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Original article

# Combination of NRP1-mediated iRGD with 5-fluorouracil suppresses proliferation, migration and invasion of gastric cancer cells

Li Zhang[a], Yanfeng Xing[b], Qi Gao[c], Xuejun Sun[c,*], Di Zhang[a], Gang Cao[a,*]

[a] Department of General Surgery, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, PR China
[b] Department of General Surgery, The Central Hospital of Tongchuan Mining Bureau, Tongchuan, Shaanxi 727000, PR China
[c] Department of General Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, PR China

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ABSTRACT

Gastric cancer is one of the most of common cancers in the world. 5-Fluorouracil (5-FU) has been identified as one of the standard first-line chemotherapy drugs for locally advanced or metastatic gastric cancer. However, poor tumor penetration, bad selectivity and toxic side effects are the major limitations for the application of chemotherapy drugs in anticancer therapy. Recently, plenty of studies demonstrate that the novel tumor-homing peptide iRGD could promote the tumor-penetrating capability of chemotherapy drugs in multiple cancers, and neuropilin-1 (NRP1) protein is the critical mediator for iRGD. Here,we found that NRP1 protein expression was significantly up-regulated in gastric cancer tissues and cell lines by Immunohistochemistry and Western blot. And elevated NRP1 was notably associated with tumor differentiation ($P=0.021$), tumor size ($P=0.004$), tumor stage($P=0.028$), lymph node metastasis($P=0.032$), TNM tumor stage ($P=0.006$) and poorer prognosis. Functionally, the data of Methyl thiazolyl tetrazolium (MTT) assay, Colony formation assay and Transwell assay revealed that NRP1 could facilitate gastric cancer cells proliferation, migration and invasion. Furthermore, iRGD could strengthen the chemotherapy effect of 5-FU on gastric cancer cells through NRP1. Taken together, NPR1 might be a promising tumor target for gastric cancer, and combination of iRGD with 5-FU may be a novel and valuable approach to improving the prognosis of gastric cancer patients.



## 1. Introduction

As one of the most common cancers in the world, gastric cancer severely threatens patients' physical and mental health [1]. Recent years, advances of targeted cancer therapy present some promising and valuable approaches to cancer treatment [1,2]. More and more molecules have been excavated to be the candidates for targeted cancer therapy, such as proteins, lncRNAs and miRNAs [3–5]. Neuropilin-1(NRP1), a transmembrane glycoprotein with five extracellular domains (a1, a2, b1, b2, and c), has been reported to be an oncogene by participating in development and progress of cancers, such as glioma, breast cancer and prostate cancer [6–8]. However, the clinical and experimental effects of NRP1 in gastric cancers remain to be further confirmed.

Chemotherapy, as an adjuvant therapy, could synergistically improve the outcomes of cancer patients, especially for these patients at middle or late tumor stage [9]. 5-Fluorouracil (5-FU) has been identified as one of the standard first-line chemotherapy drugs for locally advanced or metastatic gastric cancer [10]. However, traditional chemotherapy drugs have bad selectivity and toxic side effects,which could cause severe side effects and poor treatment effects [10]. Interestingly, studies demonstrate that, a tumor-targeting and tumor-penetrating cyclic peptide iRGD (peptide with Cys-Arg-Gly-Asp-Lys/Arg-Gly-Pro-Asp/Glu-Cys [CRGDK/RGPD/EC]), could enhance vascularity and tissue permeability in a tumor-specific and NRP1-dependent manner [11]. Briefly, in order to trigger tissue penetration of drugs, iRGD targets tumors by first binding to αv integrins and then being proteolytically cleaved in the tumor to produce CRGDK/R to interact with NRP1 [12]. Thus, when iRGD was combined with chemotherapy drugs, drugs could penetrate intensively and deeply into the parenchyma of solid tumors to exert their effects on tumor cells [12,13]. For example, previous research revealed that iRGD could enhance the antitumor efficacy of paclitaxel on cancer cells via interacting with NRP1 in peritoneal carcinoma [14]. Here, we attempted to explore whether iRGD could strengthen the

* Corresponding authors.
*E-mail addresses:* sunxy@mail.xjtu.edu.cn (X. Sun), paul5381@sina.com (G. Cao)

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chemotherapy efficacy of 5-FU on gastric cancer cell, and whether NRP1 could mediate the process.

In conclusion, we demonstrate that overexpressed NRP1 in gastric cancer was associated with malignant clinicopathological features and poorer prognosis of gastric cancer patients. Cellular experiments revealed that NRP1 could promote cell proliferation, migration and invasion. Furthermore, iRGD could strengthen the chemotherapy efficacy of 5-FU on gastric cancer cell through NRP1. Therefore, NPR1 might be a promising tumor target for gastric cancer, and combination of iRGD with 5-FU may be a novel and valuable approach to improving the prognosis of gastric cancer patients.

## 2. Materials and methods

### 2.1. Regents, cell culture and cell transfection

IRGD peptide (CRGDKGPDC)) was synthesized by GL Biochem (Shanghai, China).5-FU was purchased from Sigma-Aldrich (Taufkirchen, Germany). Four gastric cancer cell lines(NCI-N87,SGC-7901,AGS,HGC27) and human immortalized gastric epithelial cell line(GES-1) were obtained from the Chinese Academy of Sciences (Shanghai, China). NCI-N87,SGC-7901,AGS cells were cultured in RPMI-1640 (GIBICO, USA) supplemented with 10% fetal bovine serum (GIBICO,USA), 100 μg/mL streptomycin and 100 U/mL penicillin (Sigma, USA). HGC27 cells were cultured in DMEM (GIBICO, USA) supplemented with 10% fetal bovine serum (GIBICO, USA), 100 μg/mL streptomycin and 100 U/mL penicillin (Sigma, USA). All of the cells were maintained in an incubator(5% $CO_2$, 37 °C).NRP1 Human shRNA Lenti Particle for NRP1(ShRNA1/NRP1, ShRNA2/NRP1, ShRNA3/NRP1,ShRNA4/NRP1, Negative Control) (TL311093V), NRP1 Human ORF Clone Lenti Particle (Lenti-NRP1)and Negative Control (RC202952L1V) were purchased from ORIGENE(ORIGENE, USA). Cells transfections were conducted according to the product specifications.

