Overexpression of RhoGDI2 in stage II and III gastric cancer is involved in 5-fluorouracil resistance
Research Article

Wang Jinguo*, Hu Minhua, Wu Pei and Wu Jian

[1] Department of Gastrointestinal Surgery, Yijishan Hospital, Wannan Medical College, Wuhu, Anhui 241000, People's Republic of China

_____

***Correspondence**: Wang Jinguo, email: shwangjg@sina.com; Telephone: +86-553-5739558; Fax: +86-553-5738279.

Summary

Rho GDP dissociation inhibitor 2 (RhoGDI2) is known to be involved in tumor growth, metastasis and cisplatin resistance. We have further explored RhoGDI2 as a predictor of 5-fluorouracil (5-FU) treatment response in gastric cancer. Here we report that RhoGDI2 promotes tumor progression and 5-FU chemoresistance in gastric cancer. In clinical analysis, RhoGDI2 was overexpressed in 153 of 215 (71.16%) stage II and III patients who received 5-FU-based chemotherapy after radical gastrectomy. Multivariate Cox regression analysis indicated that RhoGDI2 expression was an independent prognostic factor for overall survival ($P=0.013$) and disease-free survival ($P=0.008$) of patients with gastric cancer. After being treated with 5-FU, the expression of RhoGDI2 in gastric cancer cells was increased. In RhoGDI2-overexpressing cells treated with 5-FU, cell viability decreased slower than in control cells. These findings demonstrate that RhoGDI2 is an independent prognostic factor for adjuvant 5-FU-based chemotherapy in patients with stage II and stage III gastric cancer. Targeting RhoGDI2 may thus be a useful strategy to enhance chemotherapy efficacy in gastric cancer.

I. Introduction

Gastric cancer is one of the most common causes of death from cancer worldwide (Rahman et al. 2014). The means of treating advanced gastric cancer are very limited. Many clinical trials have shown that adjuvant therapy, in particular, chemotherapy after radical gastrectomy may improve the survival of gastric cancer patients compared to surgery alone (Wu et al. 2015). The standard chemotherapy regimen used to treat gastric cancer is 5-fluorouracil (5-FU) monotherapy and its combination with other conventional therapeutics (Zhu et al. 2015; Shen et al. 2014). However, there is evidence that gastric cancer resistance to 5-FU, especially stage II and III gastric cancer, is the major cause of therapy failure, leading to disease recurrence and metastasis (Yu et al. 2014). The factors that regulate the resistance of gastric cancer to 5-FU remain poorly understood.

RhoGDI2 belongs to a family of Rho GTPase dissociate inhibitors (GDIs), and is mainly expressed in hematopoietic, endothelial and urothelial cells (Cho et al. 2010; Luo et al. 2013). Previous studies showed that RhoGDI2 inhibits bladder cancer cell metastasis and regulates it progression*in vivo*and*in vitro* (Nitz and Harding 2008). These results suggested that RhoGDI2 might be a novel tumor metastasis suppressor. In contrast, recent studies showed that RhoGDI2 is overexpressed in breast cancer, ovarian carcinoma and pancreatic cancer, and can promote the invasiveness of cancer cells *in vivo* (Moon et al. 2010; Zhen et al. 2010; Yi et al. 2014). In addition, RhoGDI2 was also reported to be implicated in cisplatin resistance (Cho et al. 2011). Therefore, the role of RhoGDI2incancerremainscontroversial.

In this study, we report the expression pattern of RhoGDI2 in gastric cancer tissues and analyze the correlation between expression levels of RhoGDI2 and the clinicopathologic characteristics of this disease. To evaluate whether RhoGDI2 can influence 5-FU-based chemotherapeutic sensitivity in gastric cancer cells,we upregulated RhoGDI2 expression in gastric cancer cells using plasmid transfection to observe cell viability after treatment with 5-FU. In addition, the relationship between RhoGDI2 expression and disease-free survival (DFS) of stage II and III gastric cancer patients who underwent surgery after 5-FU-based chemotherapy was also analyzed.

II. Materials and methods

A. Cell culture

The human gastric cancer cell lines N87 and HTB-103 were obtained from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (Shanghai, China). The gastric cancer cell lines were cultured in RPMI-1640 medium (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and 1% penicillin and streptomycin (Life Technologies) in a humidified incubator with an atmosphere of 5% CO_2 at 37°C.

