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Type

Research paper

Keywords

prognosis, neoadjuvant chemotherapy, PDGFRB, Metastatic gastric cancer

Abstract

Introduction

This study aimed to investigate the effect of PDGFRB on the efficacy of neoadjuvant chemotherapy (NAC) in metastatic gastric cancer (MGC).

Material and methods

The data from Gene Expression Omnibus (GEO) were analyzed to screen differentially expressed genes (DEGs). PDGFRB expression in cells were assessed by qRT-PCR and immunohistochemistry. Transwell, MTT and colony form assays were applied to determine the abilities of cell migration, cell invasion and cell proliferation. The association of core gene and PI3K/AKT pathway were identified through STRING database and western blot detection. Differences in overall survival (OS) among different patients were analyzed through Kaplan-Meier survival curve and prognostic factors were analyzed by Cox regression.

Results

High expression of PDGFRB and were found in gastric cancer through GEO data and MGC patients with combination chemotherapy resistance of docetaxel, cisplatin and S-1 (DCS therapy). Furthermore, the down-regulation of PDGFRB by PDGFRB shRNA or sunitinib malate could decrease cell migration, cell invasion and cell proliferation in resistant cells. Those findings were verified by in vivo experiments. Meanwhile, PDGFRB overexpression was accompany with the activation of PI3K/AKT pathway. In addition, the OS of patients in highly expressed PDGERB group were lower than that in lowly PDGFRB expressed group and PDGFRB, TNM staging and differentiation degree were the prognostic factors for MGC patients.

Conclusions

PDGFRB knockdown could rescue the efficacy of chemotherapy in MGC and PDGFRB could serve as a novel marker for the prognosis of MGC.

PDGFRB knockdown rescues the efficacy of chemotherapy in metastatic gastric cancer patients

Running title: Role of PDGFRB in MGC

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Keywords: PDGFRB; Metastatic gastric cancer; Neoadjuvant chemotherapy; Prognosis

Preprint

Introduction

In 2016, gastric cancer (GC) was ranked as the second most common cause of cancer-related death and the fourth most common cancer in the world. About 1,400,000 people worldwide are diagnosed as GC each year, and approximately 850,000 people die from GC [1]. GC is a disease caused by multiple factors, including environmental and genetic factors, such as age, sex, smoking history, family history and *Helicobacter pylori* infection [2]. With the development of clinical research, the survival rate of GC patients has been significantly improved. For example, the five-year survival rate of patients was increased from 15% to 29% from 1975 to 2016 [3]. Surgery is the best choice for the treatment of GC [4], but a simple surgical is not suitable for the therapy of metastatic gastric cancer (MGC). For MGC, chemotherapy can effectively alleviate symptoms, improve the patients' life quality and increase the survival rate. Furthermore, multidrug chemotherapy precedes monotherapy in the survival rate, remission rate and symptom control [5, 6], but the five-year survival rate of metastatic gastric cancer is still only about 5%. Seeking the markers for the diagnosis and prognosis of the diseases is significantly important [7]. It is necessary to find the biomarkers to predict the prognosis of MGC patients and regulate them for their further treatment.

Chemotherapeutic resistance refers to the resistance of tumor cells to chemotherapy drugs that often result in failure of chemotherapy. Due to the implementation of comprehensive diagnosis and treatment of tumor, chemotherapy resistance is an important cause of disease progression in patients. For example, patients with advanced

ovarian cancer have a high level of primary resistance to first-line chemotherapy drug paclitaxel, and 50 percent of patients with a multidrug resistance have relapsed. Even though the combination of two or more chemotherapeutic drugs with different mechanisms is used to minimize the incidence of drug resistance and the increase of “cross resistance”, side effects are still difficult to avoid. Chemotherapy resistance greatly limits the patient’s clinical benefit. After a period of chemotherapy drug treatment, the tumor cells were adapted to change, such as epithelial cells to interstitial phenotype transformation, and cancer stem cell (CSC) amplification, etc., which caused secondary resistance. The more evidence that EMT and CSC activation are associated with the occurrence of drug resistance [8].

Platelet derived growth factor receptor (PDGFR) as a single transmembrane glycoprotein that belongs to the type III tyrosine protein kinase family, distributes in various kinds of cells, such as smooth muscle cells [9], fibroblasts [10], endothelial cells [11], glial cells and cartilage cells, etc. Moreover, PDGF and PDGFR are expressed in many human cancers, including colon [12], Head and neck squamous cell carcinoma (HNSCC) [13, 14], prostate [15], lung [16] and breast cancer [17]. PDGFRB gene is a kind of PDGFR, and plays an important role in promoting tumor cell proliferation, invasion, and new blood vessels form, which can result in metastasis of cancers. Considering that the platelet counts of patients suffering from advanced cancer are decreasing significantly, and PDGFR is closely related with platelet, PDGERB could function as a novel biomarker for MGC through regulating downstream pathways.

Overall, we aimed to figure out the expression of PDGFRB in resistant cells of MGC, the influence on proliferation, migration and apoptosis of MGC cell lines and the reliability of PDGFRB as a prognostic marker.

Materials and methods

Microarray data processing

With “metastatic gastric cancer” and “chemotherapy” as keywords, “GSE31811” microarray data was screened as gene expression profiles for pre-analysis in the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo>). The platform was GPL6480: Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (Probe Name version). Fresh-frozen samples of tumor tissue and background gastric mucosa tissue from 19 patients with gastric cancer by endoscopic biopsy before DCS therapy (chemotherapy with combination of docetaxel, cisplatin and S-1) were obtained. The expression data were used for subsequent analysis. Differentially expressed genes (DEGs) between cancer tissues and normal tissues were screened by R program language using GEO2R with $P < 0.01$, $|\text{Fold change}| > 2$.

