent manner. The 100 ng/ml of BDNF group is most effective among the 20, 50 and 100 ng/ml of BDNF groups. *P<0.001, BDNF (100 μg/ml) group vs. control group. BDNF, brain-derived neurotrophic factor.

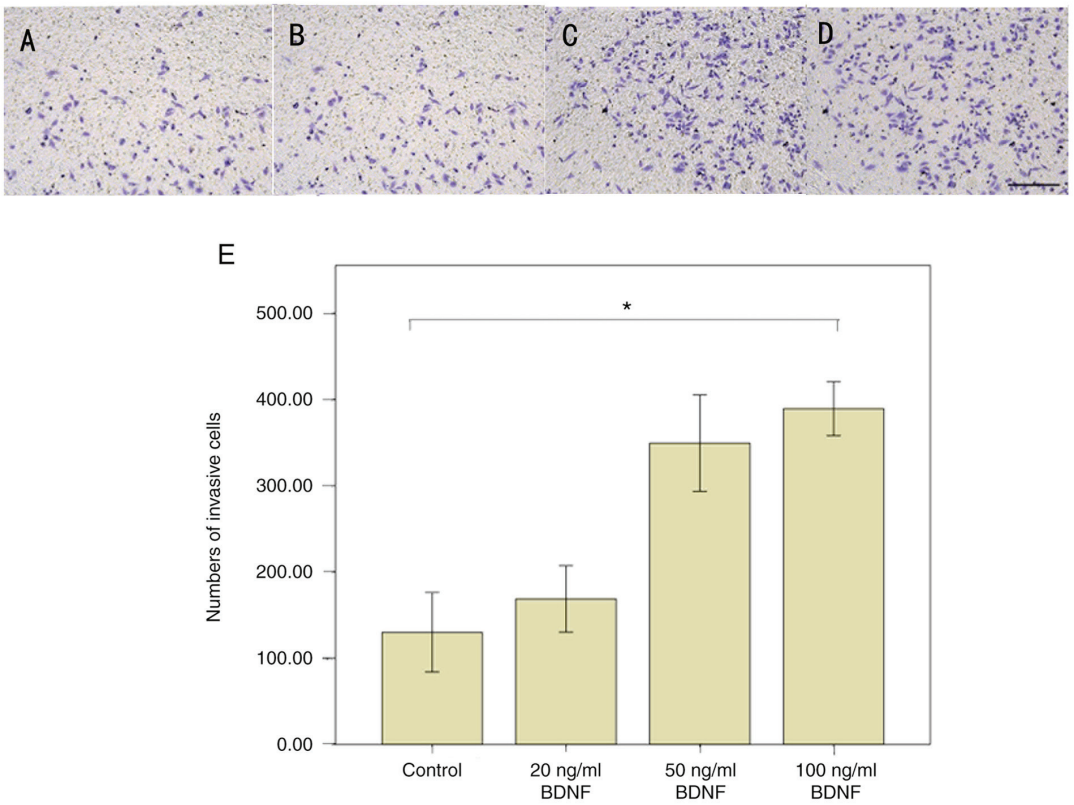

Figure 4. Transwell cellular invasion assay using SiHa cells. Starved SiHa cells were (A) treated with PBS or stimulated with BDNF at (B) 20, (C) 50 or (D) 100 μg/ml for 18 h. Subsequently, the cells were subjected to a Transwell cellular invasion assay, the invaded cells on the filters were stained and representative images are provided in the left-hand panel (scale bar, 1 mm). (E) The average numbers of invaded cells in each group (three assays) are provided in a graph. BDNF significantly promoted cellular invasion of SiHa cells in a dose-dependent manner. The 100 ng/ml of BDNF group is most effective among the 20, 50 and 100 ng/ml of BDNF groups. *P<0.001, BDNF (100 μg/ml) group vs. control group. BDNF, brain-derived neurotrophic factor.

