G-protein-coupled receptor 120 regulates the development and progression of human esophageal cancer

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Abstract. The aim of the present study was to investigate the role of G-protein coupled receptor 120 (GPR120) in esophageal cancer and explore the related mechanisms. The expression of GPR120 in esophageal cancer tissues was examined by immunohistochemistry. Correlation analysis was performed to investigate the association between the level of GPR120 and clinical parameters. The expression of GPR120 was evaluated in esophageal cancer cell lines and the effects of GPR120 on cell proliferation, clone formation, migration and invasion were evaluated in an *in vitro* cell model and an *in vivo* ectopic tumor nude mice model. In addition, the effect of GPR120 on epithelial-mesenchymal transition (EMT), PI3K and I-κB pathway, as well as angiogenesis and inflammation-related cytokines was explored in order to elucidate the underlying mechanisms. Significantly increased expression of GPR120 was observed in esophageal cancer tissues compared to normal tissues. The expression of GPR120 was significantly related with histological grade, TNM stage and lymph node metastasis. GPR120 knockdown significantly decreased cell proliferation, clone formation, migration and invasion *in vitro* and decreased tumor growth *in vivo*. Furthermore significantly increased levels of E-cadherin and decreased levels of N-cadherin and vimentin, decreased level of Akt phosphorylation and I-κB phosphorylation, as well as decreased levels of vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and cyclooxygenase-2 (Cox-2) and its corresponding protein PGE2 were observed as the underlying mechanisms. In conclusion, we observed an increased level of GPR120 in esophageal cancer tissues, which served as a positive regulator of the development and progression of esophageal cancer. Multiple mechanisms including EMT, PI3K and I-κB pathway, as well as angiogenesis and inflammation-related cytokines were involved.

Introduction

Esophageal cancer, a serious malignancy with respect to prognosis and mortality rate, accounts for more than 40,000 deaths worldwide annually (1). According to the statistical data (2-4), esophageal carcinoma is the eighth most common cancer and the sixth most common cause of cancer-related deaths worldwide with more than 80% of total cases and deaths in developing nations, while the prevalence of esophageal cancer is expected to increase by 140% by 2025. Despite many advances in diagnosis and treatment, the 5-year survival rate for patients diagnosed with esophageal cancer ranges only from 15 to 20% (5,6). Therefore, it is necessary to explore novel therapeutic targets to achieve an improved treatment. Exploration and understanding of the molecular mechanisms involved in the development and progression of esophageal cancer provide possible clues for an improved treatment goal.

Dysregulated expression of G-protein-coupled receptor (GPCR) and dysregulation of GPCR signaling have been recognized as a hallmark of cancer (7). Several studies have revealed that GPCR could affect the multiple biological processes of cancer cells, including proliferation, migration and invasion (8-10), while these processes were involved in the development and progression of cancer. Recently, several GPCRs identified as free fatty acid receptors have emerged as key players in various physiological homeostasis mechanisms, and GPR120 is one of the receptors (11). Oh *et al* (12) have demonstrated that GPR120 could function as functional ω-3 PUFA receptor that mediates potent insulin sensitizing and anti-diabetic effects *in vivo* by supressing macrophage-induced adipose tissue inflammation in obese mice. Since the inflammation effects and macrophage-related properties are cancer related (13), GPR120 was considered to play a role in tumorigenesis. However, the role of GPR120 in esophageal cancer has not yet been explored.

In the present study, we explored the relationship between GPR120 and esophageal cancer and investigated the function

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and mechanisms of GPR120 in esophageal cancer cells in order to elucidate the role of GPR120 in the development and progression of esophageal cancer.

Materials and methods

Ethical approval of the study protocol. All research involving human participants was approved by the Institutional Review Board of The First Affiliated Hospital of Bengbu Medical School (Bengbu, China). Written informed consent was obtained from the participating individuals. The study protocol on animal research was approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Bengbu Medical School which is adherent to the accepted international guidelines for animal experimentation.

Tissue collection. A total of 100 specimens surgically derived from 100 esophageal cancer patients treated from January 2012 to December 2014 at The First Affiliated Hospital of Bengbu Medical College were collected. Among them, 50 cases belonged to matched tumor and normal mucosae, which were taken at least 5-10 cm away from the edges of a tumor of the same patient. All the enrolled patients did not receive any neoadjuvant chemotherapy or radiation therapy prior to esophagectomy. Fresh tumor tissues or corresponding normal esophageal mucosae were immediately frozen in liquid nitrogen after dissection, then stored at -80˚C until further analysis. Tumor specimens were carefully microdissected to ensure that at least 90% of the analyzed tissue contained cancer cells. The clinical diagnosis, tumor stage, histological differentiation and resection margin were determined by routine histopathological examination of hematoxylin and eosin (H&E) stained specimens by an experienced pathologist.