Study patients

A total of 102 GC patients who were enrolled at Shandong Provincial Hospital Affiliated to Shandong University during January 2013 to December 2017 were included in current study. Inclusion criteria included: (1) diagnosed through pathological examination before chemotherapy; (2) ECOG ≥ 2 , Karnofsky (KPS) ≥ 80 points; (3) diagnosed with metastasis through clinical examination; (4) survival rate > 3 months; (5) signed informed consent. Exclusion criteria included: (1) incomplete clinical data; (2) with severe organ dysfunction; (3) in the phase of lactation or

pregnancy.

Therapeutic schedule

MGC patients were treated with Neoadjuvant chemotherapy (NAC). Chemotherapy with combination of docetaxel, cisplatin and S-1 (DCS therapy) was performed for 2 courses. Each course lasted for 3 weeks. S-1 (40 mg/m²) was administered orally twice daily on days 1-14, followed by a 7-day recovery period. Meanwhile, 60 mg/m² of cisplatin and docetaxel mixture was given on day 8 of every course by 120 min of intravenous infusion. Patients were sorted into two groups: response and resistant group according to tumor regression rates (TRR) based on the Response Evaluation Criteria and Solid Tumors (RECIST) [18]. The patients in response group showed TRR > 10% and the patients in resistant group showed TRR < 10% after 2 cycles of treatment.

Cell culture, reagents and transfection

After 2 cycles of treatment, patients in response and resistant group underwent biopsy from tumor. Tumor samples were treated with trypsin and then cultured in 90% RPMI-1640 with 10% FBS medium under condition of 37°C, 5% CO₂ for next cell experiments. Sunitinib malate was purchased from Bio Vision Inc. (San Francisco, CA, USA). Crenolanib was supplied by Arog Pharmaceuticals. Sorafenib tosylate was obtained from LC Laboratories (Woburn, MA). Drugs at a stock concentration of 10 μM in dimethyl sulfoxide were dissolved in RPMI-1640 to the indicated final concentrations. Resistant cells were classified into three groups. The cells in DCS+sunitinib malate were treated with sunitinib malate and cells in vehicle group were treated with equivalent dimethyl sulfoxide. The cells in DCS group were without other treatment.

The PDGFRB shRNA were synthesized (Thermo Fisher Scientific, Waltham, MA, USA) and cloned into lentiviral vector (LV3) and were then packaged with Lentivector

Packaging Plasmid mix (pGag/Pol, pRev, and pVSV-G) to establish stable cell lines as previously described. Briefly, 293T Cells were cultured in a 6-well plate. The following day, cells were transfected with PDGFRB plasmids and Lentivector Packaging Plasmids with Lipofectamine 2000 Reagent (Life Technologies). After 48 h, the transfectants were selected with puromycin (Amresco, Cleveland, OH, USA) to gain the positive stably transfected clone.

Quantitative real time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the Trizol reagent (Thermo Fisher Scientific), and the obtained RNA was reverse-transcribed into cDNA using the GoScript reverse transcription system (# A5001, Promega Corporation, WI, USA). Quantitative analysis of mRNA was performed using the QuantiTect SYBR Green PCR kit (Qiagen Company, Hilden, Germany) according to the manufacturer's instruction. The primer sequences of PDGFRB were as follows: forward 5'-GAACGCTCGCACGCCTAG-3' and reverse 5'-CATGGGAACACTGAGCTTCA-3'.

Immunohistochemistry

To detect the expression of PDGFRB, tissue sections from endoscopic biopsy were stained by LSABTM 2 Kit/HRP (DAKO, Tokyo, Japan). Briefly, after endogenous peroxidase and protein blocking reacts, the primary antibodies of PDGFRB (ab16868, Abcam, Cambridge, MA, US) were added, followed by HRP-labeled anti-rabbit IgG as the secondary antibody. And specimens were counterstained with hematoxylin.

Transwell migration and invasion assay

Transwell assay was performed for the ability of migration and invasion in the resistant cells. In short, the inserts were coated with 50 μ L Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Cells (5×10^4) were suspended in 100 μ l serum-free medium and then

seeded on the upper floor of Transwell chambers (BD Biosciences). The lower chamber was added 500 μ l serum with 20% FBS. After 48 h of incubating at 37°C with 5% CO₂, the un-invaded cells were wiped with a cotton swab, and invaded cells were fixed in methanol and stained with 0.1% crystal violet. The number was counted under microscope. Each experiment was conducted in triplicate.

MTT

With 10% FBS of medium, the cells were prepared with a single cell suspension, and then they were inoculated into 96 orifice plates; each hole was filled with a volume of 200 μ l. Constant temperature incubator with the condition of 37°C and 5% CO₂ were used to culture cells for 3 to 5 days. MTT solution (5 mg/ml prepared with PBS, pH = 7.4) of 10 μ l was added into each hole to continue incubation for 4 h, supernatant was absorbed in order to be removed from hole (suspended cells needed centrifugal suction first, then abandon supernatant). Each well plus 100 μ l DMSO oscillated for 10 min so as to dissolve the crystal. 450 nm was chosen to detect the absorption value of various apertures on the enzyme-linked immunoassay, and the results were recorded. With time as the horizontal coordinate, the absorbance value was used to draw the growth curve of the cell.

Colony formation assay

Cells with specific treatment were collected and then placed onto a fresh 6-well plate. The cells were cultured for about 7 days to form colonies. Colonies were fixed by 100% methanol and stained by 0.1% crystal violet in 20% methanol for 15 min. Colony-forming number was calculated under microscope.

