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MicroRNA-106b in cancer-associated fibroblasts from gastric cancer promotes cell migration and invasion by targeting PTEN

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ABSTRACT

It is well established that the interaction between cancer cells and microenvironment has a critical role in tumor development, but the roles of miRNAs in this interaction are rarely known. Here, we have shown that miR-106b is up-regulated in cancer associated fibroblasts compared with normal fibroblasts established from patients with gastric cancer, the expression level of miR-106b is associated with poor prognosis of patients, and CAFs with down-regulated miR-106b could significantly inhibit gastric cancer cell migration and invasion by targeting PTEN. Taken together, these data suggest that miR-106b might be a novel candidate target for the treatment of gastric cancer.

1. Introduction

Gastric carcinoma is the second most common cause of cancer-related death in the world and therefore seriously threatens human health [1,2]. Previous studies have indicated that the initiation and progression of gastric carcinoma was a long and multistep process characterized by genetic and epigenetic alterations of oncogenes and tumor suppressor genes [3], but the exact molecular mechanisms remain unclear.

Recently, it has been increasingly proved that the tumor microenvironment plays a crucial role in the development and progression of cancer [4,5]. Co-evolution and "reprogramming" of the stromal compartments are necessary for human cancer to progress into advanced stages [6,7]. Stromal interactions with cancer cells in the microenvironment determine whether cancer cells remain stable or progress into invasive and metastatic tumors [8–11].

The tumor microenvironment consists of various nonmalignant cells, such as endothelial cells, fibroblasts, immune cells, inflammatory cells, and so on. These cells are recruited to transform tumor cells by acting directly on them and/or releasing active molecules, such as growth factors and chemokines, which lead to cancer progression by promoting cell proliferation, inhibiting apoptosis and stimulating angiogenesis [12]. Cancer associated fibroblasts (CAFs) make up the major cell types in the tumor stroma, and reactive CAFs frequently accumulate in gastric cancer tissues. Furthermore, the prevalence of CAFs is related to certain clinicopathologic features, such as tumor size and invasive depth, as well as metastasis of gastric carcinoma [13]. Moreover, CAFs could stimulate tumor cell growth, migration and invasion, as well as resistance to chemotherapy [14–16].

MicroRNAs are a novel class of small non-coding regulatory RNAs that play an important role in various biological processes including cell division, differentiation, senescence and apoptosis [17,18]. Accumulating data indicate that microRNAs are involved in the dramatic changes in tumor microenvironment, particularly in the CAFs, which may contribute to activate fibroblasts and induce the progression of human tumor [19].

Abbreviations: CAFs, cancer associated fibroblasts; NFs, normal fibroblasts; miRNA, microRNA; PTEN, protein tyrosine phosphatase and tensin homologue; α-SMA, α-smooth muscle actin; FSP1, fibroblast specific protein 1; FAP, fibroblast activation protein; TGF-β, transforming growth factor-β; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; FGF-7, fibroblast growth factor-7; SDF-1, stromal cell-derived factor-1

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In the present study, we investigated the differential expression of microRNAs in the CAFs obtained from gastric cancer tissues versus matched normal gastric mucosa fibroblasts, and revealed that down-regulating miR-106b could significantly inhibit gastric cancer cell migration and invasion, which was mediated by its directly targeting the PTEN gene.

2. Materials and methods

2.1. Tissue samples and cell lines

We obtained paired samples of tumorous and adjacent normal tissue from patients with gastric cancer undergoing surgical resection at Department of General Surgery, Shanghai Tenth Peoples' Hospital Affiliated Tongji University. The protocols were approved by the Ethics Committee of Shanghai Tenth Peoples' Hospital for obtaining all tissue samples. All tissues were obtained from patients with gastric cancer who had not treated with radiation therapy or chemotherapy before surgery.

The fresh specimens were washed three times with PBS containing 100 U/ml penicillin and 100 μg/ml streptomycin and cut into small pieces and digested with collagenase I. Then the mixture was centrifugated and rinsed the cells with PBS, the pellets were resuspended in DMEM supplemented with 10% FBS and transferred to 100 mm dishes. Incubation conditions for CAFs and NFs were 37 °C in humidified air containing 5% CO_2. All cell lines were used in the experiments were in passage 3.

2.2. Immunofluorescence staining

Primary CAFs and NFs were seeded on coverslips and grown to 80% confluency, fixed in 4% paraformaldehyde for at least 15 min and permeabilized with 0.2% Triton X-100 in PBS for 5 min at room temperature. Then cell immunostaining was done with anti-α-SMA antibody and anti-fibronectin antibody at 4 °C. After being rinsed with PBS, then cells were incubated with secondary FITC-labeled goat anti-rabbit antibodies. DAPI was added to slides for counterstain. Immunofluorescent images were observed and recorded with a Nikon Eclipse 80i microscope.

2.3. MicroRNA microarray analysis

Total RNA from CAFs and NFs were extracted with the mir-Vana microRNA Isolation Kit. Then according to protocols by the manufacturer, the RNA were labeled and hybridized to Affymetrix microarray chips. GC-RMA algorithm was used to normalized raw data and the significance analysis of microarray (SAM) method was employed to identify differentially expressed genes. The NCode™ Multi-Species microRNA microarray kit V2 was used for analysis of the arrays. MicroRNAs levels with more than 1.5 folds increase or decrease changes at P value < 0.05 were considered significant ones and were studied.