### 2.2. Tissue samples

A total of 203 paired gastric cancer tissues and adjacent normal tissues were obtained from gastric cancer patients underwent curative resection at The Department of General Surgery at the Second Affiliated Hospital of Xi'an Jiaotong University during January 2008 to December 2011. All of the patients were diagnosed by postoperative pathological analysis and none of the patients had received any perioperative chemo- or radiotherapy. The protocols of this study were approved by the Second Affiliated Hospital of Xi'an Jiaotong University Ethics Committee according to the Declaration of Helsinki. Clinical samples were used after obtaining informed consent from each patient.

### 2.3. Immunohistochemistry

The sections were dewaxed, dehydrated, and rehydrated. Then, using citrate buffer to retrieve antigen, and blocked the endogenous peroxidase activity applying hydrogen peroxide (3.0%). After being blocked by 10% goat plasma, the primary antibody NRP1 (1:200) (ab81321,abcam,USA) was added to the sections and incubating at 4 °C overnight. Then applying the biotinylated secondary antibodies (Goldenbridge, Zhongshan,China) to measure the primary antibodies. Counterstained using hematoxylin and dehydrated.

### 2.4. Western blot

Cells were harvested and then lysed via radio immunoprecipitation assay lysis buffer (Thermo Fisher Scientific, MA,USA) to obtain proteins. Subsequently, separated proteins by SDS–PAGE and transferred proteins to PVDF membranes. The above PVDF membranes with corresponding antibodies NRP1 (1:1000)(ab81321, abcam,USA) and β-actin (1:500, Santa Cruz, CA, USA) and then incubated with the secondary antibodies anti-Mouse IgG-HRP (Dako, 00049039, 1:30000) and anti-Rabbit IgG-HRP (Dako, 00028856, 1:10000).

### 2.5. Methyl thiazolyl tetrazolium (MTT) assay

The viabilities of different cell groups were assessed using MTT (Sigma, St. Louis, MO, USA) assay. The cells were plated at $1 \times 10^3$/well in a 96-well plate. Each group comprised ten duplicate wells. After incubation for 0, 24, 48 and 72 h with DMEM containing 10% FBS, 5 mg/mL MTT (100 μL/well) dissolved in PBS was added to each well for 4 h. After the supernatants were removed, 100 μL DMSO was added to each well. The absorbance of at least 3 individual wells of one cell type at each time point was read using a microplate reader (Bio-Rad, USA).

### 2.6. Colony formation assay

Cells were cultured in 6-cm plate at a density of 200 cells/plate. After 14 days, colonies were washed with PBS, fixed with methanol for 30 min, and stained with crystal violet for 30 min. Clearly visible colonies (>50 cells/colony) were counted as positive for growth.

### 2.7. Transwell assay

Transwell chamber migration assay was measured using a transwell chamber with 8 μm filter inserts (BD Biosciences, USA) without Matrigel. For transwell chamber-based invasion assay, transwell inserts with 8 μm filter were pre-coated with 50 μg of Matrigel (Becton Dickinson, San Jose, CA).$5 \times 10^4$ cells were added to the inserts containing 200 μL serum-free DMEM medium. The lower chamber was filled with 600 μL DMEM medium with 10% FBS. After 24 or 48 h, the inside of the inserts was cleaned thoroughly with a cotton swab to remove any non-migrated or non-invasive cells, and the cells which had migrated through the porous membrane and invaded into the Matrigel were fixed with 4% paraformaldehyde for 20 min, and were then stained with 0.1% crystal violet for 20 min. Photographs were captured and the cells were counted at least 5 random fields.

### 2.8. Tumor treatment in nude mice

12 male BALB/c nude mice(4-week-old) were assigned to 4 groups with 3 mice in each group. Among them,two groups were subcutaneously injected into the flanks by $3 \times 10^6$ HCG27 cells, the other two groups were conducted by NCI-N87 cells. Experimental groups were intravenously injected by 5-FU (25 mg/kg) mixed with iRGD (4 μmol/kg)at every three days for 4 weeks while control groups were treated by 5-FU (25 mg/kg) mixed with PBS. And tumor volume was computed every 1 week with a digital vernier caliper using the following formula: tumor volume = (length × width$^2$)/2. The protocols of animal experiments were approved by the institutional animal care and use committee of the Second Affiliated Hospital of Xi'an Jiaotong University.

### 2.9. Statistic analysis

Statistical analysis was carried out using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). All data were represented as mean value ± S.D. The statistical methods included Chi-square test, *t* test and Kaplan-Meier and so on. $P < 0.05$ was considered to be statistically significant.