Western blot

Cold phosphate buffer saline (PBS) was used to wash the cells twice, and the total

protein was extracted with radio-immunoprecipitation assay (RIPA) lysis buffer. Concentration of the protein was then determined using the BCA kit (Thermo Fisher Scientific). Equal amounts of protein were re-dissolved in 10-14% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAG) and transferred to a closed nitrocellulose membrane (Amersham Biosciences, GE Healthcare, Milan, Italy). After that, the membrane was incubated with following antibodies: VEGFR (ab36844, 1: 1000, Abcam), PTEN (ab31392, 1: 1000, Abcam), PDGFRB (ab16868, 1: 2000, Abcam) and β -actin (ab8227, 1: 2000, Abcam) at 4°C overnight and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h. The positive protein was detected using enhanced chemiluminescence reagent (Thermo Fisher Scientific) and analyzed by the ImageQuant LAS 4000 mini (GE Healthcare, Piscataway, NJ, USA).

Tumor xenografts in nude mice

Female 5/6-week-old BALB/c nude mice were bought from center of laboratory animal science of Guangdong. Treated cells with a concentration of 1×10^6 /ml diluted in 0.1 ml PBS were injected subcutaneously on the back flank of each mouse at day 0. In the three groups, each group had 6 mice, and each one was injected with 1×10^6 /ml cell. Tumor size was gauged with a caliper every 7 days. The tumor volume was computed via the formula (Volume=length \times width² \times 0.5). After 21 days observation, mice were executed, tumors were isolated to weigh by electronic scales and perform immunohistochemistry assay and qRT-PCR assay. All experimental procedures were carried out on the basis of the ethical standards under a protocol approved by the Committee on Shandong Provincial Hospital Affiliated to Shandong University.

Statistical analysis

Measurement data were recorded in the form of average \pm standard deviation.

Enumeration data were expressed as frequencies or percentages, and comparisons among multiple groups were made through Pearson chi-square test or Fisher's exact test when appropriate. The prognostic factors of MGC patients were screened using Cox regression analysis. Difference of the survival time between patients with different expression level of PDGFRB was tested by Log-rank method. All statistical analyses were done by R 3.4.1 program language software and $P < 0.05$ signified statistical significance.

Results

PDGFB expression was elevated in DCS therapy resistant cells

The data from GSE31811 were analyzed using GEO2R to screen DEGs (differentially expressed genes) between cancer tissues and normal tissues. DEGs with $P < 0.01$, $|\text{Fold change}| > 2$ were selected, including 16 down-regulated genes and 5 up-regulated genes (Table 1). Compared with normal tissues, PDGFB was highly expressed in cancer tissues. Besides, we detected the PDGFRB expression in tumor tissues from MGC patients and investigated the correlation between PDGFRB expression and clinic-pathological features. Results revealed that expression level of PDGFRB was related to TNM staging and differentiation degree, $P < 0.05$ (Table 2).

Cells in response group and resistance group were transfected with empty vectors; resistant cells in shRNA group were transfected with PDGFRB shRNA. The expression of PDGFRB in resistant cells with DCS therapy was significantly higher than that in response group (Figure 1A). The resistant group had a higher immunohistochemical positive rate (Figure 1B). As showed in Figure 1C and 1D, we found that the invasion rate and migration rate of shRNA group was significantly lowest among three groups

($P < 0.05$), while the resistance group showed higher invasion and migration rate than response group ($P < 0.05$), which validated the up-regulated PDGFRB promoted cell migration and invasion in cancer. Subsequently, the influences of PDGFRB on the viability and proliferation of DCS therapy resistant cells were investigated. The outcomes of MTT assay indicated that DCS therapy resistance significantly promoted the proliferation of cells comparing with shRNA and response group (Figure 1E, $P < 0.05$). The colony number in resistant group were dramatically more than that in response group and approximately twice as much as shRNA group (Figure 1F, $P < 0.05$).

PDGFRB overexpression activated PI3K/AKT pathway

A pre-computed database, STRING V9.0 was used to predict the MGC-related gene/protein-protein associations. Protein-protein interaction (PPI) network of MGC-related was gained from STRING. It indicated that PDGFRB was involved in the occurrence and development of MGC and PDGFRB played an important role in PI3K/AKT pathway (Figure 2A).

Western blot experiment indicated that the expressions of three different proteins, namely VEGFR, PTEN and PDGFRB in resistant group were all higher than those in response group. And resistant cells showed low level of VEGFR, PTEN and PDGFRB protein expressions after knock-downing PDGFRB (Figure 2B, $P < 0.05$). In resistant cells, highly expressed PDGFRB was corresponding to the highly expressed VEGFR and PTEN and down-regulated PDGFRB was corresponding to the lowly expressed VEGFR and PTEN, which suggested that the AKT/PI3K pathway was activated.

Three kinds of inhibitors (sunitinib malate, creolanib, and sorafenib tosylate) were applied to treat resistant cells, we found that all of them could inhibit PDGFRB expression in different degrees compares with vehicle group (Figure 2C, $P < 0.05$).

However, sunitinib malate showed the strongest effect among three inhibitors, namely sunitinib malate could effectively suppress the expression of PDGFRB. As mentioned before, the effect of DCS therapy would decrease during the early treatment of gastric cancer, and the resistance of DCS therapy would increase in a short period, DCS plus sunitinib malate group was required to increase the effect of DCS therapy. [Figure 2D](#) confirmed again that the expression of PDGFRB in DCS + sunitinib malate group was dramatically decreased compared with DCS or vehicle group ($P<0.05$). It means that the supplement of sunitinib malate could increase the effect of DCS therapy and sensitize the drug susceptibility of cells.