2.4. Tissue microarray

Tumor cores obtained from 120 patients with gastric carcinoma from January 2006 to December 2012 in the Shanghai Tenth People's Hospital Affiliated to Tongji University were used for the tissue microarray. Briefly, after being deparaffinized with xylene and rehydrated with diluted alcohol series, the slides were digested with proteinase K. Hybridization was done with LNA-modified DIG-labeled probes for miR-106b, followed by a stringency wash. And then after blocking endogenous peroxidase activity, the slides were incubated in alkaline phosphatase conjugated anti-DIG Fab fragment solution.

Signal amplification was done with BCIP/NBT substrate, and nuclei were counterstained with Nuclear Fast Red. Independent blinded pathologists scored the intensity of staining with the scale as followed: negative = 0, weakly positive = 1, moderately positive = 2 and strongly positive = 3. Patients were dichotomized into low or high miR-106b expression groups using the median value of miR-106b as the cutoff point.

2.5. Quantitative Real-time PCR

Total RNA from tissue samples and cell lines were extracted with TRIzol reagent (Invitrogen, US). Real-time quantitative PCR was done with Real-time PCR Universal Reagent and the MX3000P Real-time PCR instrument following the manufacturers' protocols [20]. All steps were repeated for 3 times.

2.6. Western blotting

Proteins were purified with the NE-PER Nuclear and Cytoplasmic Protein Extraction Reagents Kit (Thermo Scientific) [20]. In brief, cells were lysed with lysis buffer and proteins were harvested. Proteins were resolved by 10% SDS PAGE and transferred onto PVDF membranes. The membranes were immunoblotted with primary antibodies overnight at 4 °C, and were incubated with a secondary antibodies conjugated with HRP. The expression level of proteins were analyzed with the Lab Work™ Image Acquisition and Analysis Software (UVP).

2.7. Dual-luciferase activity assay

Dual-luciferase activity assays were performed as previously described [20]. The 3′-UTR mRNA sequences of PTEN gene containing the miR-106b binding site were amplified by PCR and cloned into the Xba1-site of pGL3 vectors. The combination was named pGL3-PTEN. We also synthesized a pGL3-PTEN-mut construct with point mutations in the seed sequence using PCR method. All constructs were confirmed by DNA sequencing. Then, the cells were seeded in a 48-well plate and co-transfected with 0.5 μg of miR-106b inhibitors or control vector, 0.5 μg of pGL3-PTEN or pGL3-PTEN-mut, and 0.5 μg of a Renilla luciferase expression construct pRL-TK (Promega, WI) using Lipofectamine 2000. Luciferase activity was determined with the dual-luciferase reporter assay system after 48 h transfection, the relative luciferase activity is calculated as the ratio of Firefly luciferase activity versus Renilla luciferase activity.

2.8. Cell transfection and co-culture of fibroblasts with gastric cancer cells

Fibroblasts were transfected with miR-106b inhibitors or control plasmids with HiPerfect Transfection Reagent based on the manufacturer's instructions. Briefly, the cells were seeded onto plates with complete medium. And the transfection mixture prepared with serum-free medium, miRNA or control and HiPerfect reagent. And then they were incubated at room temperature and added onto the plates. Cells were incubated with transfection mixture for 48 h without changing medium change.

For co-culture experiments, gastric cancer cells were seeded at a density of 1×10^4 cells per well on the plates and incubated in the condition media collected from CAFs transfected with miR-106b inhibitors or empty vector control. The tumor cell growth was evaluated by MTT assay [20]. Briefly, at the end, gastric cancer cells were incubated in MTT at 37 °C and then lysed in DMSO at room temperature. Cell viability was estimated as the absorbance of 580 nm at different time points. All experiments were repeated for 3 times.

2.9. Cell migration and invasion assay

Gastric carcinoma cells with a density of 1×10^4 in serum-free media were seeded in transwell chambers with the Matrigel membrane covered or uncovered and then migration or invasion assay was performed with the condition media collected from CAFs transfected with miR-106b inhibitors or empty vector control. Cancer cells were fixed with methanol and stained with crystal violet. The cells which passed through the membrane were counted. All experiments were repeated for 3 times.

3. Results

3.1. Primary culture and characteristics of CAFs and NFs

The histopathologic grade of gastric cancer tissue sections were evaluated with H&E staining. The paired CAFs and NFs were isolated from tumor tissues and normal gastric mucosa. We determined the expression of the fibroblast markers, fibronectin (FN) and α-smooth muscle actin (SMA) to verify the purity of CAFs and NFs with immunofluorescence staining. The results showed that CAFs and NFs were all FN positive. Moreover, CAFs expressed a high level of α-SMA, which is a biomarker for myofibroblasts, but not in NFs (Fig. 1A). We have observed increased amounts of fibroblast specific protein 1 (FSP1) and fibroblast activation protein (FAP), known to be highly expressed in CAFs (Fig. 1B). Hence, we were able to culture CAFs with high purity from gastric tumor tissues and utilized them in the following assays.

To further analyze the effect of CAFs on gastric cancer cells, we carried out co-culture experiments to determine the ability of CAFs to promote the growth of gastric cancer cells. The results revealed that gastric cancer cells co-cultured with CAFs grew faster than with matched NFs (Fig. 1C). We also performed migration or invasion assays to examine the effects of CAFs on cancer cell motility, which showed that both migration and invasion capacity of gastric cancer cells were promoted by CAFs compared with NFs (Fig. 1D).