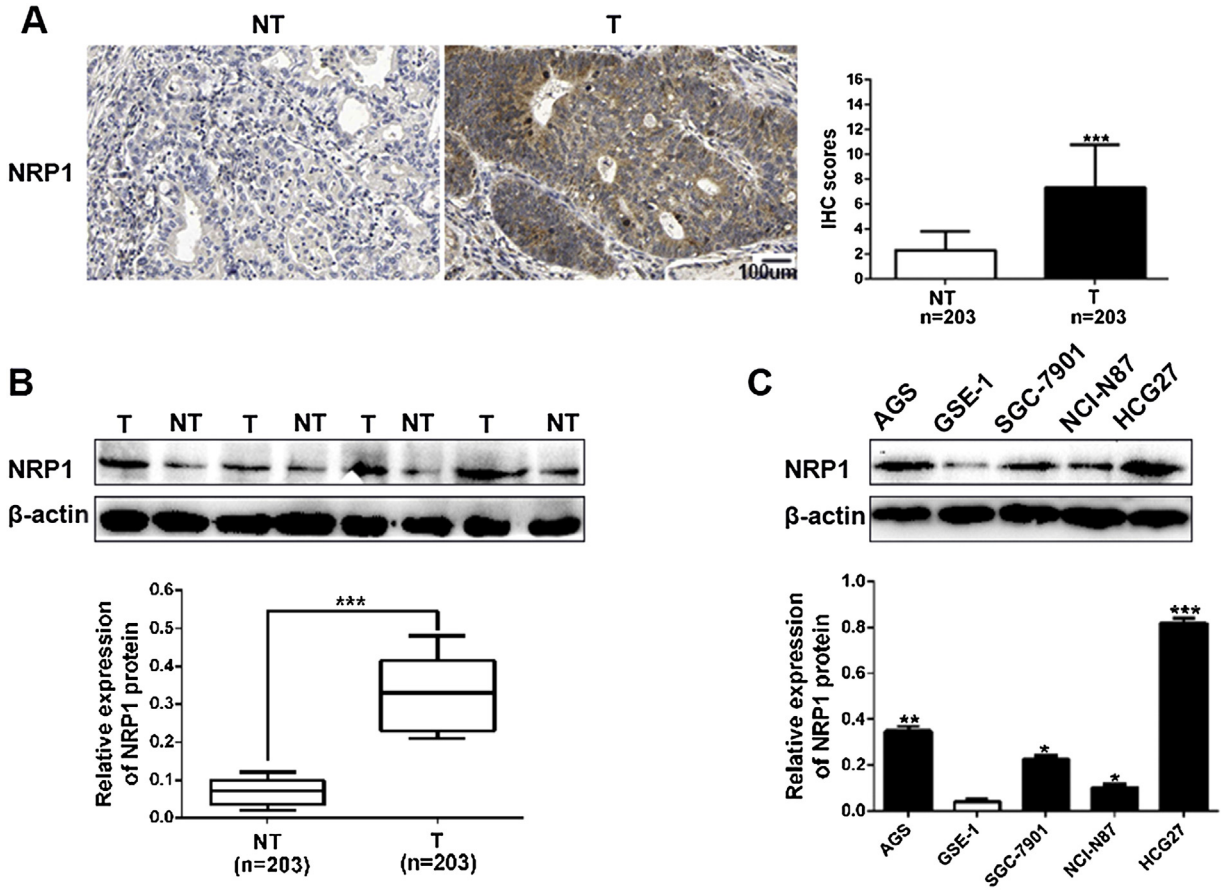


**Fig. 1.** NRP1 was up-regulated in gastric cancer. (A) Immunohistochemistry results(magnification: × 200) revealed that the expression of NRP1 in tumor tissues(T) was significantly higher than that in adjacent nontumor tissues(NT).(B) The expression of NRP1 in tumor tissues and adjacent nontumor tissues was detected by Western blot.(C) Western blot results showed that NRP1 was notably increased in gastric cancer cell lines compared to human immortalized gastric epithelial cell line(GES-1). $^{*}P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$.

## 3. Results

### 3.1. NRP1 is up-regulated in gastric cancer tissues and cell lines

Firstly, we detected the expression of NRP1 protein in 203 paired gastric cancer tissues and adjacent normal tissues by immunohistochemistry. The results revealed that the expression level of NRP1 in gastric cancer tissues was significantly higher than that in adjacent normal tissues($P < 0.05$, Fig. 1A). Subsequently, western blot was applied to measure the expression of NRP1 in gastric cancer tissues and adjacent normal tissues. Consistently, NRP1 was notably up-regulated in gastric cancer tissues ($P < 0.05$, Fig. 1B). In addition, western blot results showed that gastric cancer cell lines had markedly increased NRP1 than GES-1 cells ($P < 0.05$, Fig. 1C). HCG27 cells had the most and NCI-N87 cells had the lowest expression (Fig. 1C). Taken together, these data

**Table 1**
Correlation between expression of NRP1 and the clinicopathologic characteristics in gastric cancer($n = 203$).

| Characteristic | | *n* | Expression of NRP1 | | *P* |
|---|---|---|---|---|---|
| | | | Low ($n = 99$) | High ($n = 104$) | |
| Gender | Female | 70 | 31 | 39 | 0.354 |
| | Male | 133 | 68 | 65 | |
| Age (year) | <60 | 89 | 43 | 46 | 0.909 |
| | ≥60 | 114 | 56 | 58 | |
| Tumor differentiation | Well and moderate | 98 | 56 | 42 | **0.021** |
| | Poor | 105 | 43 | 62 | |
| Lauren's histology | Intestinal type | 123 | 62 | 61 | 0.563 |
| | Diffuse type | 80 | 37 | 43 | |
| Tumor size(cm) | <3 | 92 | 55 | 37 | **0.004** |
| | ≥3 | 111 | 44 | 67 | |
| Tumor stage | T1 + T2 | 138 | 60 | 78 | **0.028** |
| | T3 + T4 | 65 | 39 | 26 | |
| Lymph node metastasis | Negative | 114 | 48 | 66 | **0.032** |
| | Positive | 89 | 51 | 38 | |
| TNM tumor stage | I + II | 126 | 52 | 74 | **0.006** |
| | III + IV | 77 | 47 | 30 | |

demonstrated that NRP1 was increased in gastric cancer,which suggested that it might be an oncogene in gastric cancer.

### 3.2. Clinical significance of NRP1 expression in gastric cancer

According to the median expression level of NRP1 protein in gastric cancer tissues, 203 patients were sorted into low NRP1 group and high NRP1 group. The statistical results revealed that elevated NRP1 was notably correlated with tumor differentiation (P = 0.021), tumor size (P = 0.004), tumor stage(P = 0.028), lymph node metastasis(P = 0.032), and TNM tumor stage (P = 0.006) (Table 1). Subsequently, Kaplan–Meier survival curves for 5-year overall survival (Fig. 2A) and 5-year disease-free survival ((Fig. 2B) were conducted. The results showed that patients in high NRP1 group had significantly worse prognosis than the low NRP1 group patients ($P < 0.05$, respectively, Fig. 2). Taken together, the above data demonstrated that NRP1 may be a valuable and promising indicator to predict the prognosis for gastric cancer patients.