Immunohistochemistry. The paraffin specimen of each patient was stained by H&E staining and the pathological type of the tissue was analyzed by an experienced pathologist. The immunohistochemistry was performed using streptavidin-peroxidase method. The section was deparaffinated by the Leica TP1020 tissue processor (Leica Instruments, Mussloch, Germany) and pre-treated with microwave antigen retrieval procedure at 100˚C for 5 min in 10 mM citrate buffer (pH 6.0). After incubation in 3% hydrogen peroxide for 6 min, washing with phosphate-buffered saline (*PBS*) for 3 times, the slide was blocked using 50 μl goat serum at room temperature (RT) for 30 min. Subsequently, the primary anti-GPR120 antibody (dilution 1:100; cat. no. ab118757; Abcam, Cambridge, MA, USA) was added and the slide was incubated at 4˚C overnight. Subsequently, the HRP-conjugated sreptavidin was added followed by washing 3 times with PBS. To visualize the immunostaining, DAB (Dako, Carpinteria, CA, USA) was used. After the tissue turned yellow, the sections were washed, re-stained with hematoxylin, dehydrated and covered.

Assessment of the immunohistochemistry. The intensity of the immunostaining was evaluated by two pathologists without knowing the clinical history of the patients. The cells with dyed membrane or cytoplasm were considered as positive. Five representative regions of x200 magnification were selected to observe and at least 200 cancer cells were presented at that regions. Frequency and staining intensity of GPR120 by tumor cells were analyzed, and the expression of GPR120 was quantified using the modified Histo-score (H-score) (14), with a range of possible scores from 0 to 300. The expression of GPR120 was categorized into two groups according to the frequency distributions of the H-scores, using a cut-off score of >100 (H-score, 0-99=negative/low expression and 100-300=positive/high expression).

Cell culture. Human esophageal cancer cell lines Eca-109, TE-1 and KYSE450 and human colorectal cancer cell line SW480 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were maintained as monolayer cultures in cell culture flasks with RPMI-1640 medium containing 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics. Cells were cultured at 37˚C in a humidified atmosphere with 5% CO_2. All the cell culture media and additives were purchased from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Lentiviral shRNA particles. Recombinant lentiviral particles expressing GPR120 or control siRNA were obtained from GenePharma Co. Ltd. (Shanghai, China). Cells were grown to a certain degree of confluency ~40% and then infected with lentiviral particles in complete medium for 48 h. To increase infection efficiency, cells were co-treated with the cationic polymer Polybrene (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; 8 μg/ml in water). Neither shRNA nor Polybrene affected cell viability. The siRNA and shRNA had no off-target effects and at the indicated multiplicity of infection (MOI) and duration, failed to modulate cell adherence, shape and viability.

Real-time quantitative PCR. Total cellular RNA of human esophageal cancer cell line Eca-109 was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RT-PCR was performed using a One Step SYBR® PrimeScript™ RT-PCR kit (Takara Biotechnology, Co., Ltd., Dalian, China) and an iQ5 real-time PCR Detection system (Bio-Rad Laboratories, Hercules, CA, USA). The expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was assessed simultaneously in all samples as an internal control. Relative gene expression was determined by the $2^{-\Delta\Delta Ct}$ method (15). Oligonucleotide primers specific for GPR120, vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), cyclooxygenase-2 (Cox-2) and GAPDH are listed in Table I.

Western blotting. Cells obtained from the above-mentioned treatment were lysed in RIPA buffer, followed by high-speed centrifugation and protein quantification using a bicinchoninic acid assay (Thermo Fisher Scientific). Cellular proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidenedifluoride membranes. After blocking, the membranes were incubated with anti-total-(1:1,000; cat. no. 9272) or -phospho-Akt (1:1,000; cat. no. 5012), phospho-IκB (1:1,000; cat. no. 2859), E-cadherin (1:1,000; cat. no. 3195), N-cardherin (1:1,000; cat. no. 13116), vimentin (1:1,000; cat. no. 5741) (Cell Signaling Technology, Inc., Danvers, MA, USA) and GPR120 monoclonal

Table I. Primer sequences.

Gene	Forward primer	Reverse primer
VEGF	5'-TGCAATGGATCAAGGACCAGAGG-3'	5'-TGCAGCCAGCAAGAAGCATCAG-3'
IL-8	5'-CCGAGGATCTGATGACGATTA-3'	5'-GGCTCCCAGAAATAGCTTCAA-3'
Cox-2	5'-CACAGCACAGCCAGGAAGG-3'	5'-GTTCCCTGGCTCTGAGTAGTCGA-3'
GAPDH	5'-GGATTTGGTCGTATTGGG-3'	5'-GGAAGATGGTGATGGGATT-3'

VEGF, vascular endothelial growth factor; IL-8, interleukin-8; Cox-2, cyclooxygenase-2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

primary antibodies (dilution 1:1,000; cat. no. ab118757; Abcam, Cambridge, MA, USA). GAPDH (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as the loading control. Appropriate horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit HRP conjugate antibody: Dilution 1:2,000; cat. no. 7074; Cell Signaling Technology; and goat anti-mouse HRP-conjugate antibody: 1:2,000; cat. no. 7076; Cell Signaling Technology) were applied to detect labeled proteins. The protein bands were developed with SuperSignal Ultra Chemiluminescent Substrate (Pierce; Thermo Fisher Scientific) on X-ray films (Kodak Japan Ltd., Tokyo, Japan).