3.2. MicroRNA profiling

Using microarray chips to investigate the microRNA signature of the CAFs and NFs obtained from human gastric cancer tissues, we identified 11 human miRNAs differentially expressed in CAFs compared with corresponding NFs (Fig. 2A and B). Notably, miR-34b, miR-93, miR-301a and miR-106b, which have been reported to stimulate epithelial to mesenchymal transition (EMT) by targeting PTEN in invasive endometrial cancer cell lines and promote cell cycle in gastric cancer through regulating the expression of p21 and E2F5, were significantly up-regulated. Another seven miRNA, including miR-214, which have been indicated to promote gastric cancer cell proliferation, migration and invasion through regulating the expression of PTEN gene post-transcriptionally, were significantly up-regulated.

3.3. The expression of miR-106b in the gastric cancer stroma is associated with poor prognosis

The tissue microarray contains samples from 120 resected gastric cancer tissues and was stained for miR-106b using in situ hybridization. Representative images of stroma histoscore were shown in Fig. 2C. After separating the patients into groups with

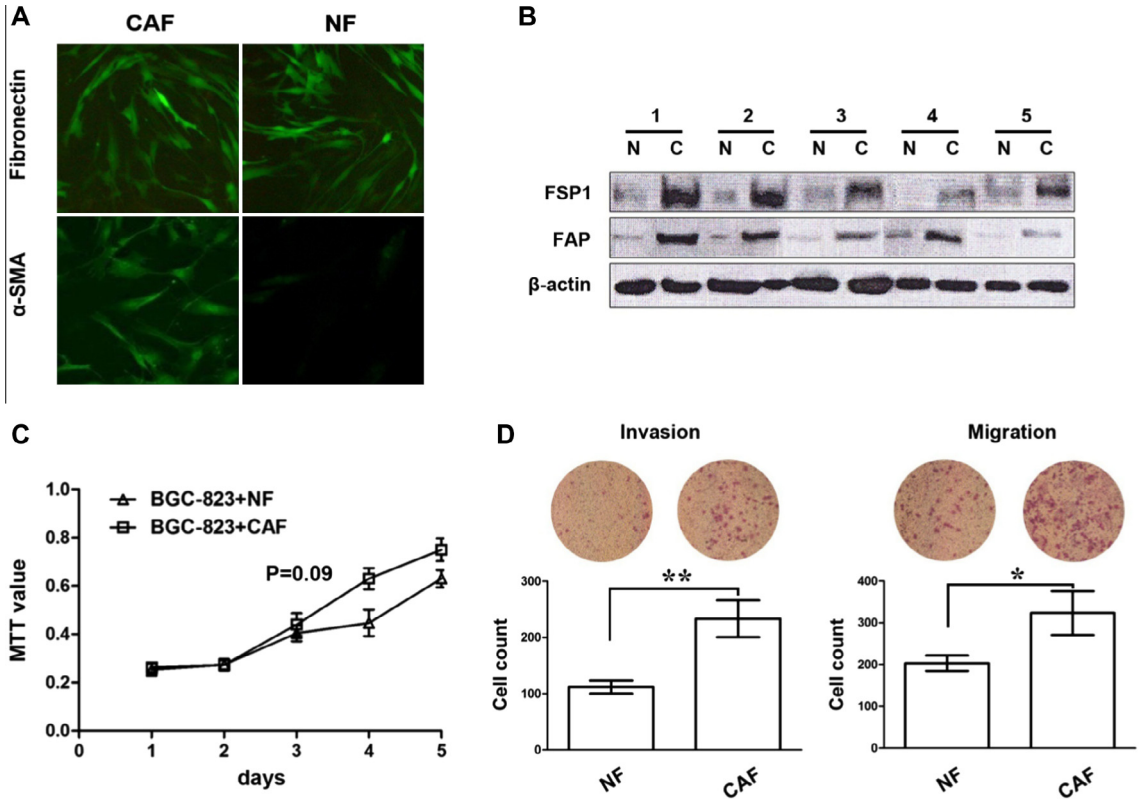

Fig. 1. Characterization of cancer-associated fibroblasts (CAFs) and normal fibroblasts (NFs). (A) The primary cultures of CAFs and NFs were isolated from the tumor tissue and the adjacent normal gastric mucosa. Fibronectin (FN), the fibroblast biomarker, is universally expression in CAFs and NFs. α-smooth muscle actin (α-SMA), the biomarker of the activate fibroblast is in high level expression in CAFs and weak expression in NF. (B) Western blot analysis of CAFs and NFs with anti-Fibroblast Specific Protein 1 (FSP1) and anti-Fibroblast Activation Protein (FAP). (C) CAFs stimulate growth of gastric cancer cell line compared to normal fibroblasts in co-culture experiments. (D) Conditioned media from CAFs stimulate gastric cancer cells transwell invasion and migration. Values in (C and D) represent average numbers for five pairs of fibroblasts ± S.E.M. *P* values were obtained by paired *t*-test ($^*P < 0.05$, $^{**}P < 0.01$).