### 3.3. NRP1 promotes viability and proliferation of gastric cancer cells

In order to explore the biological functions of NRP1 in the development of gastric cancer, we transfected HCG27 cells with shRNAs for NRP1(shRNA1/NRP1, shRNA2/NRP1, shRNA3/NRP1,

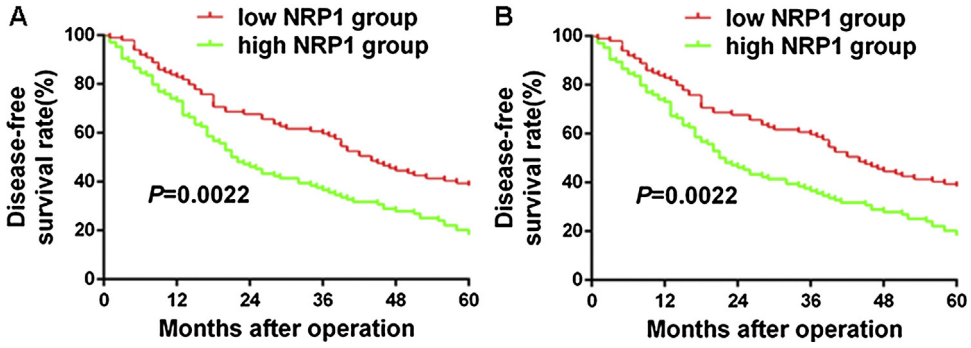


**Fig. 2.** The effects of NRP1 expression level on the prognosis of gastric cancer patients. Gastric cancer patients in high NRP1 group had worse (A) 5-year overall survival and (B) 5-year disease-free survival rate,compared to the low NRP1 group.

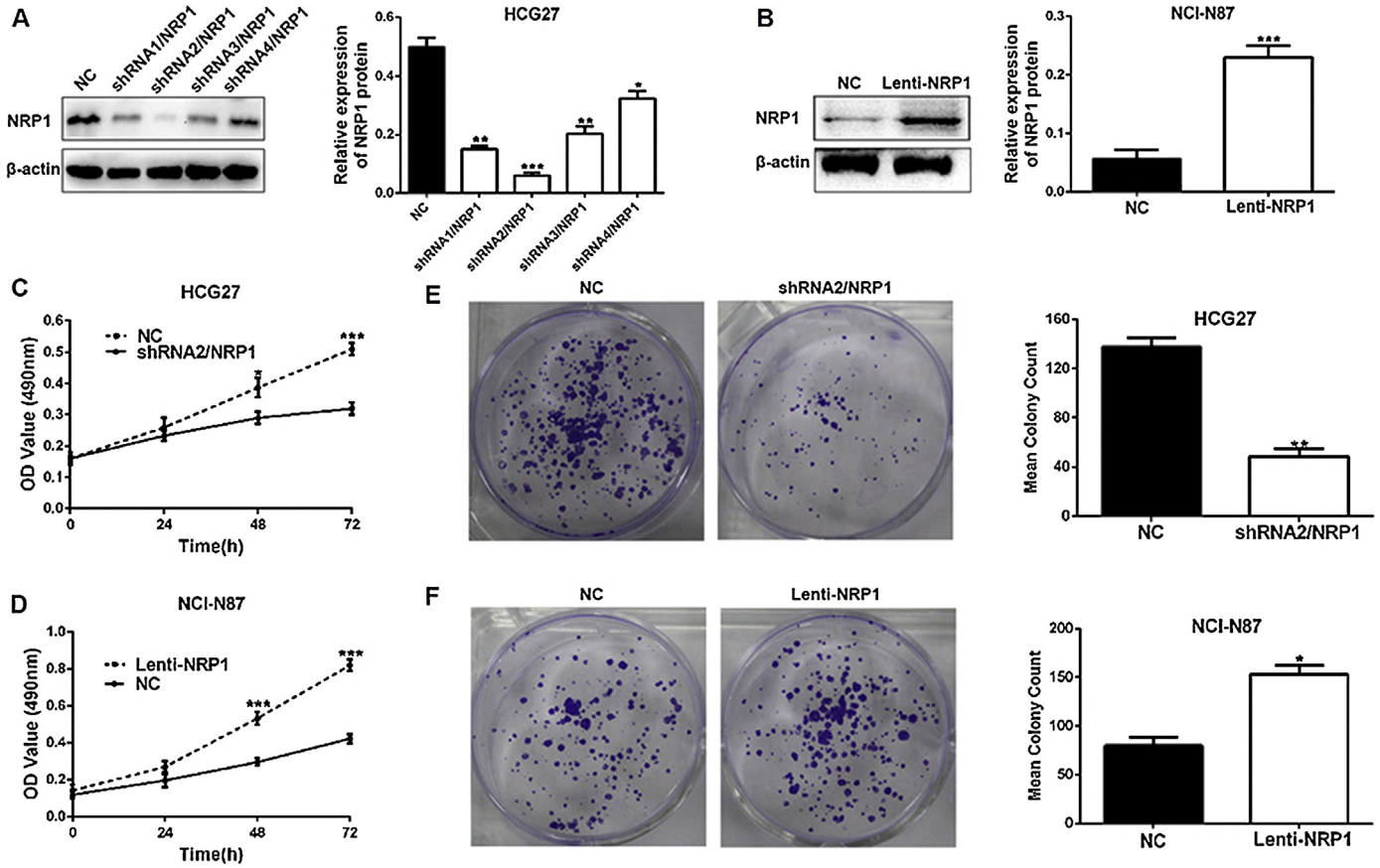


**Fig. 3.** NRP1 promoted gastric cancer cells viability, proliferation, migration and invasion. (A) Western blot results of NRP1 expression after transfecting HCG27 cells with shRNAs. (B) Western blot results of NRP1 expression after transfecting NCI-N87 cells with Lenti-NRP1. C,D) MTT assay showed that NRP1 could promote viability of gastric cancer cells. (E,F) Colony formation assay revealed that NRP1 could significantly promote proliferation of gastric cancer cells. $^{*}P < 0.05$,$^{**}P < 0.01$,$^{***}P < 0.001$.