Cell proliferation. Human esophageal cancer cell line Eca-109 ($3x10^3$ cells) were seeded in 96-well plates in complete medium and infected with GPR120 or control siRNA lentivirus particles. Two days later, cell proliferation was evaluated by Cell Counting Kit-8 (CCK-8) method according to the manufacturer's instructions using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to assess the absorbance.

Clone formation. Human esophageal cancer cell line Eca-109 (800 cells) were seeded in 6-well plates in complete medium and infected with GPR120 or control siRNA lentivirus particles. After medium replacement at 24 h post-infection, the cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 for 7 days, and then they were stained with crystal violet. The colony survival with a definition of >50 cells were counted under a light microscope (DM4000B; Leica Microsystems, Benshein, Germany). The whole process was performed 3 times to obtain a mean number of colony formation.

Scratch assay. Human esophageal cancer cell line Eca-109 infected with GPR120 or control siRNA lentivirus particles were plated at 70,000 cells/well in a 12-well plate. Cells were grown to 90% confluency and scratched once using a sterile 1-ml pipette tip, washed twice with complete medium to remove floating cells and cell components. Images were captured at a x40 magnification using a Leica inverted phase contrast microscope (DM IRB; Leica Microsystems). The area of the gap at 24 h was assessed and subtracted from that at 0 h to quantify the migrated cells. The experiments were repeated at least 3 times with similar results.

Cell invasion. A Transwell system was employed to perform the cell invasion assay. Briefly, resuspended Eca-109 cells ($2x10^5$ cells) infected with GPR120 or control siRNA lentivirus particles were seeded into the upper chamber prefilled with Matrigel and RPMI-1640 medium supplemented with 20% FBS was added to the lower chamber. After the Transwell plate was maintained in a routine cell culture incubator for a specific time-point, the upper chamber was retained and the membranes were obtained for hematoxylin staining. The cell number of each membrane was determined in 3 randomly picked fields (magnification, x200) under a light microscope. All the experiments were performed in triplicate.

Nude mice model of ectopic tumor. Athymic nude (nu/nu) 6-weeks old mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). The tumors were generated by subcutaneous injection of $2x10^6$ GPR120 or control siRNA lentivirus particles infected Eca-109 cells suspended in 50 μl PBS into the dorsal region near the thigh. Mice were then weighted and assessed for tumor size every other day by measuring tumor length and tumor width. At week 4 post-treatment, all mice were sacrificed by cervical dislocation and the tumors were excised, weighted and imaged. For histological analysis, organs from the treated groups and the control group were fixed in 4% formalin, and then conducted with paraffin-embedded sections for H&E staining. The slices were examined by a digital microscope (Leica QWin Plus v3 software; Leica Microsystems).

Enzyme-linked immunosorbent assay (ELISA). Condition medium was obtained from the above-described cell culture at 1,500 x g centrifugation for 10 min and was stored at -80˚C before further processing. Angiogenesis and inflammation-related cytokines including VEGF, IL-8 and PGE2, were determined by ELISA kit (Invitrogen; Thermo Fisher Scientific) according to the manufacturer's instructions.

Statistical analysis. All statistical analyses were performed using SPSS version 18 (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± SD. The Student's t-test or one-way analysis of variance (ANOVA) were used to examine differences between groups. A P-value of <0.05 was considered to indicate a statistically significant difference.

Results

Correlation analysis between GPR120 level and clinical parameters in esophageal cancer. In order to investigate the role of GPR120 in esophageal cancer, we firstly performed

Table II. Correlation analysis between the level of GPR-120 and the clinical parameters.