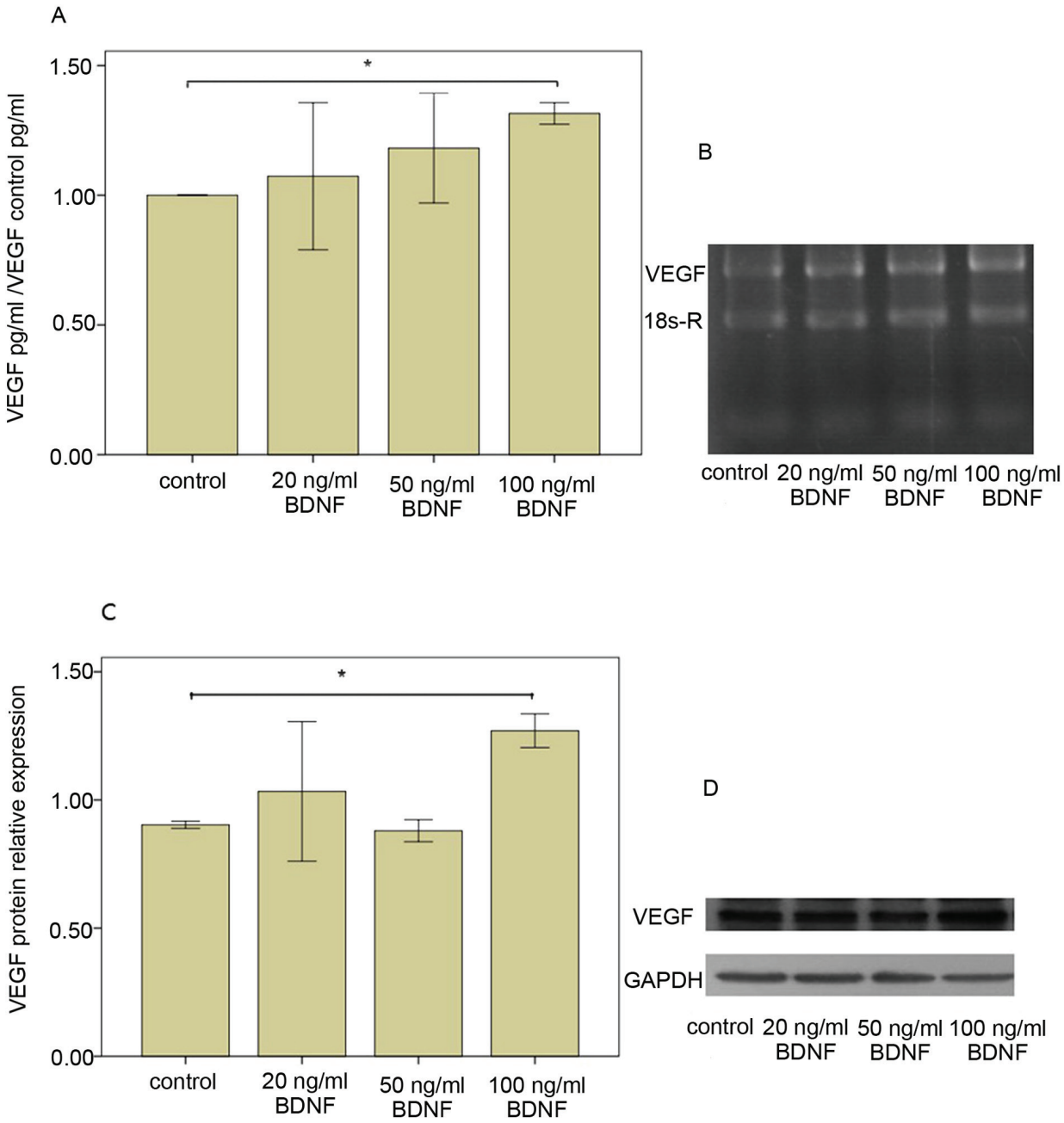

Figure 5. BDNF induces VEGF mRNA and protein expression in SiHa cells. SiHa cells stimulated with the indicated concentrations of BDNF. VEGF-A mRNA levels were detected by reverse transcription semi-quantitative PCR. The maximal expression of VEGF-A was detected in the cells treated with 100 ng/ml BDNF (P<0.05). (A) The average densitometric value for each condition is presented and a (B) representative gel with the PCR amplicons. The protein levels of VEGF were analyzed by western blotting. The maximal expression of VEGF-A was detected in the 100 ng/ml BDNF-treated cells (P<0.05). (C) The average densitometric value of the VEGF-A protein for each assay condition and (D) representative bands are presented. Values are expressed as the mean ± the standard error of the mean from three independent assays. BDNF stimulation of SiHa cells enhanced VEGF-A expression significantly in the protein and RNA level. *P<0.05, BDNF (100 μg/ml) group vs. control group. VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor.

size (P<0.05) and positive LVSI (P<0.05). No statistically significant correlation was identified between the expression of BDNF, TrkB and VEGF, as well as the MVD (estimated by CD105 stain), and other clinicopathological parameters, including age and DSI (P>0.05; Tables I and II).