PDGFRB inhibition could suppress the invasion, migration and proliferation of MGC cells

The experimental results of Transwell showed that resistant cells treated with sunitinib malate (the group of DSC + sunitinib malate) showed decreased invasion and migration abilities compares with resistant cells in vehicle or DSC group ($P<0.05$, [Figure 3A, B](#)). Actually, it indicated that the inhibition of PDGFRB by adding sunitinib malate in resistant cells inhibited cell migration and invasion. In addition, MTT and colony formation assays were utilized to assess the cell viability and proliferation ability in three groups that prepared before. Results showed that cells viability in the DCS plus sunitinib malate group was lower than that in the DCS or vehicle group ([Figure 3C](#), $P<0.05$). Meanwhile, the colony numbers in DCS + sunitinib malate group were fewer than that in the DCS or vehicle group ([Figure 3D](#), $P<0.05$). These findings indicated that the addition of sunitinib malate could enhance the influence of DCS in metastatic gastric cancer.

PDGFRB inhibition restrained tumor growth and predicted better prognosis

To learn more about the effect of PDGFRB on cell function, *in vivo* experiments were performed. Resistant cells with different treatments were subcutaneously injected into the back flank of mice. Through continuous observation for 21 days, we found that the volume of tumor in DCS + sunitinib malate group was smaller compared with other two groups (Figure 4A, $P<0.05$). Similarly, the tumor weight in DCS + sunitinib malate group was lower than that in vehicle or DCS group (Figure 4B, $P<0.05$). Immunohistochemistry assay was carried out to detect the PDGFRB protein expression in tumor tissues of three groups' mice. Results showed that protein expression of the PDGFRB in DCS + sunitinib malate group was the lowest ($P<0.05$, when compared with vehicle or DCS group) (Figure 4C). Furthermore, qRT-PCR assay confirmed the results (Figure 4D, $P<0.05$).

Figure 4E and 4F showed comparison of the survival rate between patients with high expressed PDGFRB and low expressed PDGFRB in two groups (with or without chemotherapy groups). As for the patients without chemotherapy, high expressed PDGFRB showed lower survival rate but the difference was not statistically significant ($P=0.25$). As for the patients with chemotherapy, low expressed PDGFRB showed significantly higher survival rate ($P=0.00017$). Additionally, COX regression analysis showed that the prognostic factors for MGC patients included TNM staging, differentiation degree and PDGFRB expression (Table 3). Above-mentioned results suggested that PDGFRB could be served as a prognostic indicator for patients with chemotherapy.

Discussion

Chemotherapy, especially the comprehensive treatment plays an important role in

therapy of advanced cancer, including metastatic gastric cancer. There is a kind of membrane transporters, they bear the mass exchange between the inside and outside the cell membrane, substance metabolism, protect the normal physiological function of cells. In tumor cells, membrane pump function of the high expression of these proteins will reduce the antitumor drug "pump" out of cells or vesicles isolation, which results in the decrease of intracellular drug concentration or drug distribution changes leading to drug resistance of tumor cells. Gastric cancer resistance phenomenon has been a thorny problem in clinical treatment. At present, an increasing number of researches were aimed at settling drug resistance, including basing on microRNA [19, 20] or mRNA [21]. If gastric cancer drug resistance mechanism were thoroughly clarified, which will certainly promote the development of new drugs, increase the survival rate and improve the quality of life in patients with gastric cancer. As a result, the studies on basic and clinical resistance in gastric cancer are necessary. In our research, we aimed to figure out prognosis of metastatic gastric cancer and explore the effect of PDGFRB on chemotherapy in patients with metastatic gastric cancer.

PDGFRB is class III receptor tyrosine kinases (RTK) functioning in the development of normal connective tissue cells. In non-small cell lung cancer (NSCLC), alterations in PDGFR are markers of poor prognosis and efficient targets of these receptors are yet to be achieved [22]. Aberrant activation of PDGFR signaling due to gene amplification, translocation and activating mutation, has been found in various solid tumors [23]. PDGFR A and B often form heterodimers to activate downstream signaling [23]. The

expression of PDGFRB mRNA was higher in colorectal adenoma than that in normal tissues, suggesting that PDGFRB has a role in the diagnosis of rectal adenocarcinoma [24]. When the cervical intraepithelial neoplasia develops to the micro-invasion cancer, PDGFRB protein expression is increased, which is beneficial to the pathological diagnosis and prognosis evaluation [25]. In addition, PDGFR in the tumor stroma have attracted attention as interesting drug target due to their function as regulators of tumor transvascular transport, tumor interstitial fluid pressure and tumor drug uptake [26]. PDGFRB expression induced by inflammatory cytokines could promote growth of intestinal smooth muscle cells and control of PDGFRB expression might be beneficial in chronic intestinal inflammation [9]. We found PDGFB expression was elevated in DCS therapy resistant cells. This result was similar to the study of Kodama M *et al.* that PDGFRB expression was associated with lymphatic metastasis in gastric carcinoma [11]. Combing with our research results, PDGFRB could be considered as a new biomarker in MGC prognosis.

Various signaling pathways were involved in progress of cancer. Jedroszka D *et al.* reported that the expression of Notch pathway members could be considered as useful biomarkers for predicting prognosis in renal carcinoma and specific member of the Notch pathway was related to the specific subtype of renal carcinoma [27]. In current study, we also found that PDGFB overexpression activated PI3K/AKT pathway and PDGFRB inhibition could suppress the invasion and migration of MGC cells. Consistent to our findings, Wang H *et al.* reported the over-expression of the PDGFRB

in endothelial progenitor cells (EPCs) enhanced the PDGF-BB-induced proliferation, migration and angiogenesis of EPCs through the PI3K/AKT signaling pathway. Moreover, the PI3K/AKT signaling pathway was required for PDGFRB over-expression to enhance these PDGF-BB-induced phenotypes [28]. Similarly, PDGFB inhibited apoptosis in the pulmonary arterial endothelial cells by stimulating the phosphorylation of both AKT and STAT3, and the PI3K/AKT signaling pathway served as an up-stream participant in the STAT3 activation stimulated by PDGFB [29].