Parameters	Cases (n=100)	GPR-120		χ^2	P-value
		Low	High		
Age (years)				0.219	0.640
≤60	43	11	32		
>60	57	17	40		
Sex				0.118	0.732
Female	42	11	31		
Male	58	17	41		
Tissue type				112.5	<0.001
Normal	100	100	0		
Tumor	100	28	72		
Size (cm)				0.842	0.359
≤5	57	18	39		
>5	43	10	33		
Histological grade				43.96	<0.001
I	16	15	1		
II	64	13	51		
III	20	0	20		
Location				0.121	0.942
Neck and upperthoracic	10	3	7		
Mid-thoracic	48	14	34		
Lower-thoracic	42	11	31		
Lymph node metastasis				8.73	0.003
Yes	45	6	39		
No	55	22	33		
Metastasis depth				16.053	<0.001
T1+T2	31	17	14		
T3+T4	69	11	58		

GPR-120, G-protein coupled protein-120.

the correlation analysis between the expression level of GPR120 and the clinical parameters of the esophageal cancer patients. As displayed in Table II, the expression level of GPR120 was significantly elevated in esophageal cancer tissues and correlated with histological grade (P<0.001), lymph node metastasis (P=0.003) and metastasis depth (P<0.001). These results indicated that GPR120 affected the progression of esophageal cancer.

Effects of GPR120 on cell proliferation, clone formation, cell migration and invasion in esophageal cancer. Since the expression of GPR120 in esophageal cancer was elevated, we obtained some esophageal cancer cell lines to investigate the biological function of GPR120. In order to mimic the clinical status, we first examined the expression level of GPR120 in esophageal cancer cell lines. As displayed in Fig. 1, significantly increased level of GPR120 expression was found in Eca-109 cells compared to TE-1 and KYSE450 cells. Therefore, Eca-109 cell line was selected as the cell line for the following experiments. According to the results displayed in Fig. 2,

GPR120-knockdown esophageal cancer cell line Eca-109 exhibited a significantly decreased degree of cell proliferation, clone formation, cell migration and invasion compared to the control cells. These results indicated that GPR120 affected the biological function of esophageal cancer cells via proliferation, clone formation, migration and invasion.

Effects of GPR120 on tumor growth in vivo. Due to the tumor-promoting effects of GPR120 observed *in vitro*, we further established an ectopic tumor nude mice model to evaluate the effects of GPR120 *in vivo*. As displayed in Fig. 3A and B, GPR120-knockdown esophageal cancer cell line Eca-109 exhibited a decreased level of tumor growth *in vivo* according to tumor size and weight. Furthermore, the immunostaining results also confirmed the effects of GPR120 knockdown in esophageal cancer cells (Fig. 3C).