shRNA4/NRP1) and Negative Control(NC).The data showed that shRNA2/NRP1 presented to be the most efficient shRNA to knockdown NRP1($P < 0.05$, Fig. 3A). Meanwhile, lenti-NRP1 was used to notably up-regulate NRP1 expression of NCI-N87 cells ($P < 0.01$, Fig. 3B)). Then we conducted MTT assay to measure the cell viability. Results revealed that cells with increased NRP1 had more viability while cells with decreased NRP1 had less viability over time compared to the NC groups ($P < 0.05$, Fig. 3C,D). Consistently, colony formation assay confirmed that NRP1 could significantly promoted proliferation of gastric cancer cells ($P < 0.05$, Fig. 3E,F). We observed that NRP1 could markedly suppress cell apoptosis. Therefore, we demonstrated that **N**RP1 could promote viability and proliferation of gastric cancer cells.

### 3.4. NRP1 promotes gastric cells migration and invasion

Then, we explored the functions of NRP1 in migration and invasion of gastric cancer cells by Transwell assay. The results showed that down-regulated NRP1 suppressed the migration and invasion abilities of HCG27 cells ($P < 0.05$, Fig. 4A). In contrast, increased NRP1 could reinforce the abilities of NCI-N87 cells ($P < 0.05$, Fig. 4B). Taken together, these data revealed that NRP1 could promote gastric cells migration and invasion.

### 3.5. IRGD strengthens the chemotherapy efficacy of 5-FU on gastric cancer cells

To determine whether the therapeutic efficacy of 5-FU was enhanced when combination of 5-FU with iRGD was applied to treat HCG27 and NCI-N87 cells. Firstly, we investigated whether only iRGD could exert any influences on the viability, proliferation, migration and invasion of gastric cancer cells. Then we found that the cells treated with only iRGD or only PBS had no significant difference in the abilities of cell viability, proliferation, migration and invasion(Data not shown), The data meant that when gastric cancer cells were treated with iRGD only, the biological behaviours had not any change. Subsequently, MTT assay were performed to measure the viability of HCG27 and NCI-N87 cells treated with iRGD (0.3 μmol/ml) in combination with the gradient concentration of 5-FU. The survival rate curves revealed that the percentage of viable cell declined gradually with the increasing concentrations of 5-FU in both groups, and faster in the 5-FU + iGRD group