A

miRNA	Location	CAF/NF	Fold change	p-value
hsa-miR-34b	11q23.1	Up	1.66	1.87E-03
has-miR-301a	19q32	Up	1.74	2.51E-02
hsa-miR-106b	7q22.1	Up	2.25	3.24E-02
hsa-miR-93	7q22.1	Up	2.42	2.45E-02
hsa-miR-483-3P	11p15.5	Down	-1.54	1.78E-02
hsa-miR-26a	3p22.2	Down	-1.56	2.12E-02
hsa-let-7g	5q32	Down	-1.58	1.45E-02
hsa-miR-148a	7p15.2	Down	-1.73	2.32E-02
hsa-miR-145	1p36.33	Down	-1.73	2.21E-02
hsa-miR-424	Xq26.3	Down	-1.88	1.34E-02
hsa-miR-214	1q24.3	Down	-2.34	3.21E-03

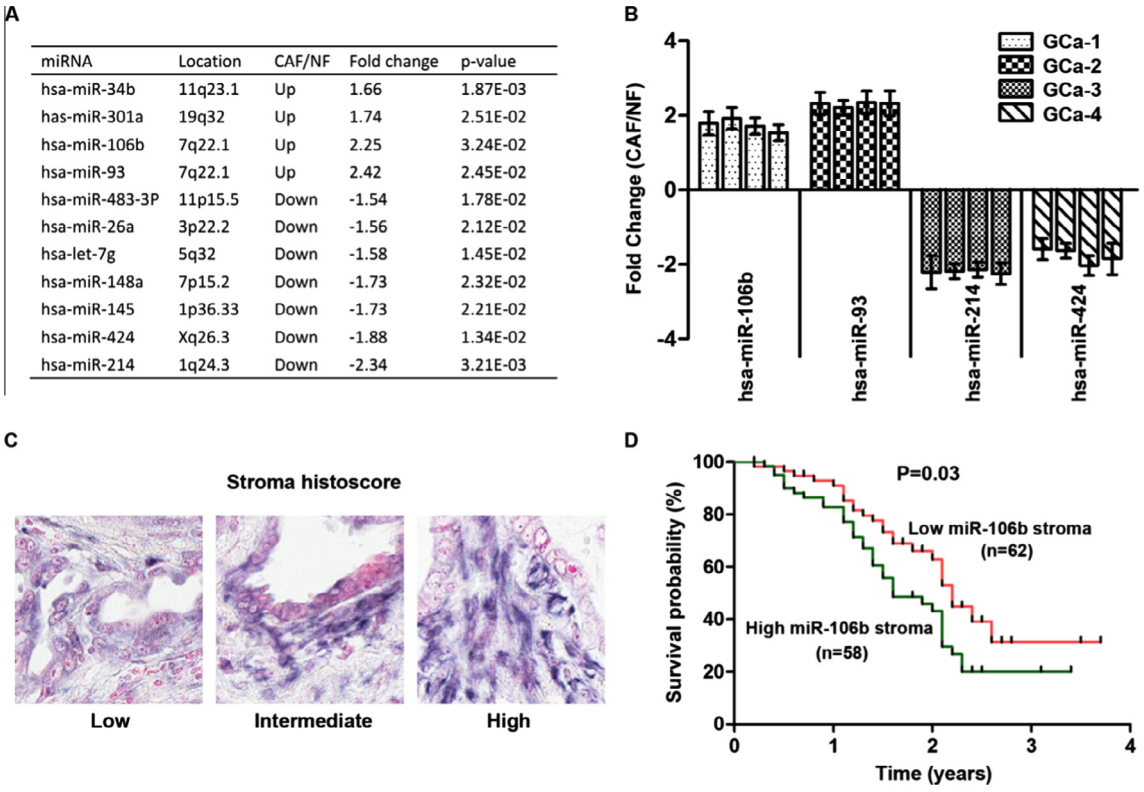

Fig. 2. MicroRNA analysis of paired CAFs and NFs. (A) List of microRNAs differentially expressed in CAFs relative to normal fibroblasts. (B) Four of randomly selected microRNA were analyzed by qRT-PCR in CAFs and NFs derived from gastric cancer patients. Data are shown as fold change between CAFs and NFs. (C) Representative images of histoscores for miR-106b in situ hybridization in gastric cancer tissues stroma. These cellular compartments were scored as 0 negative, 1 weakly positive, 2 moderately positive, 3 strongly positive. (D) Kaplan–Meier analysis shows that high miR-106b stromal expression is associated with decreased overall survival ($P = 0.03$). miR-106b expression intensity was dichotomized into high versus low based on the median score of all tumors.

high or low miR-106b expression according to the median histoscore, the results indicated that high miR-106b stromal expression were associated with shorter overall survival ($P = 0.03$, Fig. 2D) from Kaplan–Meier survival analysis, which suggested that miR-106b in tumor stroma might be related to the pathological process of gastric cancer.

3.4. Knockdown of miR-106b in fibroblasts could inhibit the migration and invasion of gastric cancer cell lines

To investigate the function of miR-106b in fibroblasts, we investigated whether knockdown of miR-106b in gastric cancer CAFs could inhibit cancer cell migration and invasion. We transfected miR-106b inhibitors into CAFs to inhibit the expression of miR-106b or the control lentiviral vector into CAFs. The conditioned medium from fibroblasts with low expression of miR-106b could reduce the migration and invasion of gastric cancer cells (Fig. 3A and B). However, MTT assay showed that there was no significant difference in growth rates of gastric cancer cells when co-cultured with CAFs transfected with miR-106b and vector control (Fig. 3D).