Mechanism involved in the effects of GPR120. We further explored the mechanism involved in the effects of GPR120 in esophageal cancer. Our observations indicated that GPR120
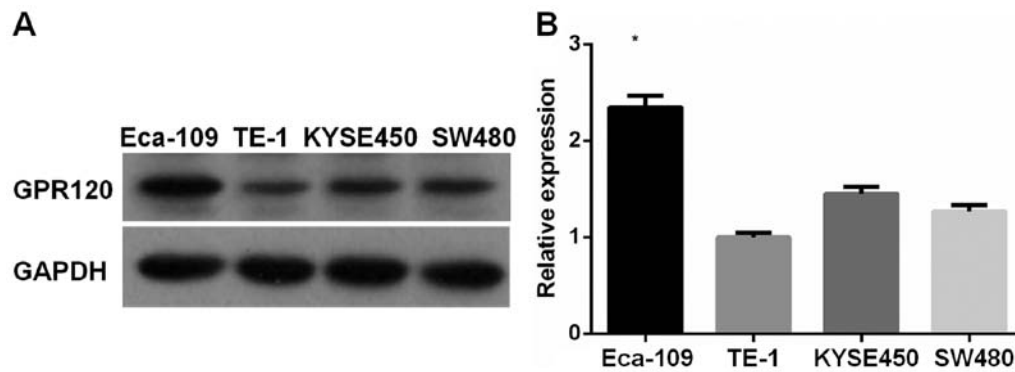
Figure 1. The expression of GPR120 in esophageal cancer cell lines. (A) Western blotting was used to evaluate the expression of GPR120 in esophageal cancer cell lines, Eca-109, TE-1 and KYSE450, while SW480 cell line was used as a positive control. Protein quantification was performed using ImageJ software (https://imagej.nih.gov/ij/download.html; Provided by the National Institutes of Health, Bethesda, MD, USA). (B) Significantly increased level of GPR120 was observed in esophageal cancer cell line Eca-109. *P<0.05 compared to TE-1. GPR120, G-protein coupled receptor 120.
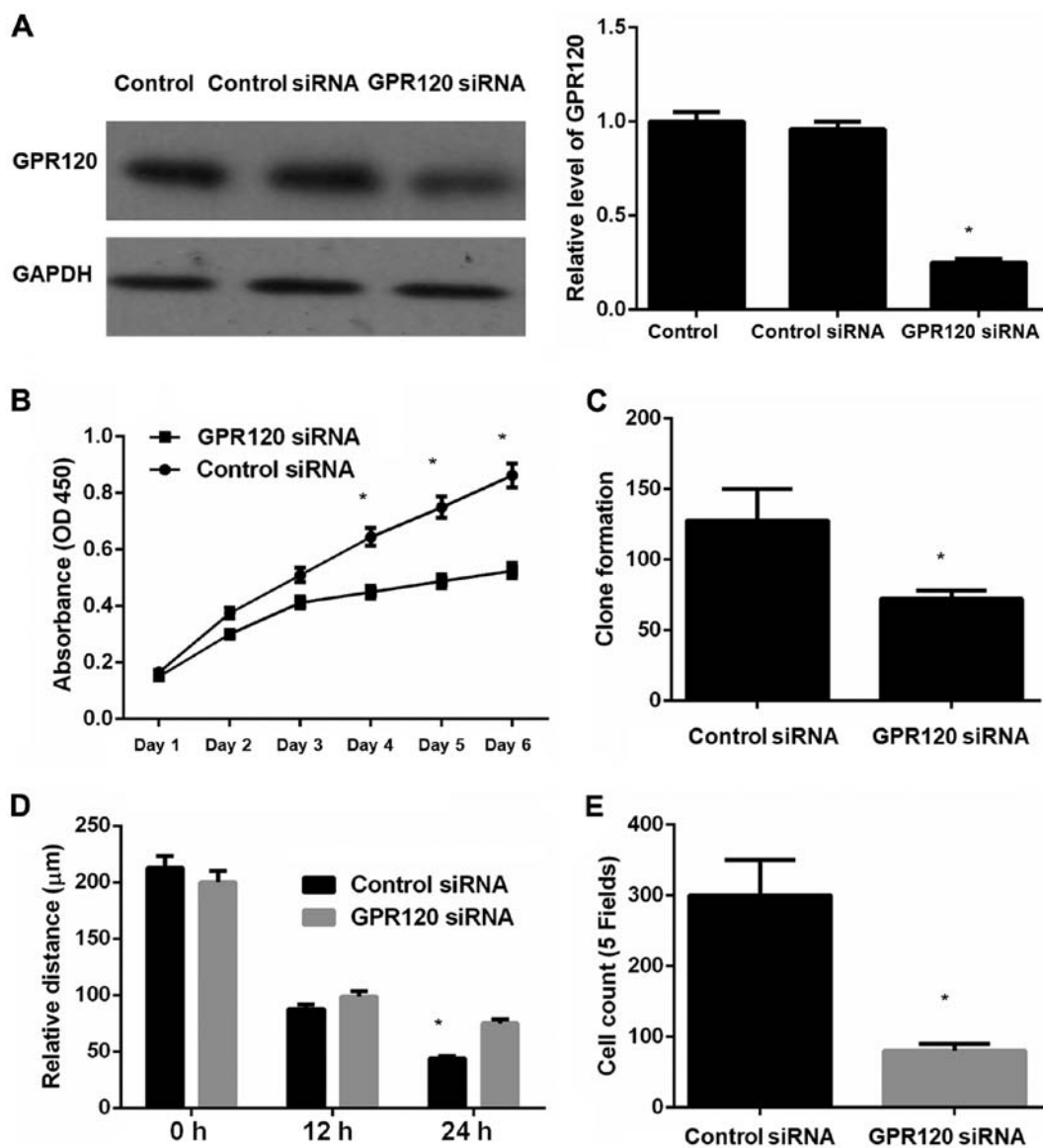