B.Patients and tissues

A total of 215 tumor specimens were obtained from stage II and III gastric cancer patients who underwent radical resection and received 5-FU based chemotherapy after radical gastrectomy at the Department of Gastrointestinal

Surgery, Yijishan Hospital, Wannan Medical College (Wuhu, Anhui province, China) from Jan 1 2007 to Dec 31 2009. Patients who were administered neoadjuvant chemotherapy and radiotherapy before surgery were excluded. The pathologic tumor staging was determined according to the American Joint Committee on Cancer's tumor-node-metastasis classification (2010) (Washington 2010). The patients were followed by history and physical examination every 3 months for 2 years, then every 6 months for up to 5 years. The study was approved by the Ethics Committee for clinical research of Yijishan Hospital, Wannan Medical College. The sections of specimens were fixed with formalin for immunohistochemistry (IHC) tests.

C. Immunohistochemistry (IHC)

The gastric cancer tissues and adjacent normal tissues from the same patient were fixed in formalin and embedded into the same paraffin block. IHC was performed as described by Wang et al. (Wang et al. 2012). RhoGDI2 expression was determined by assessing the intensity of stained tumor cells. The staining intensity was scored as 0 (no staining), 1 (mild staining), 2 (moderate staining), or 3 (intense staining). The stained area was scored as 0 ($<$5%), 1 (5%–25%), 2 (26%–50%), 3 (51%–75%), or 4 (76%–100%) according to the percentage of positively-stained cells. The final staining scores, calculated by the sum of the intensity and area scores, were divided into three groups as follows: 0–1, negative expression; 2, weak expression; and 3–4, strong expression.

D. Antibodies and western blotting

Anti-RhoGDI2, and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies were purchased from Spring Bioscience (Pleasanton, CA, USA). Western blotting was performed as previously described (wang et al. 2012). Briefly, cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. The blot was then probed with anti-RhoGDI2 at a dilution of 1:1000, followed by an incubation with a specific secondary antibody for 1 h at room temperature. Protein bands were visualized by the ECL detection system (Amersham Biosciences AB, Piscataway, NJ, USA). Autoradiogramswere quantified by densitometry (Quantity One software; Bio-Rad, Hercules, CA, USA). As a loading control, GAPDH specific antibody was used. Relative protein levels were calculated by reference to the amount of GAPDH protein.

E. RNA extraction and qPCR

Total RNA was extracted from cultured cells using RNeasy Mini Kit (Qiagen, Hilden, Germany) following manufacturer'sinstructions. Real-time quantitative polymerase chain reaction (qPCR) analysis was performed as previously described (wang et al. 2012) on the cells and on 40 frozen tumor tissue and corresponding normal tissue specimens. qPCR analysis of RhoGDI2 expression was conducted using 2 μL of cDNA. The forward primer sequence was 5'-ATGACTGAAAAAGCCCCA-3' and the antisense primer sequence was 5'-TCATTCTGTCCACTCCTT-3'. GAPDH was used as an internal control: 5'-ACAACTTTGGTATCGTGGAAGG-3' (forward) and 5'-GCCATCACGCCACAG TTTC-3' (reverse). All of the experiments were performed at least in triplicate.

F. RhoGDI2-expressing plasmid

Human RhoGDI2 cDNA was amplified by PCR using the primers: forward 5'-CATACTCGAGCGGACAGAGACGTGAAGCAC-3' and reverse 5'-GAAAAGGGTGGGACA GTGCGACTAGGTCAC-3'. PCR products were cloned into pEGFP-C3 (Clontech Laboratories, Mountain View, CA, USA). N87 and HTB-103 cells were transfected with pEGFP-C3-RhoGDI2 or pEGFP-C3 using Lipofectamine reagent (Life Technologies) according to the manufacturer's instructions. Stable plasmid-transfected clones were selected using 800 μg/ml G418 (Life Technologies) for two weeks, isolated colonies were picked up with tips and the cells were further cultured in the presence of 400 μg/ml G418.

G. Cell viability assay

To analyze the viability of cells, 1×10^4 cells/well were seeded in 96-well microplates containing 0.2 mL of RPMI-1640. After treatment, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) was added to each well and incubated at 37°C for 2 h. Then the culture medium was replaced with dimethyl sulfoxide (Sigma-Aldrich). The absorbance at 490 nm (A490) of each well was detected on a microplate reader (Bio-Rad) after shaking at ×2000 rpm for 10 min at room temperature. Cell viability was calculated from the following formula: cell viability (%) = A490 (sample)/A490 (control) ×100.

H. Statistical analysis

The significance of the *in vitro* data was determined using the Student's *t*-test. The χ^2 test and Fisher exact test were used to determine the statistical significance of covariate differences. Survival rates were calculated using the Kaplan–Meier method and the log-rank test was used to compare the survival curves. A *P* value of less than 0.05 was considered to be statistically significant. All statistical analyses were carried out using the SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL, USA).