3.5. The PTEN 3′-untranslated region is a target of miR-106b

The results of luciferase reporter assay showed that co-transfection of miR-106b inhibitors with the reporter containing PTEN 3′UTR could significantly decrease the luciferase activity, while co-transfection with miR-106b inhibitors did not affect the luciferase activity of the empty vector control (Fig. 4A). In addition, mutations of miR-106b binding site could not reduce luciferase activity of cells expressing miR-106b inhibitors (Fig. 4B). All these results indicate that PTEN is a direct target of miR-106b.

We further investigated the PTEN protein and mRNA expression of CAFs transfected with anti-miR-106b or control vector using Western blotting and quantitative RT-PCR. As shown in Fig. 4C and D, the PTEN protein expression was significantly up-regulated in cells transfected with anti-miR-106b as compared to the cells treated with control vector. However, there was no significant difference between the two groups in the expression of PTEN mRNA (Fig. 4E). All above results demonstrate that PTEN is a target gene of miR-106b in CAFs.

3.6. Function of PTEN in fibroblasts of gastric cancer

The Western blot analysis confirmed that the expression of PTEN was lower in CAFs of gastric cancer than in NFs (Fig. 5A and B). Moreover, we also examined the prognostic value of PTEN expression in CAFs, the results of Kaplan–Meier analysis indicated that low expression of PTEN in CAFs was correlated with short survival time (Fig. 5C and D).

To further evaluate the contribution of PTEN in CAFs or NFs to gastric cancer cell migration or invasion, we did several experiments using CAFs in which the expression levels of PTEN were lower than those in NFs. Overexpression of PTEN in CAFs could promote migration and invasion of gastric cancer cells (Fig. 5E and F). While the migration and invasion of gastric cancer cells co-cultured with PTEN-depleted NFs were decreased compared with control vector (Fig. 5G and H).

Our results showed that up-regulation of PTEN expression in CAFs could significantly stimulate gastric cancer cell migration and invasion, which indicated that down-regulation of miR-106 in CAFs could inhibit migration and invasion of gastric cancer cells through PTEN-mediated signaling pathway.