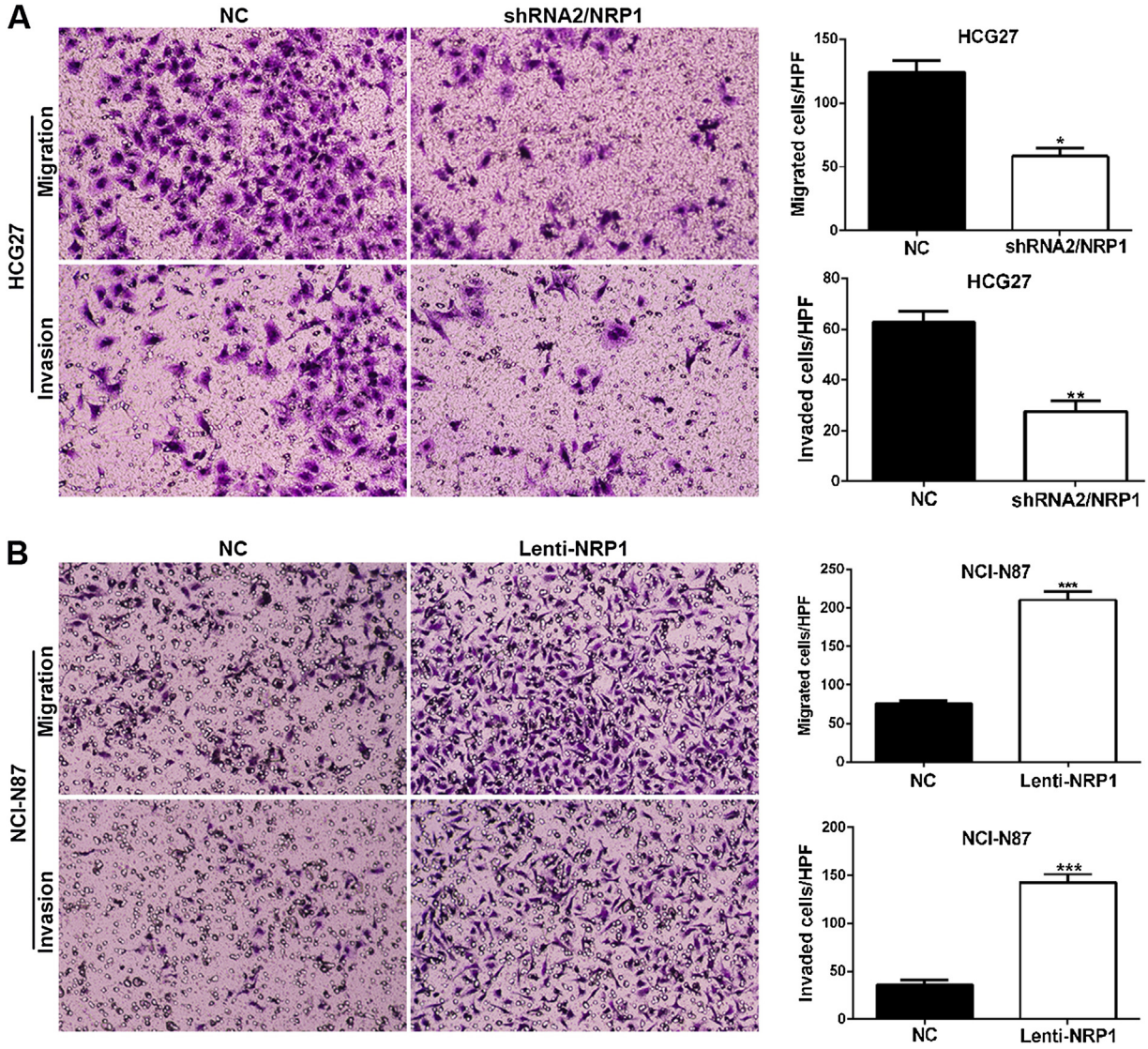


**Fig. 4.** NRP1 promoted migration and invasion of gastric cancer cells. (A) Transwell assay (magnification: × 200) showed that HCG27 cells with shRNA2/NRP1 had notably attenuated abilities of migration and invasion compared to NC group.(B) Transwell assay (magnification: × 200) showed that NCI-N87 cells with Lenti-NRP1 had significantly strengthened abilities of migration and invasion compared to NC group. Data represent means ± SD (n = 3, each).*$P < 0.05$,**$P < 0.01$,***$P < 0.001$.

($P < 0.05$, Fig. 5A, B). Interesting enough, all of the chemotherapy effects reached to a therapeutic plateau when 5-FU concentration was 60 ug/mL (Fig. 5A, B). Then we chose the concentration 60ug/ml to continue the further exploration. A series of experiments were conducted to explore the effects of 5-FU in combination with iRGD on cell viability, proliferation, migration and invasion. The results showed that the cells treated with 5-FU + iGRD presented notably attenuated abilities of cell viability($P < 0.05$, Fig. 5C, D), proliferation($P < 0.05$, Fig. 5E, F), migration($P < 0.05$, Fig. 5G, I) and invasion($P < 0.05$, Fig. 5H, J).

In order to conform the functions of the above results in gastric cancer, we established the subcutaneous xenotransplanted tumor models of human gastric cancer in nude mice. Then the tumor growth-curve of tumor volume according time in each group was conducted. The data showed that the tumor growth was markedly slower in the group treated with 5-FU + iRGD ($P < 0.05$, Fig. 6A,B). Therefore, we concluded that iRGD could enhance the therapeutic efficacy of 5-FU on gastric cancer cells both in *vitro* and in *vivo*.

### *3.6. IRGD strengthens the chemotherapy efficacy of 5-FU on gastric cancer cells through NRP1*

It has been reported that iRGD could promote the permeation of drugs into tumor cells, and NRP1 plays a critical role in the process. Thus, we attempted to explore whether NRP1 could mediate the promoting therapeutic effects of iRGD on 5-FU in gastric cancer cells. 5-FU in combination with iRGD was applied to treat the two kinds of cells(HCG27-shRNA2/NRP1 and NCI-N87-Lenti-NRP1), whose NRP1 expression had been stably altered. In HCG27-shRNA2/NRP1 cells, results of MTT assay ($P > 0.05$, Fig. 7A), Colony formation assay ($P > 0.05$, Fig. 7C)and Transwell assay ($P > 0.05$, Fig. 7E,F) revealed that there was no significant difference between 5-FU + iRGD group and 5-FU + PBS group in terms of the chemotherapy efficacy of 5-FU. These data suggested that when NRP1 of HCG27 cells was silenced,iGRD could not exert its functions. In contrast, in NCI-N87-Lenti-NRP1 cells, the chemotherapy efficacy was notably strengthened by 5-FU + iRGD($P < 0.05$, Fig. 7B,D,G,H). Thus, these data demonstrate that NRP1 could act as a mediator for iRGD in gastric cancer cells.

## 4. Discussion

Gastric cancer is one kind of malignant tumors with high mortality, morbidity and recurrence rates, and the patients are liable to have a poor prognosis [15]. For decades, researchers have been working on determining some clinical practical tumor markers for gastric cancer [16]. At present, more and more potential biomarkers have been explored [16,17]. For example, research reported that high expression of STAT3 and EZH2 in gastric cancer is associated with the TNM stage, and STAT3 signaling could enhance EZH2 promoter activity in GC cells [18]. Increased EphA3 in gastric cancer is associated with metastasis and poor survival [19]. And it has been found that NRP1 was overexpressed in multiple malignant tumors, such as prostate