III. Results

A. Aberrant overexpression of RhoGDI2 in gastric cancer tissues

To detect the expression of RhoGDI2 in normal human gastric mucosa and in gastric cancer tissues, IHC was used. One hundred and fifty-three of 215 (71.16%) stage II and III patients' gastric cancer tissues were shown to be positive for RhoGDI2 (Table 1). Typical immunostainings of RhoGDI2 in normal gastric mucosa and in gastric cancer specimens were shown in Figure.1A. Subsequently, the 40 randomly selected paired cases were used to evaluate RhoGDI2 mRNA and protein expression by qPCR and western blotting respectively. Using qPCR, it was shownthat 27 of 40 (67.5%) cases had at least a 2-fold increase in RhoGDI2 mRNA levels compared to adjacent normal tissues. This difference in RhoGDI2 mRNA expression was significant (*P*<0.05, Figure.1B). Western blotting also confirmed that the level of RhoGDI2 protein was higher in gastric cancer samples than in the matched adjacent normal tissues (Figure. 1C).

B. Association between RhoGDI2 expression and the clinical features of gastriccancer

To determine the correlation between RhoGDI2 expression level and clinicopathological factors in gastric cancer, the 153 malignant tumors with positive expression (in any degree) of RhoGDI2 from stage II and III patients were analyzed. The clinicopathological characteristics of 153

patients with gastric cancer are shown in Table 1. The data from Table 1 showed that the expression of RhoGDI2 was correlated with tumor differentiation (*P*=0.000) and recurrence (*P*=0.019). However, no significant correlation was found between the staining intensity of RhoGDI2 and gender, tumor size and lymphatic invasion. These data indicate that RhoGDI2 may be involved in gastric cancer progression.

C. RhoGDI2 expression is associated with a poor clinical outcome in stage II and III gastric cancer patients

Results from the IHC analysis of 153 stage II and III tissues showed that there was a significant difference between the RhoGDI2-positive and RhoGDI2-negative groups in the number of patients who developed primary gastric cancer recurrence after 5-FU-based chemotherapy (*P*=0.019). More of the patients with RhoGDI2-positive tumors underwent subsequent recurrence than the patients with RhoGDI2-negative tumors, positive RhoGDI2 expression (weak and strong) was associated with a 5.757-fold increased risk of recurrence (hazard ratio (HR), 5.757; 95% confidence interval (CI), 2.769–11.968; *P*<0.001). We used the Kaplan–Meier analysis to show that the expression of RhoGDI2 was significantly correlated with the DFS of gastric cancer patients (log-rank test, *P*<0.05; Figure.2A). Patients with RhoGDI2-positive tumors had significantly lower 5-year overall survival (OS) than those with RhoGDI2-negative tumors (*P*<0.05; Figure. 2B). In multivariate analysis using clinicopathological variables such as differentiation grade, the expression of RhoGDI2 was an independent prognostic marker to predict patient outcomes (Table 2).This suggests that RhoGDI2 is of clinical significance in the diagnosis and prognosis of patients with stage II and III gastric carcinomas who are treated with 5-FU-based chemotherapy.

D. 5-FU induces upregulation of RhoGDI2 expression

To investigate the role of RhoGDI2 in mediating sensitivity to gastric cancer chemotherapy, we used two human gastric cancer cell lines, N87 and HTB-103. Western blot analysis showed that both untreated cell lines expressed RhoGDI2 protein. Following treatment with 10 μM 5-FU for 24, 48 and 72 h, a gradual increase in the RhoGDI2 mRNA levels was observed in both N87 and HTB-103 cells (Figure. 3A). To examine whether RhoGDI2 protein levels were also altered after 5-FU treatment, western blot analysis was performed. As shown in Figure. 3B, RhoGDI2 levels increased following 5-FU treatment in both cell lines, which was similar to our qPCR data (Figure. 3A).

E. RhoGDI2 increases cell viability

Western blot analysis showed that the expression of RhoGDI2 in N87 and HTB-103 cells was significantly increased after being transfected with aRhoGDI2-overexpressing plasmid (Figure. 3C). To determine whether RhoGDI2 could enhance gastric cancer cell viability after treatment with 5-FU, MTT assays were used. Figure. 4A shows that cell viability was reduced after being incubated with 10 μM 5-FU for 0, 24, 48, and 72 h. However, RhoGDI2-overexpressing cell lines showed a significant enhancement in cell viability with all three incubation times (24, 48, and 72 h) compared to control cells. In addition, cell viability was reduced by 5-FU in a dose-dependent manner (Figure. 4B). In summary, overexpression of RhoGDI2 increased chemoresistance in N87 and HTB-103

cells. These data indicatethat RhoGDI2 mediates chemoresistance in gastric cancer cells.