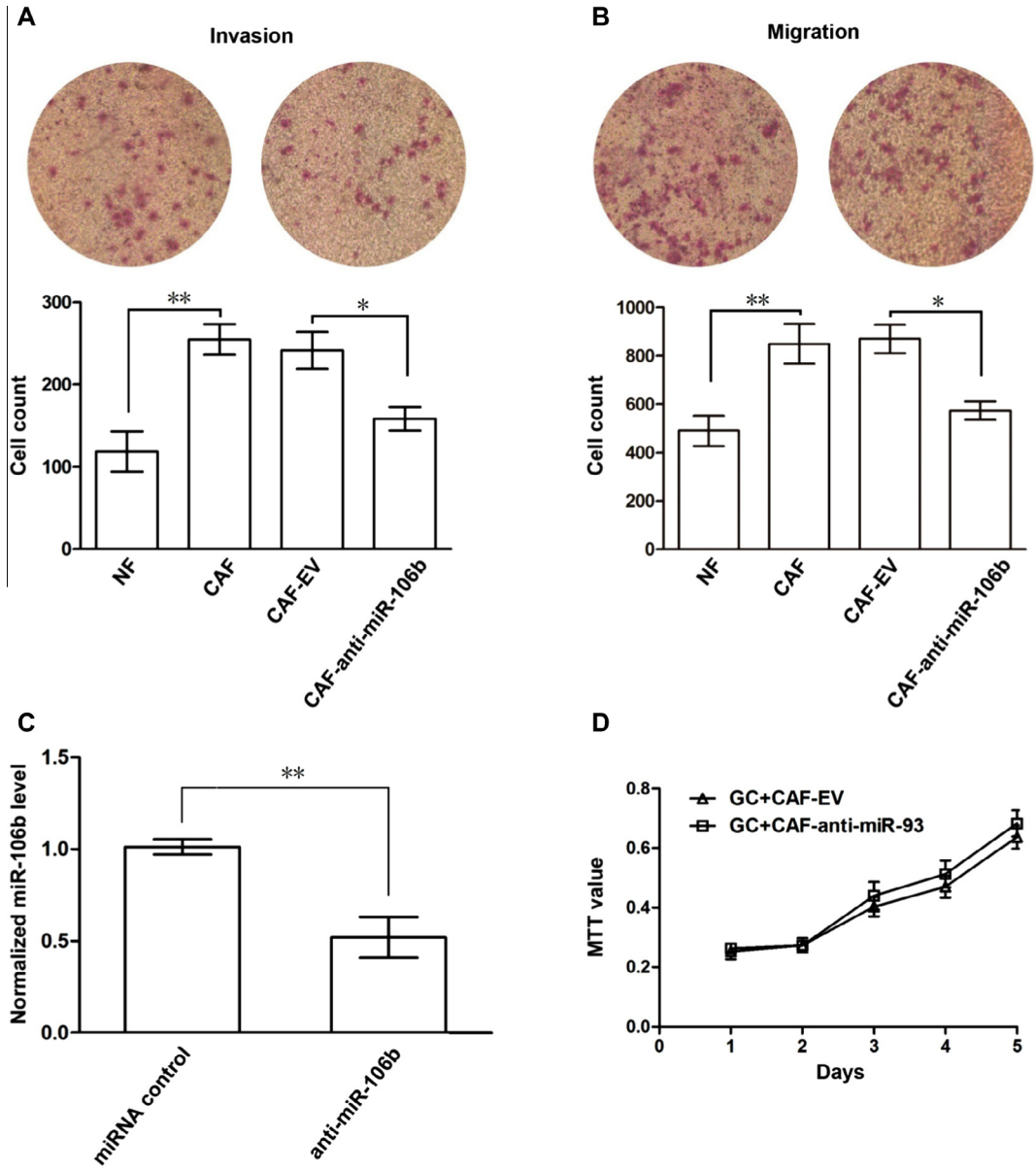

Fig. 3. Inhibition of miR-106b expression in CAFs could decreased tumor cell migration and invasion. (A and B) Transfection of anti-miR-106b in CAFs significantly decreased migration and invasion tumor cells in co-cultured experiment. (C) Detection of miR-106b mRNA in CAFs by qRT-PCR using U6 snRNA for normalization. Data are presented as mean ± S.E.M. Experiments were performed in triplicate and repeated at least three times using CAFs from two different patients. *P* values were obtained by paired *t*-test ($^{*}P < 0.05$, $^{**}P < 0.01$). (D) Inhibition of miR-106b expression in CAFs do not affect gastric cancer cell growth in co-culture experiments.

4. Discussion

Increasingly more evidence has shown that the initiation and progression of cancer are related to the interactions between both tumor and stromal cells in the tumor microenvironment [21]. Cancer associated fibroblasts are the most abundant cells in tumor stroma, and are well known to differ from normal fibroblasts in their expression profiles and behavior, as well as in their influence on cancer cells [22,23]. However, the exact molecular mechanism underlying aberrant expression remains poorly understood.

Several previous studies have suggested that microRNAs play an important role in regulation of specific genes present in the cells of the tumor microenvironment, which plays a vital role in tumorigenesis of various human cancer including stomach carcinoma [24–26]. For instance, Naito et al. [27] have shown that miR-143 is highly expressed in stromal fibroblasts of scirrhous type gastric cancer, where it may promote tumor progression by regulating the expression of collagen type III through TGF-β/SMAD signaling pathway.

Here, we examined whether microRNA expression differed substantially between NFs and CAFs with gastric cancer and whether these differences regulated fibroblast behavior. The results of our present study have revealed that miR-106b was significantly up-regulated in CAFs of gastric cancer, which was previously reported to be frequently overexpressed in many human malignant tumors, such as colorectal cancer [28], gastric cancer [29], hepatocellular carcinoma [30] and head and neck squamous cell carcinomas [31]. In addition, up-regulation of miR-106b was also related to enhancing the proliferation, migration and invasion of tumor cells in human cancers. Moreover, our present study indicated that overexpression of miR-106b in the gastric cancer tissue stroma is associated with poor prognosis. All these findings together with those presented here suggest that miR-106b expression in CAFs might play an important role in biological process of human gastric cancer.