High MVD is an independent predictor of poor prognosis in IB2-stage SCC patients. Among the 79 SCC patients, 23 patients died as the consequence of cervical cancer in the follow-up time of 13 years; the 5-year overall survival (OS) rate of the SCC patients was 70.9%. According to the univariate analysis of the association of factors with survival, age >35 years, as well as higher expression of VEGF and MVD were associated with a poorer prognosis regarding OS (P<0.05; Tables SIII and SIV). However, Cox multivariate regression analysis revealed that only high MVD was an independent prognostic factor for OS (hazard ratio, 2.723; 95% CI: 1.097-6.762; P<0.05; Fig. 2; Table SV), the expression of BDNF and VEGF were not independent prognostic factors for OS in SCC patients (P>0.05).

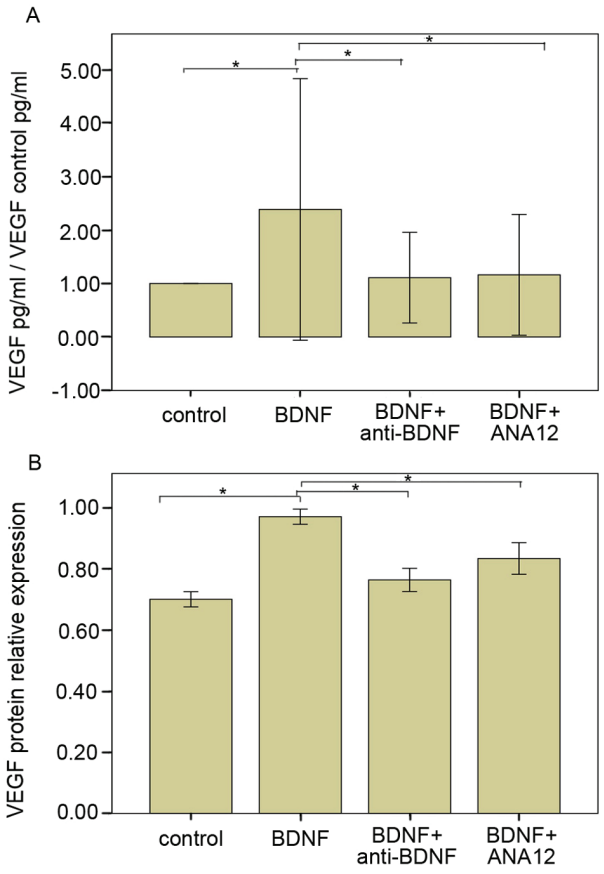

Figure 6. BDNF antibodies or BDNF antagonist ANA12 prevent BDNF-induced VEGF-A expression. BDNF antibodies (BDNF + BDNF antibody) or TrkB antagonist ANA12 (BDNF + ANA12) significantly prevented the 100 ng/ml BDNF-induced (A) VEGF mRNA and (B) VEGF protein expression (P<0.05). Values are expressed as the mean ± the standard error of the mean from three independent assays. *P<0.05. VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B.

BDNF enhances the cellular migratory and invasive potential of SiHa cells. The above results indicated that BDNF is closely linked to the invasive metastatic ability of SCC cells. Therefore, the effect of BDNF on the cellular migratory and invasive abilities of the SiHa cell line was assessed by using Transwell assays. The results indicated that BDNF significantly promoted cellular migration (Fig. 3) and invasion (Fig. 4) of SiHa cells in a dose-dependent manner. The maximal effective dose was 100 ng/ml BDNF in the two assays among the 20, 50, and 100 ng/ml BDNF groups (P<0.001).

Effect of BDNF-TrkB pathway on VEGF-A regulation. It was indicated that exogenous BDNF stimulation of SiHa cells enhanced VEGF-A protein expression. This response reached a maximum when the concentration of BDNF was 100 ng/ml (P<0.05). Furthermore, a similar trend was observed regarding the mRNA expression of VEGF-A (P<0.05; Fig. 5).

Addition of anti-BDNF antibody or inhibitor of tyrosine kinase receptor (ANA12, an inhibitor of TrkB receptors) obviously prevented the BDNF-induced VEGF-A expression in SiHa cells (P<0.05), indicating that VEGF-A induction by BDNF was mediated through TrkB receptor binding specifically. Similar results were obtained from the western blot detection of VEGF-A (P<0.05; Fig. 6).