IV. Discussion

RhoGDI2 belongs to a family of Rho GDIs which are known to inhibit the activity of Rho GTPase (Griner and Theodorescu 2012). RhoGDI2 has been identified as a metastasis suppressor gene in bladder cancer. Decreased RhoGDI2 expression was associated with a more invasive variant of the HRAS mutation-positive T24 bladder cancer cell line (Wu et al. 2007). This phenomenon was also observed in ovarian cancer cells. Suppression of RhoGDI2 expression increased ovarian cancer cell growth and invasion *in vitro* (Stevens et al. 2011). However, Zhang et al. (2009) reported that RhoGDI2 was overexpressed in MDA-MB-231 breast cancer cells and promoted cell migration. In colorectal cancer, RhoGDI2 was found to be overexpressed and increased cancer cells proliferation, motility, and invasion (Li et al. 2012). This is consistent with our trial results. In our study, RhoGDI2 was also found to be overexpressed in gastric cancer tissues compared to their normal counterparts. And RhoGDI2 expression in gastric cancer tissues correlated with tumor differentiation and recurrence. Our data show that RhoGDI2 expression may be implicated in gastric cancer progression.

To further understand how RhoGDI2 contributes to gastric cancer progression, multivariate Cox regression analysis was used. We found that more gastric cancer patients with RhoGDI2-positive tumors underwent subsequent recurrence than patients with RhoGDI2-negative tumors after 5-FU based chemotherapy. Kaplan–Meier analysis also showed that patients with RhoGDI2-positive tumors had a significantly lower 5-year DFS and OS than those with RhoGDI2-negative tumors. This data shows that RhoGDI2 may be a predictor of 5-FU treatment response in gastric cancer.

As a commonly used chemotherapeutic agent 5-FU can interfere with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell apoptosis (Longley et al. 2003). Resistance to 5-FU can result from various causes, including alteration of drug influx and efflux, enhancement of drug inactivation and mutation of the drug target (Longley and Johnston 2005). High expression of thymidylate synthase (Kadota et al. 2011), increased activity of deoxyuridine triphosphatase (Ladner et al. 2000), methylation of the CDKN2A and CCNA1 genes (Klajic et al. 2014), and overexpression of Bcl-2 (Wu et al. 2015), NF-𝜘B (Uetsuka et al. 2003), ERK (Ahn et al. 2013), and Akt (You et al. 2009) proteins have all been reported to be relevant to 5-FU resistance. Therefore, multiple factors might contribute to 5-FU resistance, and perhapsthe exact mechanism of 5-FU resistance remains to be further elucidated. The present study has shown that the expression of RhoGDI2 is significantly increased in both N87 and HTB-103 cells following 5-FU treatment. Moreover, with upregulated RhoGDI2 expression in gastric cancer cells by plasmid transfection to observe cell viability, cell viability was reduced by 5-FU treatment in a time and dose-dependent manner. However, compared to control cells, the cell viability of RhoGDI2-overexpressing cells decreased more slowly after treatment with 5-FU. Therefore, RhoGDI2 expression in gastric cancer may contribute to 5-FU chemoresistance.

In conclusion, our study indicates that RhoGDI2 decreases 5-FU chemosensitivity in gastric cancer cells and is an independent prognostic factor for adjuvant 5-FU-based chemotherapy in patients with stage II and III gastric cancer. RhoGDI2 could be an effective target for sensitization in gastric cancer although further research is required to confirm our findings.