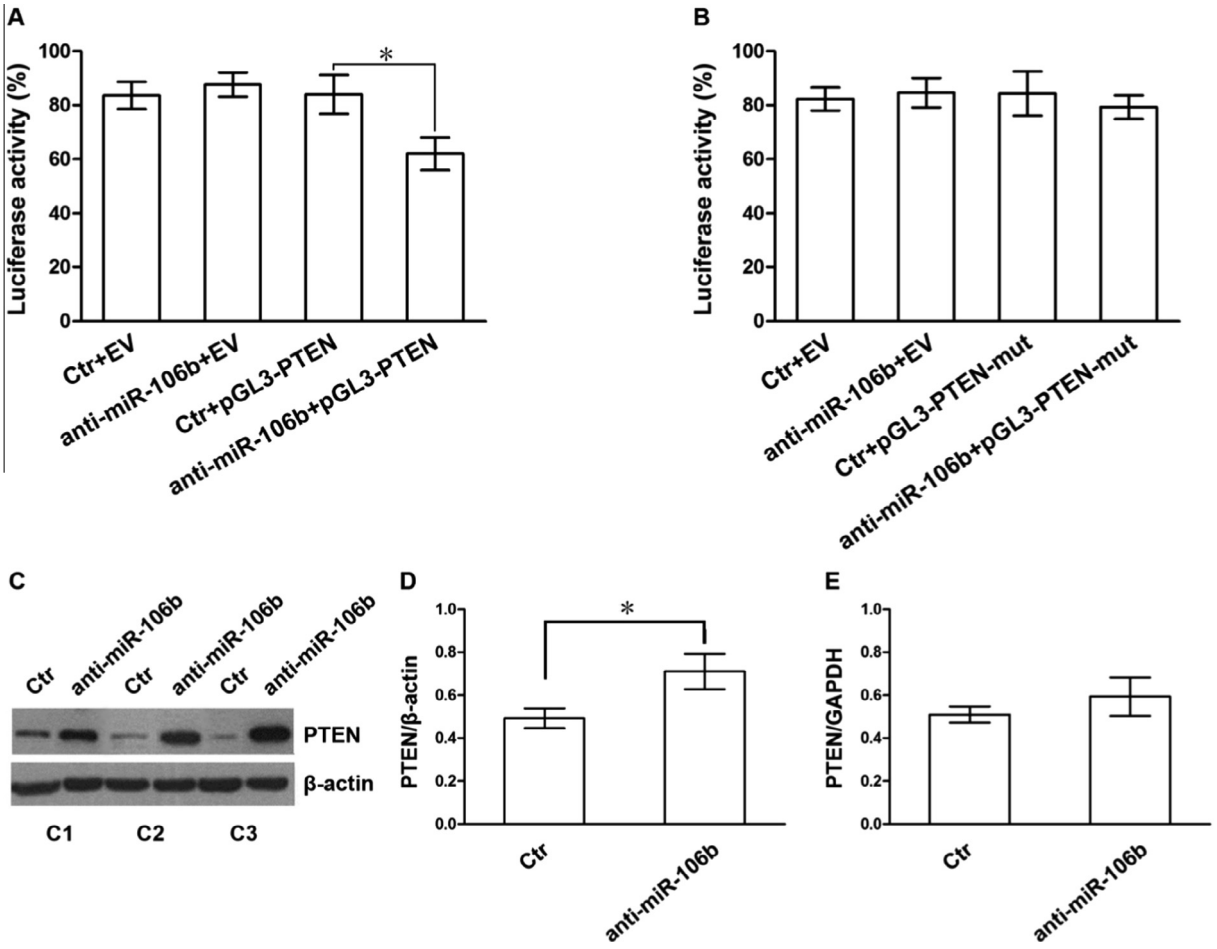

Fig. 4. PTEN is a direct target of miR-106b. (A and B) Luciferase reporter assay were performed at 48 h post-transfection. CAFs were transfected respectively with the Renilla luciferase expression construct pRL-TK and pGL3-PTEN-3′-UTR firefly luciferase expression construct, along with either anti-miR-106b or control anti-miRNA. Ctr, non-targeting control; EV, empty vector. Results showed that cells co-transfected with miR-106b and pGL3-PTEN plasmid exhibited a significant increase of reporter activity in comparison with those co-transfected with the control anti-miRNA and pGL3-PTEN plasmid. However, the reporter activity of cells co-transfected with anti-miR-106b and pGL3-PTEN-mut plasmid showed no significant difference with that of cells co-transfected with control microRNA and pGL3-PTEN-mut plasmid. (C and D) The expression level of PTEN protein was detected by Western Blot at 48 h post-transfection and normalized to that of β-actin. Results showed that the level of PTEN protein was significantly increased in cells transfected with anti-miR-106b as compared to the cells transfected with control anti-miRNA. (E) The expression level of PTEN mRNA was detected by qRT-PCR at 48 h posttransfection and normalized to that of GAPDH. Results showed that the expression level of PTEN mRNA exhibited no significantly difference between cells transfected with anti-miR-106b and those transfected with control anti-miRNA. Data represent mean ± S.E.M. from three independent experiments; $^{*}P < 0.05$ by t test, $^{**}P < 0.01$ by t test.

Interestingly, we have shown that down-regulated miR-106b expression in CAFs could inhibit gastric cancer cell migration and invasion in co-culture experiments. In addition, our studies also revealed that PTEN is regulated by miR-106b through binding of its 3′-UTR, which proved that PTEN is a direct target of miR-106b. Both miR-106b and PTEN can affect gastric cancer cell migration and invasion but in opposite directions. Namely, miR-106b promotes and PTEN inhibits migration and invasion of cancer cells.

Recent studies have shown that miR-106b could regulate diverse important signaling pathways including PTEN and TGF-β signaling pathways [32,33], which are also involved in cell cycle progression mediated by CDKN1A [34] and RB [35]. Here, our experimental data suggest that down-regulation of miR-106b expression may inhibit gastric cancer cell migration and invasion by targeting PTEN.

PTEN was identified as a tumor suppressor gene, which is involved in cancer cell proliferation, migration, invasion and apoptosis, as well as angiogenesis through several signal pathways [36,37]. The function of PTEN in tumor cells is extensively studied but its role in tumor microenvironment is not well-known. Recent researches have demonstrated that PTEN deficiency in mammary stromal fibroblasts of mice could stimulate the progression and malignant transformation of mammary epithelial tumors by activating the transcription factor ETS2. They identified the Pten-miR-320-Ets2 regulatory axis as a critical stroma-specific signaling pathway important in the complicated network of communication in tumor microenvironment, which was responsible for the pathological molecular events investigated in human mammary tumors [38,39].

Cancer associated fibroblasts are most important components of tumor microenvironment in the majority of human cancers, which secrete various growth factors and chemokines, such as transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), fibroblast growth factor-7 (FGF-7), and stromal cell-derived factor-1 (SDF-1) into the tumor microenvironment that can stimulate tumor cell proliferation, invasion, metastasis and angiogenesis[40–43].

Zhi et al. [13] have previously revealed that the prevalence of CAFs is closely correlated with the potential of gastric cancer metastasis. Moreover, TGF-β and FGF-7 produced from fibroblasts could increase scirrhous gastric cancer cell proliferation and invasiveness [42]. Wu et al. [44] also demonstrated that HGF is an