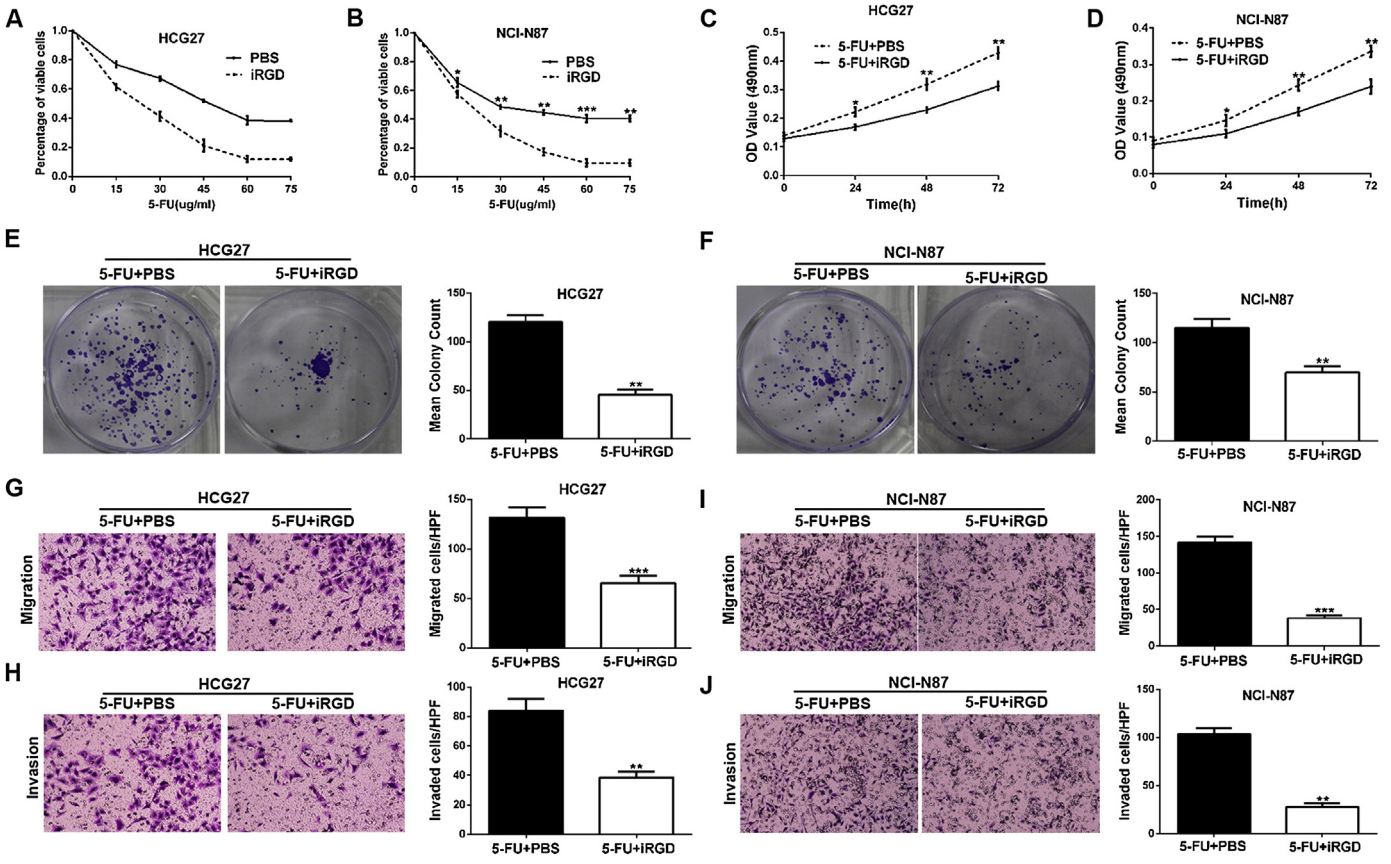


**Fig. 5.** IRGD could strengthen the chemotherapy efficacy of 5-FU on gastric cancer cells in vitro. (A,B) MTT assay revealed that the percentage of viable cell declined gradually with the increasing concentrations of 5-FU, but faster in the 5-FU + iGRD group. (C,D)MTT assay showed that iRGD in combination with 5-FU could promote viability of gastric cancer cells compared to 5-FU + PBS. (E,F) Colony formation assay revealed that 5-FU + iGRD could significantly inhibit proliferation of gastric cancer cells. (G,H,I,J) Transwell assay (magnification: × 200) showed that HCG27 cells or NCI-N87 cells treated with 5-FU + iGRD had notably attenuated abilities of migration and invasion compared to 5-FU + PBS group. Data represent means ± SD (n = 3, each).*$P < 0.05$,**$P < 0.01$,***$P < 0.001$.

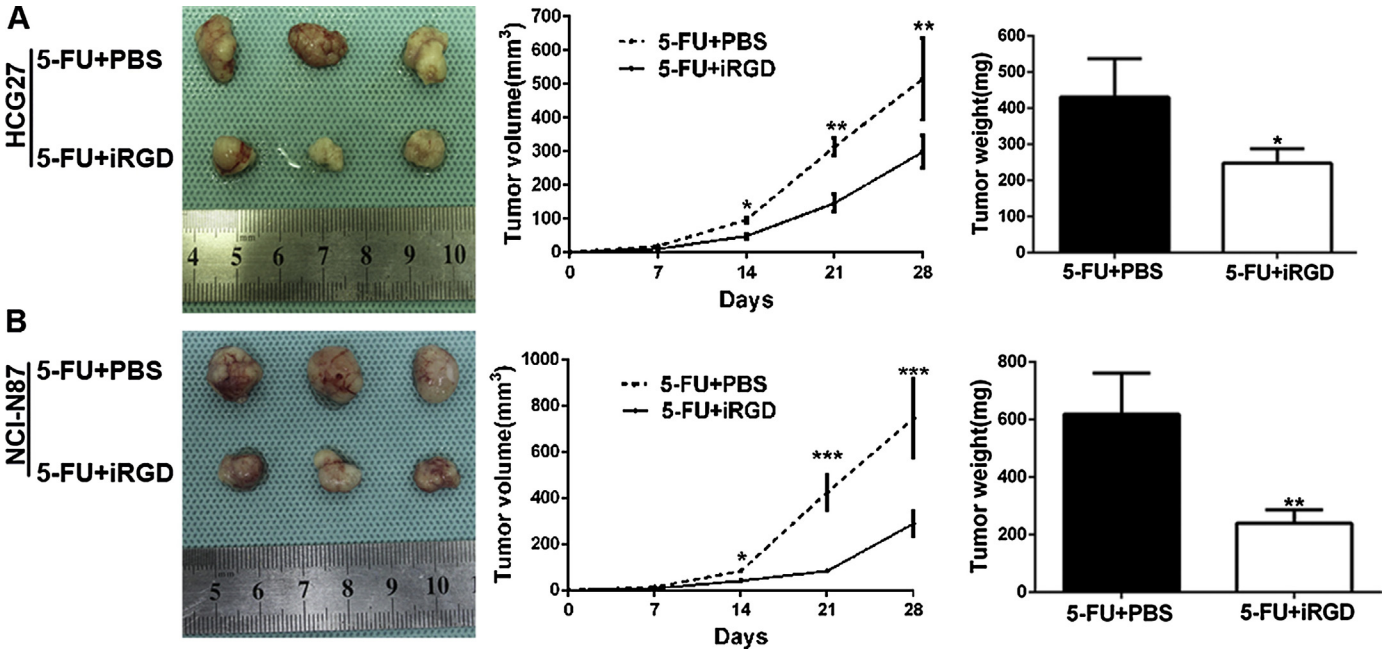


**Fig. 6.** IRGD could strengthen the chemotherapy efficacy of 5-FU on gastric cancer cells in vivo. (A)The growth and weight of HCG27 induced subcutaneous tumors was markedly slower and lighter in the group treated with 5-FU + iRGD. (B) The growth and weight of NCI-N87 induced subcutaneous tumors was markedly slower and lighter in the group treated with 5-FU + iRGD. $^{*}P < 0.05$,$^{**}P < 0.01$,$^{***}P < 0.001$.