Acknowledgements

Conflict of interest
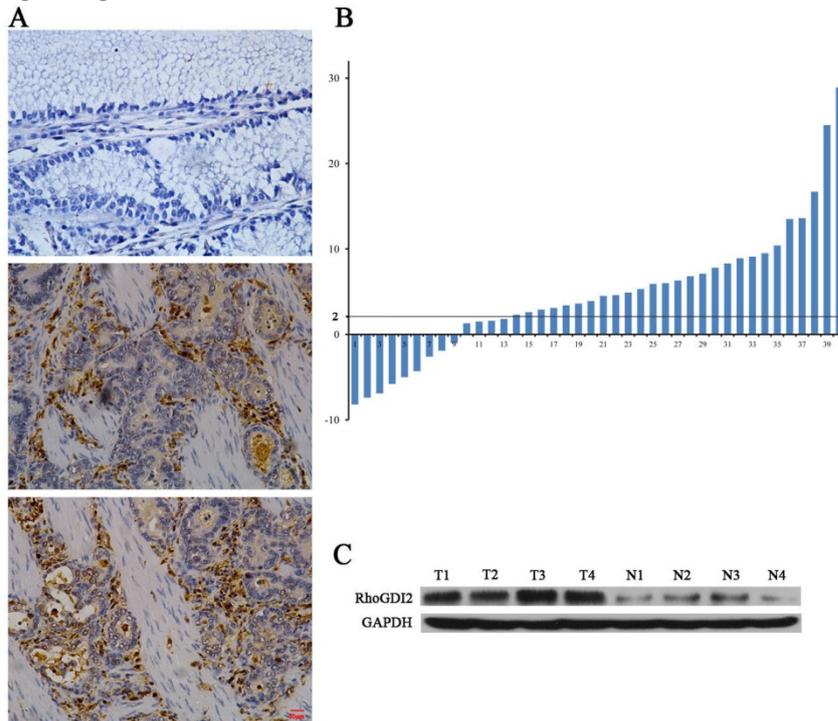
None.

Figure legends

Figure. 1

RhoGDI2 was overexpressed in gastric cancer.A Immunohistochemical staining for RhoGDI2 expression in normal and gastric cancer tissues (200×, Bar=50 μm). **B** At least a 2-fold increase in RhoGDI2 mRNA levels compared to noncancerous tissue was observed in 27 (67.5%) of 40 gastric cancer samples. The logarithmic scale $2^{-\Delta\Delta Ct}$ was used to represent the fold change in the qPCR analysis. **C** Western blotting acknowledged that higher RhoGDI2 expression was observed in gastric cancer samples compared to noncancerous tissues.
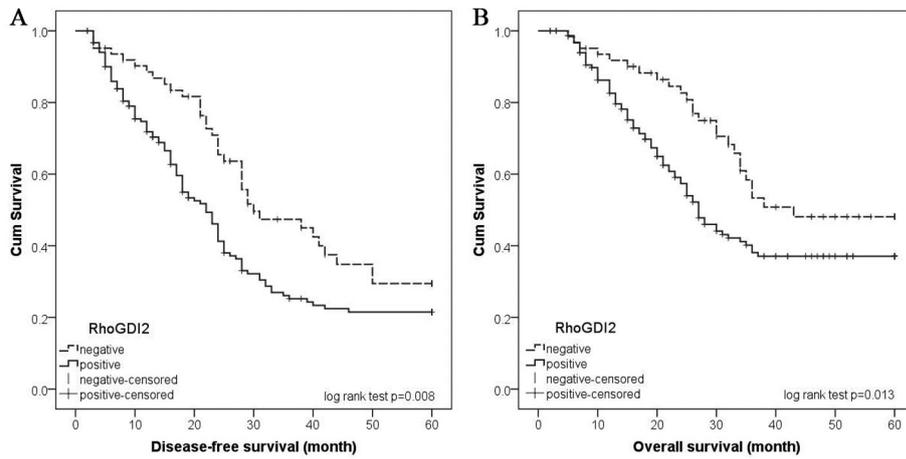
Figure. 2
Kaplan–Meier and log-rank test analysis of survival for 215 stage II and III gastric cancer patients who received 5-FU-based chemotherapy after surgery. A Overall survival (OS) was significantly better in patients with RhoGDI2-negative tumors than in patients with RhoGDI2-positive tumors (*P*<0.05). **B** Lower disease-free survival was observed in patients with higher RhoGDI2 expression (*P*<0.05).
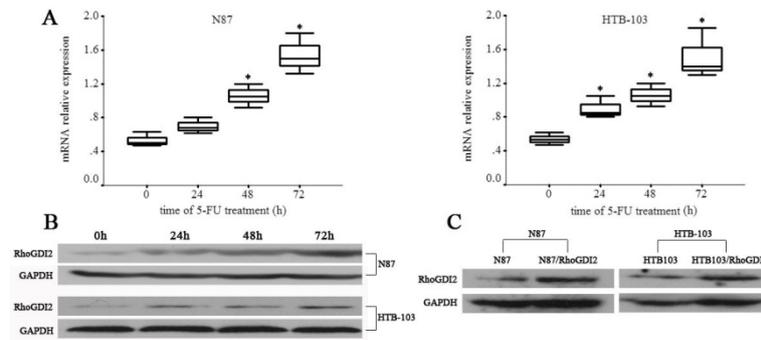
Figure. 3