Figure 2. GPR120 knockdown decreases cell proliferation, clone formation, cell migration and invasion in esophageal cancer cells. (A) The efficiency of GPR120 knockdown was verified by western blotting. The relative expression of GPR120 siRNA cell line and control siRNA cell line was ~0.25. (B) Cell proliferation assay was performed using CCK-8 assay. Significantly decreased cell proliferation was observed at day 4 to day 6. (C) Clone formation assay. GPR120 knockdown significantly decreased clone formation. (D) Scratch assay for cell migration. GPR120 knockdown significantly decreased cell migration at 24 h. (E) Transwell cell invasion assay. GPR120 knockdown significantly decreased cell invasion at 24 h. *P<0.05 compared to control group. GPR120, G-protein coupled receptor 120.
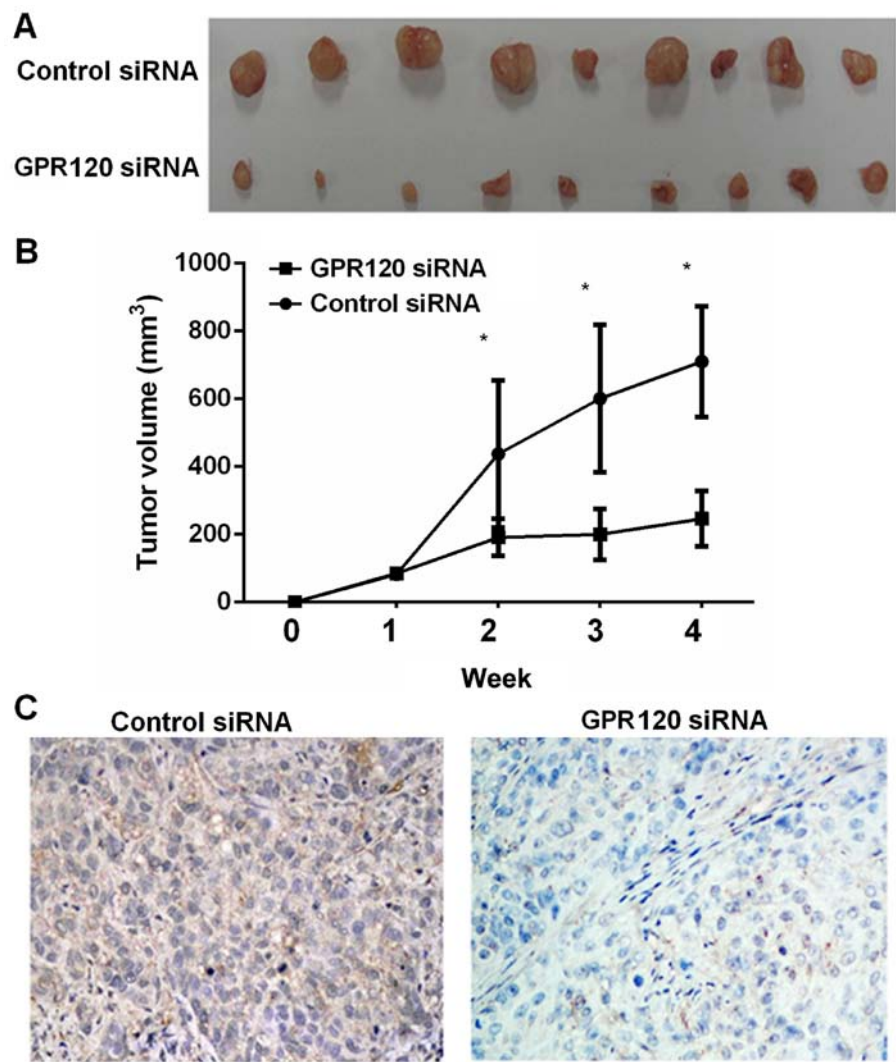
Figure 3. GPR120 knockdown decreases tumor growth *in vivo*. (A) Significantly decreased tumor size was observed in GPR120-knockdown cells compared to control cells. (B) Time-course monitoring of the tumor size. Significantly decreased tumor size was observed at week 2 to week 4. (C) Expression of GPR120 in esophageal cancer tissues from xenografts derived from GPR120-knockdown cells and control cells. Immunostaining was employed for the evaluation of the expression of GPR120. GPR120, G-protein coupled receptor 120.
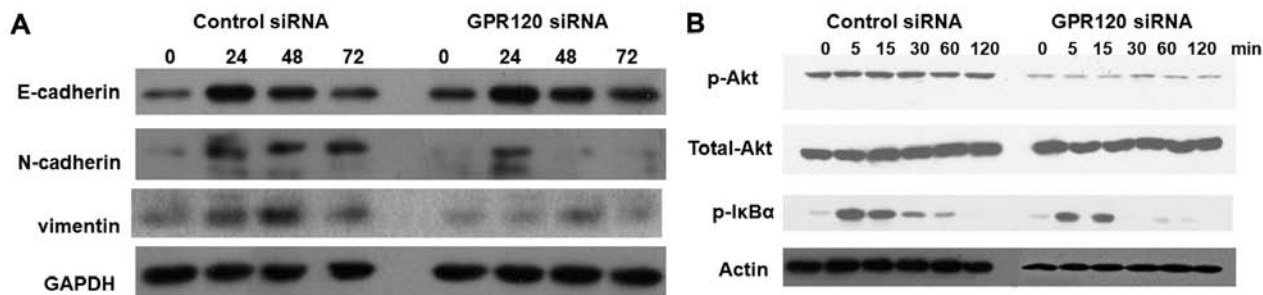

Figure 4. GPR120 regulates tumor growth via epithelial mesenchymal transition (EMT), PI3K and I-κB pathway. (A) Significantly increased level of E-cadherin and decreased level of N-cadherin and vimentin was observed in GPR120-knockdown esophageal cancer cell line compared to control cell line. (B) Decreased level of Akt and I-κB phosphorylation in GPR120-knockdown esophageal cancer cell line compared to the control cell line. GPR120, G-protein coupled receptor 120.