Though PDGFRB was expressed in various cancers, this study was firstly revealed PDGFRB was highly expressed in DCS therapy resistant cells compared to in DCS therapy response cells. Furthermore, follow-up study of MGC patients confirmed that for MGC patients with chemotherapy, lowly expressed PDGFRB indicated higher survival rate compared with highly expressed PDGFRB. Obviously, more and more genes associated with prognosis were discovered [30]. For example, Feng W *et al.* found high expression of lncRNA RMRP was related with a relatively poor prognosis in glioma patients [31]. Certainly, there are still several limitations in this study. Firstly, we could further study the influence of PDGFRB on chemotherapy resistance of MGC cells directly by knock-downing PDGFRB replacing PDGFRB inhibitor. Secondly, the concrete functional mechanisms between PDGFRB and the PI3K/AKT pathway need to further be investigated.

In conclusion, PDGFRB expression was elevated in DCS therapy resistant cells, which can activate the PI3K/AKT signaling pathway. PDGFB inhibition suppressed metastasis

and growth of resistant cells. PDGFRB, as a novel molecular marker, can predict the prognosis of metastatic gastric cancer patients treated with neoadjuvant chemotherapy.

Conclusion

A new gene PDGFRB in MGC is explored in current study. This study verifies PDGFRB knockdown can rescue the efficacy of chemotherapy in MGC and finds low expression of PDGFRB is corresponding to a higher survival rate, indicating PDGFRB can serve as a novel marker for the prognosis of MGC.

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Conflict of interest

The authors have declared that no competing interests exist.

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Figure Legends

Figure 1 PDGFRB expression is up-regulated in resistant MGC and PDGFRB promotes cell invasion, migration and proliferation. (A) Analysis of PDGFRB expressions through qRT-PCR in response or resistant MGC cells. (B) Immunohistochemical staining for PDGFRB in the specimens from response or resistant MGC patients. (C-D) Invasion and migration assays in response, restraint and PDGFRB shRNA resistant MGC cells. (E-F) MTT and colony formation assays showed that cell viability and proliferation ability in resistant group were stronger than that in response group and were inhibited by PDGFRB knock-down. $*P < 0.05$ and $**P < 0.01$, compared with response group. $\#P < 0.05$, compared with resistant group.

Figure 2 PDGFRB overexpression activates PI3K/AKT pathway and PDGFRB can be inhibited by treating with sunitinib malate in resistant MGC cells. (A) Network figure of MGC genes. The PPI data of MGC was gained from STRING. It indicated that PDGFRB involved in the occurrence and development of MGC. (B) The expressions of VEGFR, PTEN, PDGFRB were detected by Western blot in three groups (cells in response group and resistance group were transfected with empty vectors, resistant cells in shRNA group transfected with plasmid that PDGFRB gene was knocking down), all the expression levels were compared with β -actin expression. Results are indicated as mean \pm SD (n=3). $*P < 0.05$, compared with response group. $\#P < 0.05$, compared with resistant group. (C) PDGERB expression level in resistant cells treated with three different inhibitors: sunitinib malate, crenolanib and sorafenib tosylate, comparing with

vehicle were measured by qRT-PCR. $*P < 0.05$, compared with vehicle group. (D) PDGFRB expression level in resistant cells with various treatments were measured by qRT-PCR. $*P < 0.05$, compared with vehicle group. $\#P < 0.05$, compared with DCS group.

Figure 3 Inhibition of PDGFRB suppress the invasion and migration of cancer cells. (A) Transwell invasion assay showed that the invasion level of DCS + sunitinib malate group was decreased compared with vehicle group as well DCS group. (B) Wound healing assay showed the migration ability in DCS + sunitinib malate group was lowest among three groups. (C) MTT assay for measuring the cell viability of cancer cells in three groups. (D) Colony forming assay showed that the colony numbers in DCS + sunitinib malate group was the lowest compared with other two groups. $*P < 0.05$, compared with vehicle group. $\#P < 0.05$, compared with DCS group.

Figure 4 Animal experiments for validating the effect of PDGFRB in metastatic gastric cancer. (A) Tumor volume in DCS + sunitinib malate group was smallest among three groups after 21-day observation. (B) The tumor weight in DCS + sunitinib malate group was also the lowest among three groups. (C) Immunohistochemical staining for PDGFRB revealed the protein expression in three groups. (D) qRT-PCR was performed to detect PDGFRB in excised tumor tissues. (E) and (F) Survival rate of MGC patients that treated with or without chemotherapy was assessed.

Table 1 Differentially expressed genes between metastasis gastric cancer tissues and normal gastric tissues.

Gene symbol	P. Value	t	B	LogFC
A1CF	0.000359	-3.9	-4.38	-2.11
MLN	0.000606	-3.72	-4.39	-2.56
ALDOB	0.000749	-3.65	-4.4	-2.65
FABP2	0.00103	-3.54	-4.41	-2.45
ERICH4	0.001031	-3.54	-4.41	-2.62
CXCL8	0.001378	3.44	-4.42	2.41
SLC28A2	0.001988	-3.31	-4.43	-2.42
CLCA1	0.002327	-3.25	-4.43	-2.97
CHST5	0.002575	-3.21	-4.44	-2.38
S100A8	0.004543	3.01	-4.46	2.3
OTC	0.00512	-2.96	-4.46	-2.51
KLK10	0.005151	2.96	-4.46	3.13
GKN1	0.005696	-2.92	-4.46	-4.41
ZG16	0.006172	-2.89	-4.47	-2.23
PDGFB	0.007054	2.84	-4.47	2.1
SI	0.007502	-2.82	-4.47	-2.72
CHP2	0.007885	-2.8	-4.47	-2.86
TRIB3	0.008729	2.76	-4.48	2.4
ALDOB	0.008874	-2.75	-4.48	-2.75
FCGBP	0.00909	-2.74	-4.48	-2.45
KRT20	0.00926	-2.73	-4.48	-2.57

FC, fold change referred to the change in metastasis gastric cancer tissues relative to normal gastric tissues.