   5-FU treatment induces RhoGDI2 expression. A qPCR analysis indicated that RhoGDI2 mRNA was increased in both N87 and HTB-103 cells treated with 10 μM of 5-FU for 24, 48, and 72 h. **B** The level of RhoGDI2 protein was upregulated in N87 and HTB-103 cells following 5-FU treatment. **C** Western blotting confirmed that RhoGDI2 expression was increased in N87 and HTB-103 cell lines after being transfected with RhoGDI2-expressing plasmid. Values are means ± the standard error of the mean (SEM; * P<0.05).
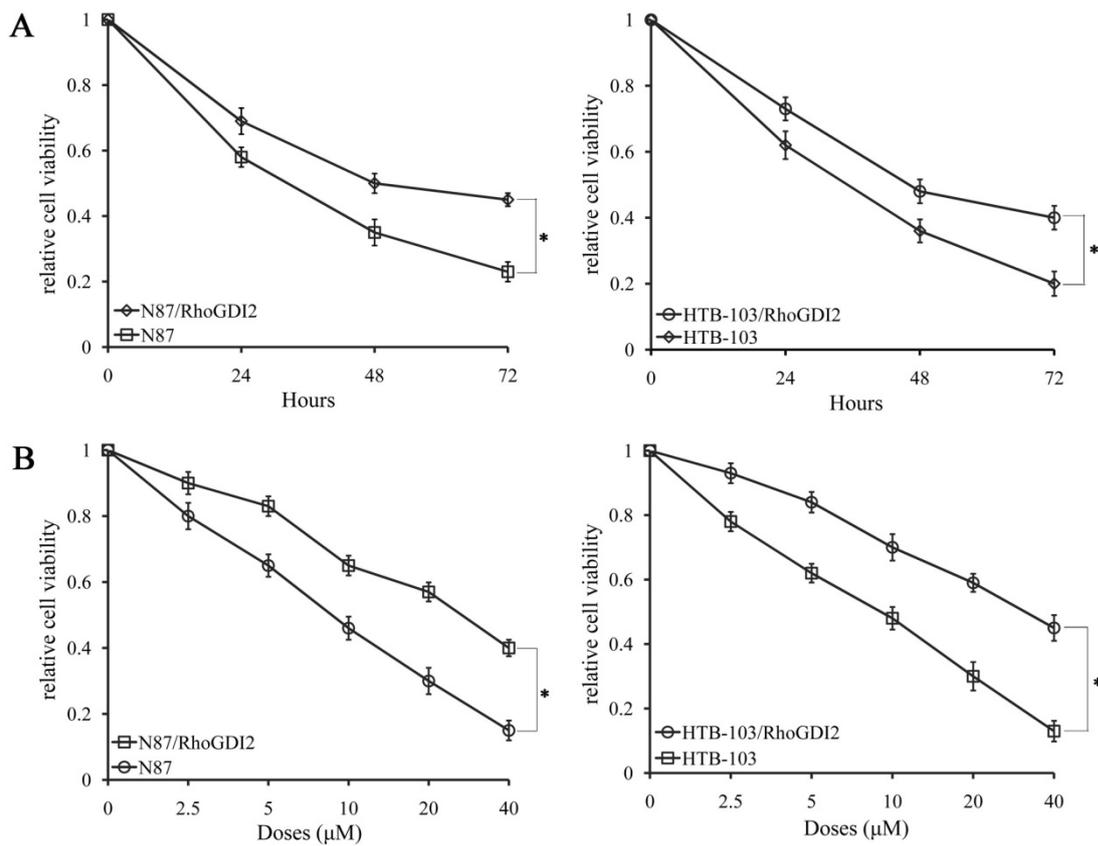
Figure. 4

RhoGDI2 increases cell viability. A RhoGDI2-overexpressing cell lines show less cell viability reduction at three time points (24, 48, and 72 h) following 10 μM 5-FU treatment (*P<0.05). **B** Cell viability was reduced by 5-FU treatment in a dose-dependent manner. Cells were treated with six concentrations of 5-FU (0, 2.5, 5, 10, 20, and 40 μM) for 48 h. Half-maximal inhibitory concentrations (IC50s) of 5-FU were determined from threeindependent experiments performed in quadruplicate.