knockdown in esophageal cancer cell line Eca-109 resulted in increased level of the EMT marker E-cadherin and decreased level of N-cadherin and vimentin, decreased level of Akt phosphorylation and I-κB phosphorylation compared to the control cells (Fig. 4). These results indicated the possible involvement of EMT process, PI3K/Akt pathway and NF-κB in the role of GPR120 in esophageal cancer. Furthermore, we also examined the possible role of angiogenesis and inflammatory cytokines on the effects of GPR120 and we observed decreased mRNA and protein levels of angiogenesis cytokine
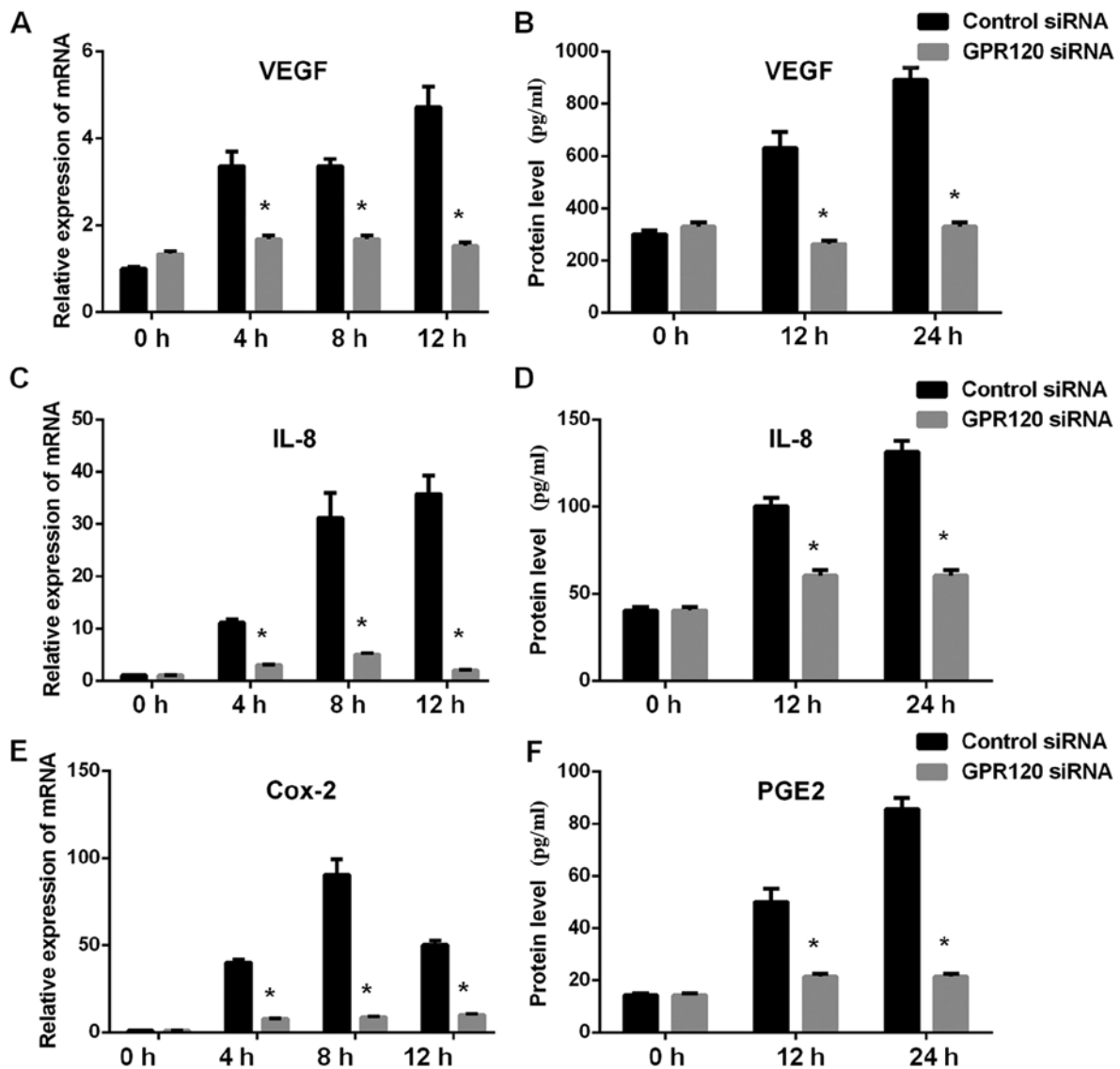
Figure 5. GPR120 regulates tumor growth via angiogenesis and inflammation-related cytokines. Significantly decreased mRNA and protein level of angiogenesis and inflammation-related cytokines in GPR120-knockdown esophageal cancer cell line compared to control cell line, concerning (A and B) vascular endothelial growth factor (VEGF), (C and D) interleukin-8 (IL-8) and (E and F) cyclooxygenase-2 (Cox-2) and its corresponding protein prostaglandin E2 (PGE2). GPR120, G-protein coupled receptor 120.

VEGF, inflammatory cytokine IL-8 and Cox-2 (protein PGE2) in GPR120-knockdown Eca-109 cells compared to control cells (Fig. 5).