Table 2 Correlation between PDGFRB expression and clinic-pathological features.

Variables	High (n = 71)	Low (n = 31)	X^2	<i>P</i>
Age(y)				
≥ 50	41 (57.75)	17 (54.84)	0.074	> 0.05
< 50	30 (42.25)	14 (45.16)		
TNM				
Stage I~II	21 (29.58)	22 (70.97)	15.16	< 0.05
Stage III~IV	50 (70.42)	9 (29.03)		
Differentiation degree				
High degree	12 (16.90)	24 (77.42)	34.802	< 0.05
Middle degree	18 (25.35)	3 (9.68)		
Low degree	41 (57.75)	4 (12.90)		
Tumor size (cm)				
< 5	34 (47.89)	17 (54.84)	0.417	> 0.05
≥ 5	37 (52.11)	14 (45.16)		

Table 3 COX regression analysis of the prognostic factors for MGC patients

Variables	RR	95% CI	<i>P</i>
TNM staging	75.411	1.428~3.105	0.013
PDGFRB expression	101.425	2.428~10.121	0.002
Differentiation degree	81.401	2.716~4.379	0.007

Note: RR, relative risk; CI, confidence interval; MGC, metastatic gastric cancer.

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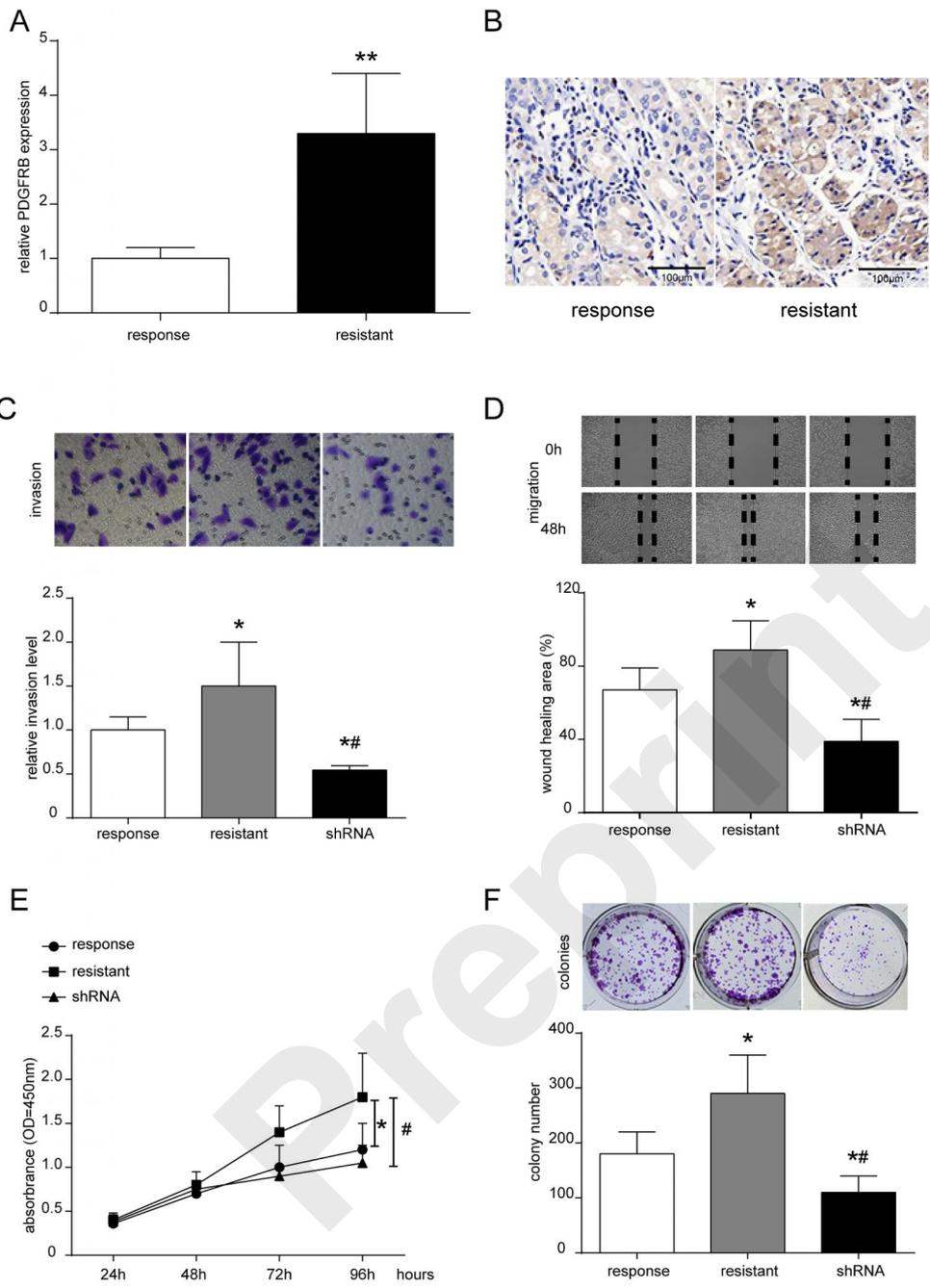