Table 1
RhoGDI2 immunohistochemical staining for protein in gastric cancer

variable	n =215	RhoGDI2 expression			p value
		Negative	Weak	Positive	
Age					0.781
<60	119	32	26	61	
≥60	96	30	20	46	
Gender					0.939
Male	120	34	25	61	
Female	95	28	21	46	
Differentiation					0.000*
High	77	40	19	18	
Moderate or Low	138	22	27	89	
T stage					0.839
T1+T2	79	21	18	40	
T3+T4	136	41	28	67	
N stage					0.069
N0	64	17	20	27	
N1+N2+N3	151	45	26	80	
TNM stage					0.193
II	85	27	22	36	
III	130	35	24	71	
Metastasis					0.051
No	132	43	32	57	
Yes	83	19	14	50	
Tumor Recurrence					0.019*
No	124	37	34	53	
Yes	91	25	12	54	

*$p<0.05$ indicates a significant association among the variables.

Table 2

Multivariate Cox proportional hazards models for disease-free survival(DFS) and overall survival (OS)

	Multivariate analysis (DFS)		Multivariate analysis (OS)	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Gender (Female vs. Male)	0.708	0.925(0.616,1.391)	0.719	0.928(0.617,1.395)
Age (<60 years vs.≥60 years)	0.951	1.013(0.664,1.546)	0.973	1.007(0.659,1.539)
T stage (T1+T2 vs. T3+T4)	0.307	1.255(0.811,1.942)	0.323	1.246(0.806,1.928)
N stage (N0 vs. N1+N2+N3)	0.666	1.148(0.613,2.150)	0.680	1.141(0.610,2.132)
TNM stage (II vs. III)	0.178	1.454(0.843,2.508)	0.167	1.467(0.852,2.526)
Differentiation(High vs.Low or Moderate)	0.035*	1.653(1.035,2.641)	0.030*	1.685(1.051,2.966)
RhoGDI2 (negative vs. positive)	0.000*	5.757(2.769,11.968)	0.000*	5.825(2.803,12.105)

HR: Hazard ratio; CI: Confidence interval

*$p<0.05$ indicates that 95% CI of HR was not including 1.

References

Ahn HJ, Kim G, Park KS (2013) Ell3 stimulates proliferation, drug resistance, and cancer stem cell properties of breast cancer cells via a MEK/ERK-dependent signaling pathway. Biochem Biophys Res Commun 437: 557-564.

Cho HJ, Baek KE, Park SM, Kim IK, Nam IK, Choi YL, Park SH, Im MJ, Choi J, Ryu J, Kim JW, Lee CK, Kang SS, Yoo J (2011) RhoGDI2 confers gastric cancer cells resistance against cisplatin-induced apoptosis by upregulation of Bcl-2 expression. Cancer Lett 311: 48-56.

Cho HJ, Baek KE, Yoo J (2010) RhoGDI2 as a therapeutic target in cancer. Expert Opin Ther Targets 14: 67-75.

Griner EM, Theodorescu D (2012) The faces and friends of RhoGDI2. Cancer Metastasis Rev 31: 519-528.

Kadota K, Huang CL, Liu D, Yokomise H, Haba R, Wada H (2011) Combined therapy with a thymidylate synthase-inhibiting vector and S-1 has effective antitumor activity against 5-FU-resistant tumors. Int J Oncol 38: 355-363.

Klajic J, Busato F, Edvardsen H, Touleimat N, Fleischer T, Bukholm I, Børresen-Dale AL, Lønning PE, Tost J, Kristensen VN (2014) DNA methylation status of key cell-cycle regulators such as CDKNA2/p16 and CCNA1 correlates with treatment response to doxorubicin and 5-fluorouracil in locally advanced breast tumors. Clin Cancer Res 20: 6357-6366.

Ladner RD, Lynch FJ, Groshen S, Xiong YP, Sherrod A, Caradonna SJ, Stoehlmacher J, Lenz HJ (2000) dUTP nucleotidohydrolase isoform expression in normal and neoplastic tissues: association with survival and response to 5-fluorouracil in colorectal cancer. Cancer Res 60: 3493-3503.

Li X, Wang J, Zhang X, Zeng Y, Liang L, Ding Y (2012) Overexpression of RhoGDI2 correlates with tumor progression and poor prognosis in colorectal carcinoma. Ann Surg Oncol 19: 145-153.

Longley DB, Harkin DP, Johnston PG (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3: 330-338.

Longley DB, Johnston PG (2005) Molecular mechanisms of drug resistance. J Pathol 205: 275-292.

Luo J, Xu T, Li C, Ba X, Wang X, Jiang Y, Zeng X (2013) p85-RhoGDI2, a novel complex, is required for PSGL-1-induced β1 integrin-mediated lymphocyte adhesion to VCAM-1. Int J Biochem Cell Biol 45: 2764-2773.