cancer, glioma and esophageal cancer [6,7,20]. Li X et al. indicate that nordihydroguaiaretic acid suppresses NRP1 function by downregulating its expression, which leads to attenuated cell motility, cell adhesion to ECM and FAK signaling in prostate cancer cells [7]. Miyauchi JT et al. showed that pharmacological inhibition of NRP1 constitutes a potential strategy for suppressing glioma progression [6]. And Shi F et al. suggest that NRP1 is a critical downstream effector of calreticulin in promoting cell migration and invasion, which might contribute to the metastasis of esophageal squamous cell carcinomas [20].

In this study, NRP1 was identified to be overexpressed in gastric cancer tissues and cell lines. Clinically, elevated NRP1 was closely associated with the tumor differentiation, tumor size, tumor stage, lymph node metastasis, and TNM tumor stage. In addition, patients with highly expressed NRP1 had worse prognosis. Functionally, a serious of results revealed that NRP1 could enhance proliferation, migration and invasion of gastric cancer cells. These data suggested that NRP1 could be a potential and valuable tumor marker for gastric cancer.

Previous studies have reported that the peptide iRGD could increase vascular and tumor cell permeability by interacting with the permeability-regulating receptor, NRP1 [21,22]. For example, iRGD could enhance the effects of co-administered cetuximab in a non-small cell lung cancer model [22]. And another research demonstrates that the iRGD modified nanoparticles loaded with ICG and TPZ could notably improve the penetration in breast tumors both in vivo and vitro [23]. In addition, 5-FU has been identified as one of the standard first-line chemotherapy drugs for

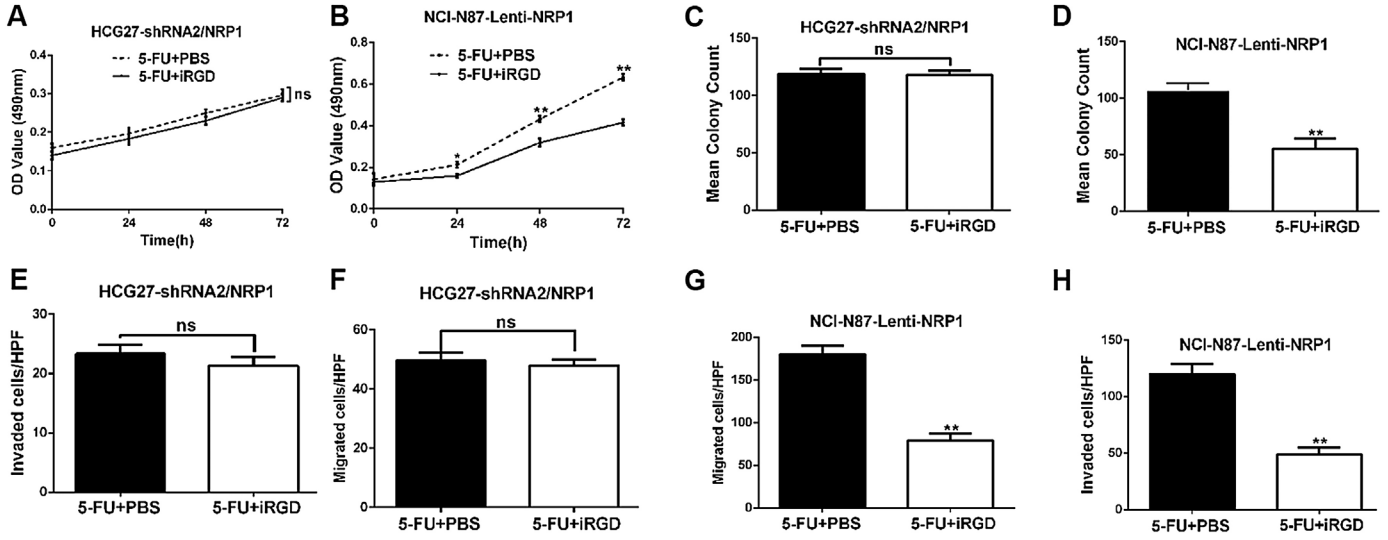


**Fig. 7.** IRGD strengthened the chemotherapy efficacy of 5-FU on gastric cancer cells through NRP1. (A,C,E,F) MTT assay, Colony formation assay and Transwell assay showed that when NRP1 of HCG27 cells was silenced,iRGD could not exert its functions. (B,D,G,H)In NCI-N87-Lenti-NRP1 cells, the chemotherapy efficacy was notably strengthened by 5-FU + iRGD Data represent means ± SD (n = 3, each). $^{*}P < 0.05$,$^{**}P < 0.01$,$^{***}P < 0.001$.

locally advanced or metastatic gastric cancer [10,24]. However, whether iRGD could strengthen the chemotherapy effect of 5-FU on gastric cancer cells and the potential mechanism remains to be explored. In this study, our data demonstrated that the cells treated with 5-FU + iRGD had diminished abilities of proliferation, migration and invasion. Consistently, 5-FU + Irgd could markedly inhibit tumor growth in vivo. Furthermore, we found that NRP1 was a mediator for iRGD in gastric cancer cells. Thus, we concluded that iRGD could enhance the therapeutic efficacy of 5-FU on gastric cancer cells through NRP1.

In summary, our results demonstrate that NRP1 may work as an early biomarker and a novel therapeutic target for gastric cancer. Furthermore, iRGD could enhance the therapeutic efficacy of 5-FU to gastric cancer cells through NRP1. These data potentially provide novel therapies to complement the existing interventions.

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## Conflicts of interest

No conflicts of interest exist.

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