Discussion

Pelvic lymph node metastasis is thought to be one of the most important sources of metastasis in cervical cancer. According to the National Comprehensive Cancer Network guidelines from 2015, it is a high-risk factor in cervical cancer patients (17).

BDNF reportedly promotes VEGF-C expression in bladder and cervical tumors. VEGF-C was verified to be positively correlated with pelvic lymph node metastasis in cervical cancer (18,19). Lin *et al* (20) reported that upregulation of BDNF is always accompanied with increased VEGF-C expression. Furthermore, they indicated that BDNF promoted VEGF-C-associated lymphangiogenesis via the mitogen-activated protein kinase kinase/ERK/mTOR signaling pathway, and further induced lymphatic metastasis. These activities were profoundly inhibited by BDNF knockdown *in vivo*.

In the present study, BDNF, TrkB, VEGF and CD105 expression was analyzed in the tumor samples of 79 patients with IB2-stage SCC of the cervix, and it was revealed that the BDNF level in cervical cancer samples was significantly associated with LVSI, pelvic lymph node metastasis. The ability of BDNF to stimulate aggressive behavior was further verified *in vitro* via cellular migration and invasion assays. Macdonald *et al* (21) investigated data from a large database, indicating that positive lymphatic metastasis is a predictor for poor prognosis in cervical cancer, which is negatively associated with the number of nodes involved. According to this previous study, radical hysterectomy and lymphadenectomy is the classic and standardized type of surgery for certain cervical cancer patients. However, Ferrandina *et al* (22) reported that the pelvic lymph node metastasis rate of locally advanced cervical cancer was only 10.9%, which means that a large proportion of patients with locally advanced cervical cancer without lymph node metastasis underwent unnecessary pelvic lymph node dissection.

The results of the present study indicated that the expression of BDNF is closely linked to positive LVSI and pelvic lymph node metastasis, suggesting that BDNF may be an effective predictor of lymph node metastasis. Furthermore, BDNF may be a useful indicator to accurately determine the status of lymph node metastasis prior to surgery and to facilitate the selection of appropriate candidates for pelvic lymph node dissection.

The results of the present study also revealed that VEGF expression was associated with tumor size and positive LVSI. In addition, MVD was an independent prognostic factor for OS of patients with SCC of the cervix. Indeed, Duff *et al* (23) reported a similar correlation of MVD (CD105-positive cells) or VEGF with cervical cancer. Barbu *et al* (24) reported on the upregulation of VEGF and angiogenesis in cervical adenocarcinomas, which was mainly distributed at the invasion front and indicated poor outcome. The results of a mechanistic *in vitro* experiment of the present study indicated that exogenous BDNF acting on SiHa cells enhanced VEGF-A expression, and this induction may be blocked by BDNF antibodies or an antagonist of its receptor TrkB (25). Therefore, it may be speculated that BDNF promotes neovascularization

by induction of VEGF-A expression through binding to its high-affinity receptor TrkB, which indirectly contributes to the progression of the tumor. Therefore, most studies have mainly focused on vascular targeting therapies for cervical cancers, including sunitinib, malate and sorafenib (26). In 2014, the US Food and Drug Administration approved bevacizumab for the clinical treatment of cervical cancer (27). However, anti-vascular targeted therapies are usually accompanied by considerable side effects: 40% of cervical cancer patients who received vascular targeting therapy had complications, including genitourinary tract spasm and thrombotic disorder, and 2.8% of the patients died as a result (28). An experiment using animal pancreatic cancer xenografts in a study by Zhao *et al* (29) revealed that TrkB antagonist significantly reduced the volume of xenografts. Based on the present results, anti-BDNF-TrkA treatment combined with anti-VEGF may be a potential strategy for the clinical treatment of cervical cancers.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YQ and WL contributed to the study design/planning, data collection/entry, data analysis/statistics and data interpretation. YQ contributed to the preparation of the manuscript and literature analysis/search. MH, WW, SK, LC, BL, ZC, CL, JH and XC contributed to the data collection/entry. CC and PL contributed to the study design/planning, data collection/entry, data interpretation and funds collection. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was part of a retrospective clinical research project based on a multicenter study of cervical cancer in China. It was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University (Guangzhou, China; code: NEEC-2017-135). Written informed consent for the use of specimens for scientific research was provided by all patients included in the current study.

Patients' consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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