Moon HG, Jeong SH, Ju YT, Jeong CY, Lee JS, Lee YJ, Hong SC, Choi SK, Ha WS, Park ST, Jung EJ (2010) Up-regulation of RhoGDI2 in human breast cancer and its prognostic implications. Cancer Res Treat 42: 151-156.

Nitz MD, Harding MA, Theodorescu D (2008) Invasion and metastasis models for studying RhoGDI2 in bladder cancer. Methods Enzymol 439: 219-233.

Rahman R, Asombang AW, Ibdah JA (2014) Characteristics of gastric cancer in Asia. World J Gastroenterol 20: 4483-4490.

Shen YC, Li CP, Yen CJ, Hsu C, Lin YL, Lin ZZ, Chen LT, Su WC, Chao Y, Yeh KH, Cheng AL (2014). Phase II multicentered study of low-dose everolimus plus cisplatin and weekly 24-hour infusion of high-dose 5-fluorouracil and leucovorin as first-line treatment for patients with advanced gastric cancer. Oncology 87: 104-113

Stevens EV, Banet N, Onesto C, Plachco A, Alan JK, Nikolaishvili-Feinberg N, Midkiff BR, Kuan PF, Liu J, Miller CR, Vigil D, Graves LM, Der CJ (2011) RhoGDI2 antagonizes ovarian carcinoma growth, invasion and metastasis. Small GTPases 2: 202-210.

Uetsuka H, Haisa M, Kimura M, Gunduz M, Kaneda Y, Ohkawa T, Takaoka M, Murata T, Nobuhisa T, Yamatsuji T, Matsuoka J, Tanaka N, Naomoto Y (2003) Inhibition of inducible NF-kappaB activity reduces

chemoresistance to 5-fluorouracil in human stomach cancer cell line. Exp Cell Res 289: 27-35.

Wang J, Liu B, Chen X, Su L, Wu P, Zhu Z (2012) PTP1B expression contributes to gastric cancer progression. Med Oncol 29: 948-956.

Washington K (2010) 7th edition of the AJCC cancer staging manual: stomach. Ann Surg Oncol 17: 3077-3079.

Wu DW, Huang CC, Chang SW, Chen TH, Lee H (2015) Bcl-2 stabilization by paxillin confers 5-fluorouracil resistance in colorectal cancer. Cell Death Differ 22: 779-789.

Wu FL, Lu DC, Ying YP, Huang JJ, Zhou AM, Jiang DK, Chen MW, Yang X, Zhou J, Huang HQ, Zeng HY (2015) A Meta-analysis Reveals S-1-based Chemotherapy Improves the Survival of Patients With Advanced Gastric Cancer. Medicine (Baltimore) 94: e652.

Wu Y, McRoberts K, Berr SS, Frierson HFJr, Conaway M, Theodorescu D (2007) Neuromedin U is regulated by the metastasis suppressor RhoGDI2 and is a novel promoter of tumor formation, lung metastasis and cancer cachexia. Oncogene. 26: 765-773.

Yi B, Hu Y, Qin G, Gu W, Zhu X, He S, Zhou J, Li D (2014) Depletion of RhoGDI2 expression inhibits the ability of invasion and migration in pancreatic carcinoma. Int J Mol Med 34: 205-212.

You F, Aoki K, Ito Y, Nakashima S (2009) AKT plays a pivotal role in the acquisition of resistance to 5-fluorouracil in human squamous carcinoma cells. Mol Med Rep 2: 609-613.

Yu P, Du Y, Cheng X, Yu Q, Huang L, Dong R (2014) Expression of multidrug resistance-associated proteins and their relation to postoperative individualized chemotherapy in gastric cancer. World J Surg Oncol 12: 307.

Zhang Y, Rivera Rosado LA, Moon SY, Zhang B (2009) Silencing of D4-GDI inhibits growth and invasive behavior in MDA-MB-231 cells by activation of Rac-dependent p38 and JNK signaling. J Biol Chem 284: 12956-12965.

Zhen H, Yang S, Wu H, Wang S, Lv J, Ma L, Zhang X (2010) LyGDI is a promising biomarker for ovarian cancer. Int J Gynecol Cancer 20: 316-322.

Zhu X, Zhao X, Peng W, Sun S, Cao J, Ji D, Liu X, Wang C, Huang M, Yu H, Guo W, Li J, Yin J (2015) Epirubicin combined with oxaliplatin and 5-day continuous infusion of 5-fluorouracil as a first-line treatment for metastatic gastric cancer: treatment outcomes and analysis of prognostic factors. J Cancer Res Clin Oncol 141: 109-118.