Discussion

Practical strategies have been proposed to prevent the harmful sequelae of the worldwide obesity epidemic in order to reduce the future medical burden to society. Research has indicated the association between obesity and the overall risk for multiple cancers, including endometrial, colorectal, prostate, pancreatic and postmenopausal breast cancer (16-20). However, the exact role of obesity in cancer risk has not been fully explored. According to a previous study (21), the physiological effects related to obesity, including increased tissue inflammation, insulin resistance and/or hyperinsulinemia are considered to play a critical role in cancer risk. Therefore, dietary intervention is a potential mean to decrease this type

of risk in our daily life. Altering the balance between dietary ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) has been considered as an approach for disease prevention (22,23) and several epidemiological and preclinical studies have revealed an antitumor effect of ω-3 PUFAs in cancer patients (24-26). The detailed mechanisms mediated by which ω-3 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert their anticancer effects are not well understood despite multiple targets regulating cell proliferation and survival, inflammation, angiogenesis and metastasis may be involved (27). Recently, several GPCRs identified as free fatty acid receptors have emerged as key players in various physiological homeostasis mechanisms, and GPR120 has been demonstrated to function as a receptor for ω-3 PUFAs (11), and molecular and cellular effects could be generated following the ligand-receptor interaction.

In the present study, we firstly evaluated the expression of GPR120 in esophageal cancer tissue and observed significantly

increased GPR120 in esophageal cancer tissues compared to the normal tissues. Based on this observation, we performed *in vitro* and *in vivo* experiments to investigate the role of GPR120 in esophageal cancer development and progression. Our results indicated that GPR120 served as tumor-promoting regulator in esophageal cancer according to cell model and nude mice ectopic model. In addition, the investigation of the underlying mechanism indicated that EMT, PI3K and I-κB pathway, as well as angiogenesis and inflammation-related cytokines secretion attributed to the phenotype resulted by GPR120. To the best of our knowledge, this is the first study to elucidate the role of GPR120 in esophageal cancer.

Oh *et al* (12) have demonstrated that GPR120 is a functional ω-3 PUFA receptor that mediates potent insulin sensitizing and anti-diabetic effects *in vivo* by suppressing macrophage-induced adipose tissue inflammation in obese mice. The state of chronic, low grade inflammation arising in obesity is characterized by infiltration of M1-type adipose tissue macrophages, cells that secrete high levels of proinflammatory cytokines, including TNF-α, IL1β and IL-6, which are considered to be major contributors to tissue inflammation and insulin resistance in obesity (28,29). In cancer patients, increased inflammation levels are positively correlated with tumor cell proliferation, tumor stage and lymph node metastasis (30,31). In the present study, we also demonstrated that GPR120 promoted tumor cell proliferation, migration and invasion, and its expression level was associated with tumor stage and lymph node metastasis.

According to a previous study, the PI3K and NFκB pathways are involved in the inflammatory signaling pathway and ω-3 PUFAs can inhibit these pathways by sequestering TAB1 in obese mice (32). In the presents study, we also demonstrated that GPR120 knockdown resulted in decreased activity of Akt and I-κB phosphorylation. In a study by Wu *et al* (33), they also revealed that GPR120 exerted its functions via the PI3K and NFκB pathways in colorectal cancer. However, controversial results also existed. In a recently published study, Chung *et al* (34) demonstrated that obesity promoted mammary tumor progression in a model of postmenopausal breast cancer and that ω-3 PUFAs inhibited mammary tumor progression in obese mice, independently of GPR120. We believe that the differences may be attributed to the type of cancer.

Besides the aforementioned signaling pathways, GPR120 is considered to enhance cell motility by inducing EMT. In the present study, we observed significantly increased levels of E-cadherin and decreased level of N-cadherin and vimentin in GPR120 knockdown esophageal cancer cells compared to control cells, which is consistent with previous research.

In conclusion, in the present study, we demonstrated that increased level of GPR120 in esophageal cancer tissues, functioned as a positive regulator of the development and progression of esophageal cancer. Furthermore, multiple mechanisms including EMT, PI3K and I-κB pathway, as well as and angiogenesis and inflammation-related cytokines secretion were involved.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

ZC, DL, JL and HJ conceived and designed the experiments; ZC, DL, JL, YZ, HX, HY, HL, GW, HC, LZ and SY performed the experiments; ZC, DL, JL, YZ, HX, HY, HL, GW, HC, LZ, SY and HJ analyzed the data; ZC, DL, JL, YZ, HX, HY, HL, GW, HC, LZ, SY and HJ contributed reagents/materials/analysis tools; ZC, DL, JL and HJ contributed to the writing of the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All experimental protocols were approved by the Institutional Review Board of The First Affiliated Hospital of Bengbu Medical School (Bengbu, China).

Patient consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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