Figure 1

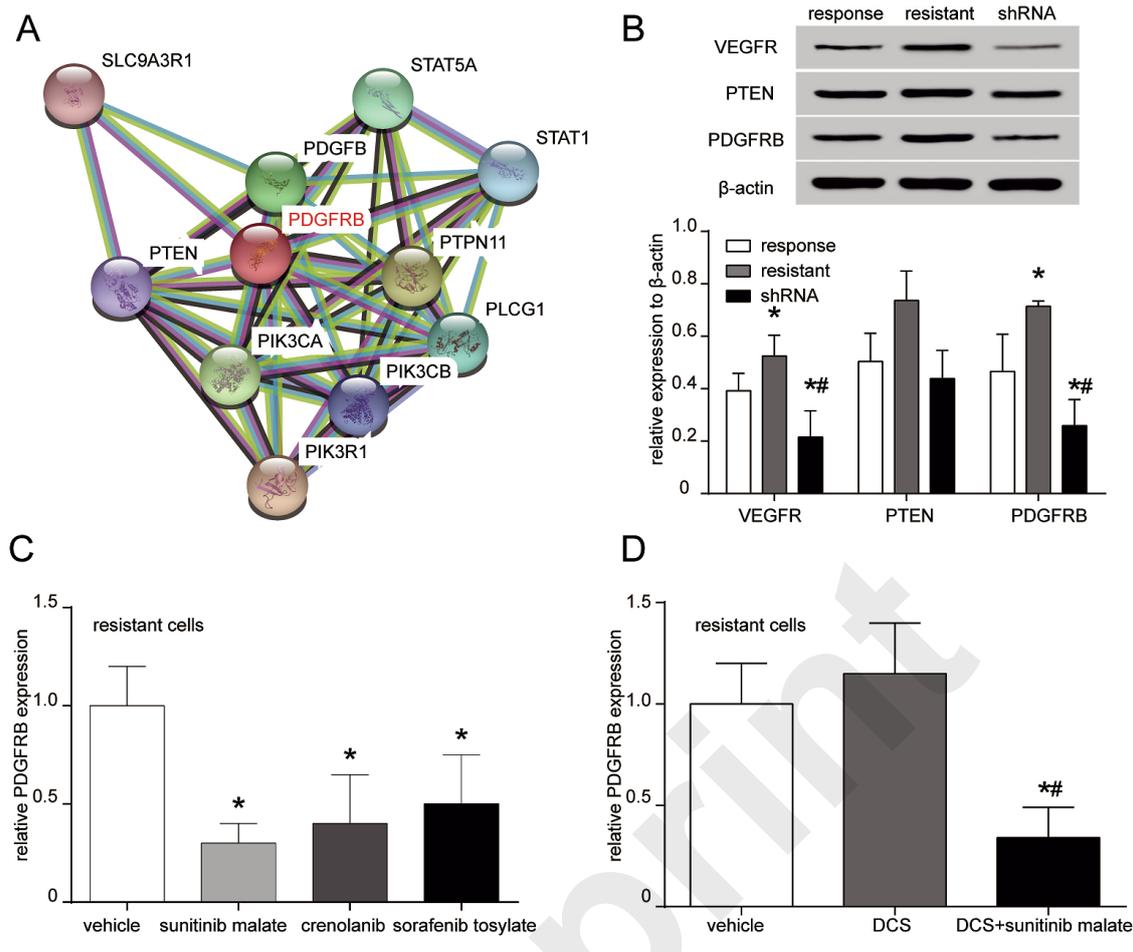


Figure 2

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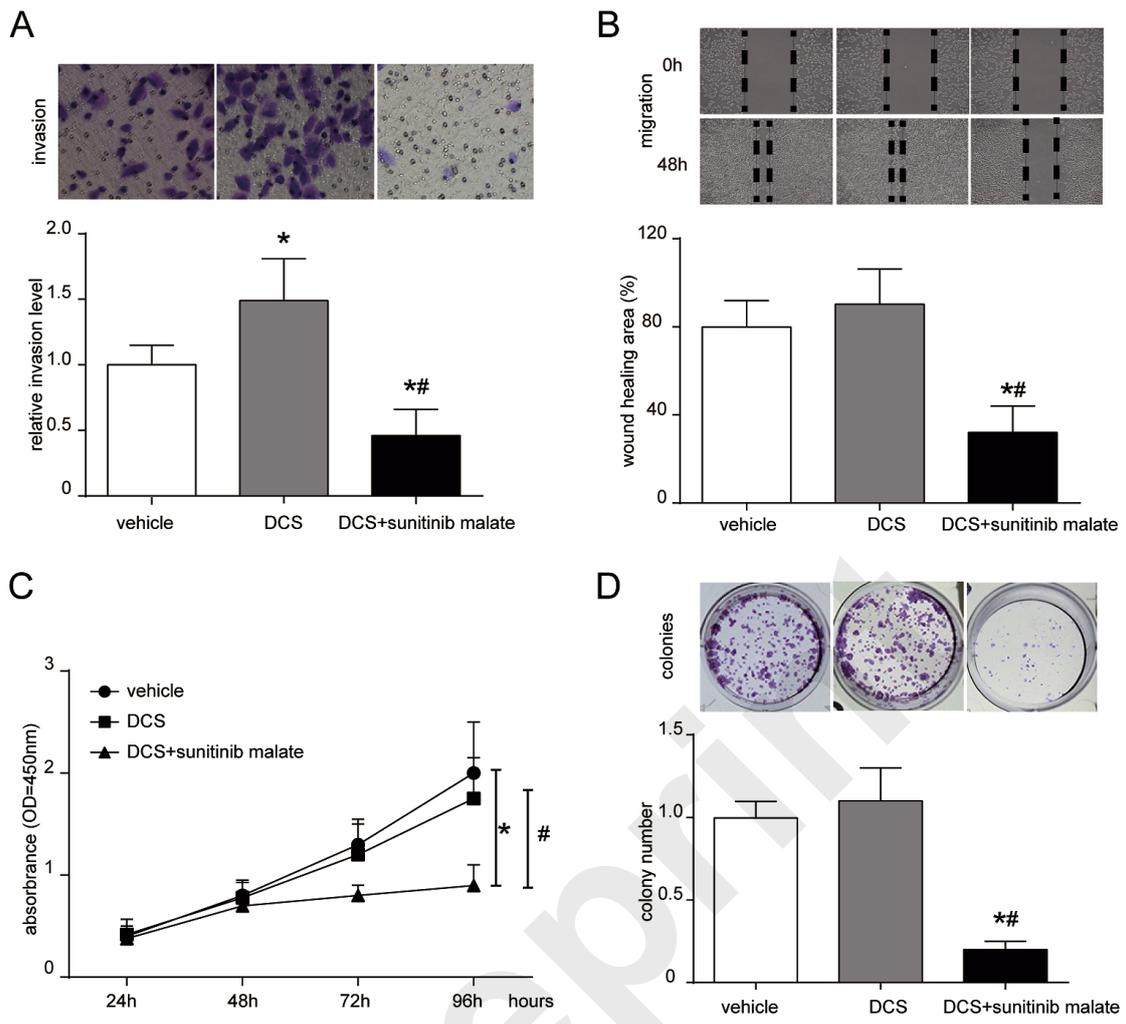