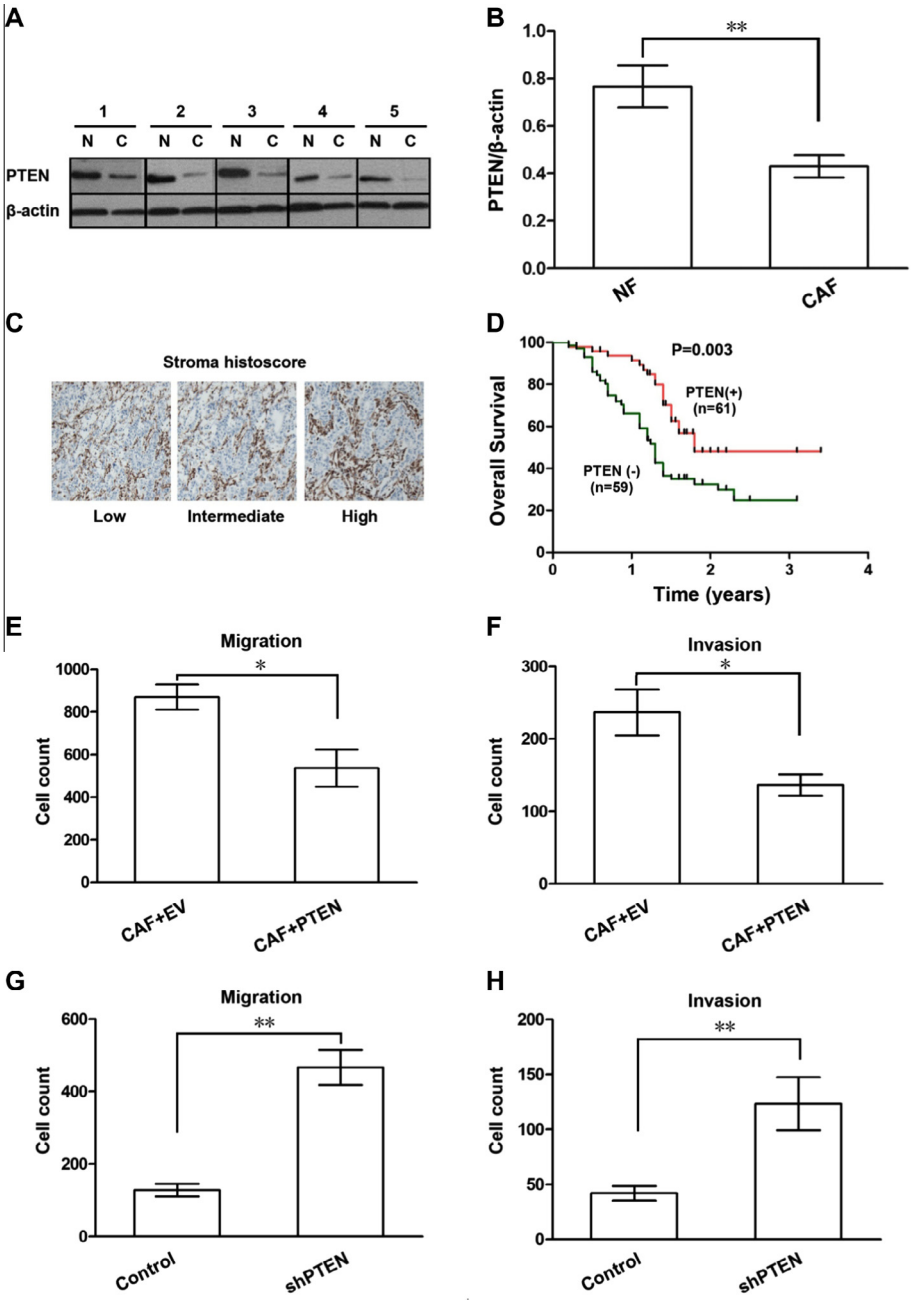

Fig. 5. Up-regulation of PTEN expression in CAFs could inhibit gastric cancer migration and invasion. (A and B) Western blot analysis of PTEN protein expression in five pairs of CAFs and NFs, the results shows that the expression of PTEN was significantly lower in CAFs than in NFs. (C and D) Representative images of H&E staining histoscores for PTEN protein in gastric cancer tissues stroma, furthermore, Kaplan–Meier analysis and the log-rank test showed that low expression of PTEN in CAFs predicted poor survival. (E and F) Transfection of PTEN plasmid in CAFs significantly inhibit migration and invasion tumor cells in co-cultured experiment. (G and H) The PTEN expression of NFs was knockdown by stable expression of lentiviral vector with shPTEN, the results indicated that down-regulation of PTEN in NFs could promote migration and invasion of gastric cancer cells. Data represent mean ± S.E.M. from three independent experiments; $^{*}P < 0.05$ by t test, $^{**}P < 0.01$ by t test.

important factor secreted from CAFs that could accelerate tumorigenesis in gastric cancer in a paracrine manner. Furthermore, PTEN signaling is also closely associated with growth factors and chemokines from CAFs. PTEN can modulate glioma response to the inhibition of HGF/c-Met signaling pathway [45], and PTEN loss amplifies c-Met-induced glioblastoma malignancy [46]. In addition, PTEN loss also induces autocrine FGF signaling to promote skin tumorigenesis [47], and lack of PTEN in osteoprogenitor cells resulted in increased osteoblast numbers and expanded bone matrix by stimulating FGF signaling [48]. Thus, in our study, up-regulation of PTEN expression in CAFs inhibited gastric cancer cell migration and invasion, which is probably mediated through regulating the expression and secretion of these growth factors and chemokines.

In summary, our results showed that miR-106b is overexpressed in CAFs of gastric cancer, and knockdown of miR-106b in CAFs could significantly inhibit gastric cancer cell migration and invasion through PTEN-mediated signal pathway, which has not been reported in previous studies. Based on these findings, we suggest that miR-106b in CAFs might be regarded as a new promising therapeutic agent for gastric cancer.

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