Figure 3

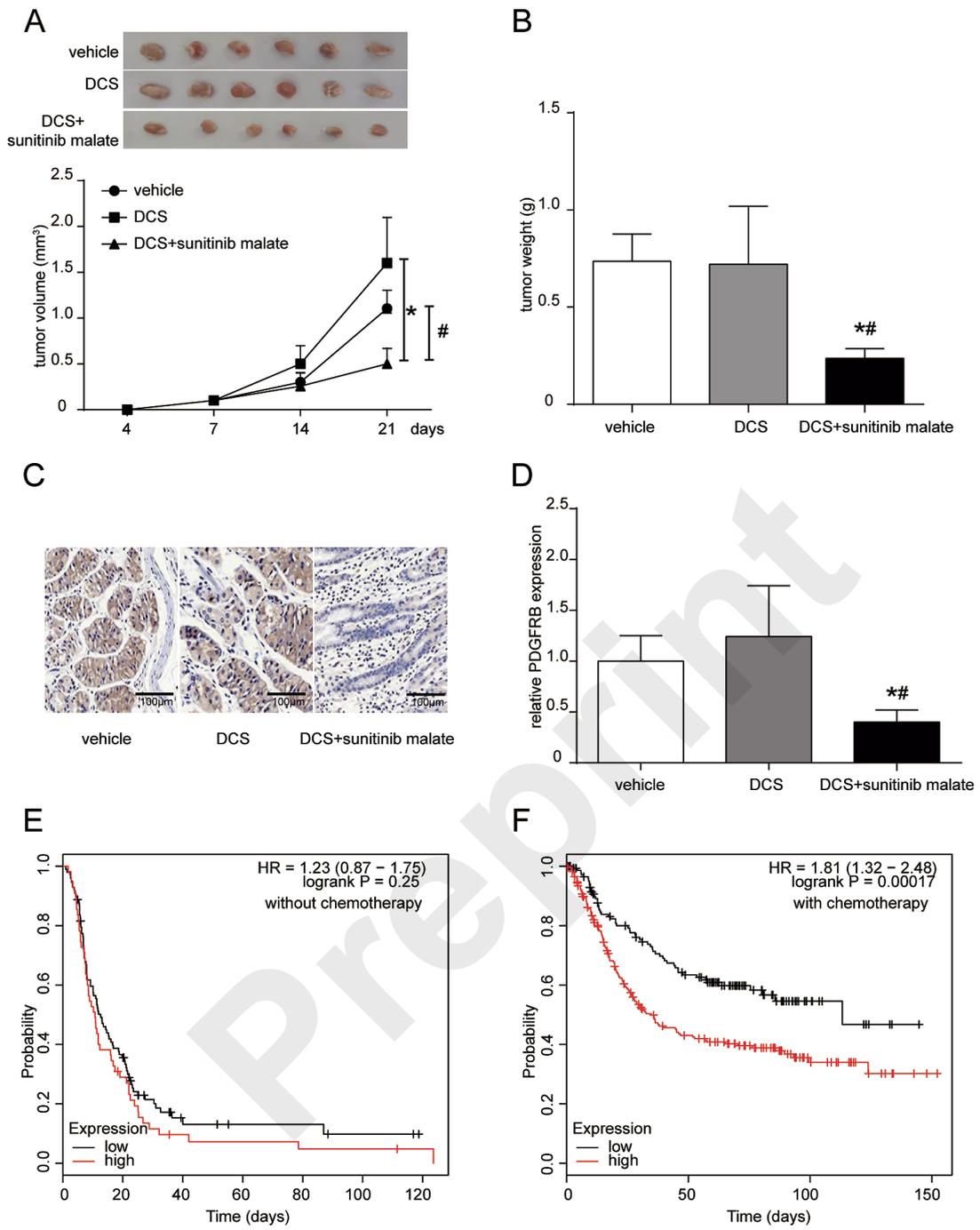


Figure 4