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ORIGINAL ARTICLE

Prdx2 Upregulation Promotes the Growth and Survival of Gastric Cancer Cells

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Abstract

Peroxiredoxins (Prdxs) play important roles in cell proliferation, differentiation, and the mediation of intracellular signalling pathways. Prdx2 is an important member of the peroxiredoxin family and is upregulated in many cancers. Until now, the biological functions of Prdx2 in gastric cancer have not been completely understood, and the underlying mechanisms remain elusive. The aim of this study was to identify the role of Prdx2 on the growth of gastric cancer cells and the underlying mechanisms. We demonstrated that Prdx2 was highly expressed in gastric cancer tissues and cell lines and that the overexpression of Prdx2 correlated with the progression of gastric cancer. Further, Prdx2 was silenced with a specific, lentiviral vector-mediated shRNA, and this suppressed the proliferation of gastric cancer cells and promoted the apoptosis of gastric cancer cells. Finally, the knockdown of Prdx2 contributed to the attenuated gastric cancer growth in BALB/c nude mice. In conclusion, these findings demonstrate that Prdx2 may participate in the carcinogenesis and development of gastric cancer.

Keywords Peroxiredoxin2 · Gastric cancer · Carcinogenesis

Introduction

Gastric cancer (GC) is one of the most common malignancies that threatens human health, with nearly one million new cases annually worldwide.[1] Although remarkable progress has been made in the diagnosis and treatment of GC, it remains to be one of the most deadly diseases with a poor prognosis, and the molecular mechanism of GC is still elusive.[2, 3] Therefore, it is particularly important to explore the molecular mechanisms and new therapeutic targets for gastric cancer.

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), have been well known for being both beneficial and deleterious.[4, 5] The elevated levels of ROS play important roles in cellular signalling pathways and biological activities.[6] As important scavengers of ROS and main antioxidant enzymes, peroxiredoxins (Prdxs) also play important roles in cell proliferation, differentiation, and the mediation of the intracellular signalling pathways involved in apoptosis.[7] Prdxs are upregulated in many cancers, including cancers of the bladder, brain, and breasts.[8–10] As an important member of peroxiredoxins, Peroxiredoxin2 (Prdx2) is upregulated in many cancers, such as colorectal cancer.[11] Prdx2 induces cancer cell proliferation and prevents ROS-induced cell apoptosis.[12] Prdx2 silencing sensitizes head and neck cancer cells to radiation.[13] The elevated levels of Prdx2 have been associated with resistance to cancer therapy and have been shown to promote tumour cell aggression.[14] Prdx2 promotes the growth of colorectal cancer cells by protecting cells from oxidative stress.[15] Prdx2 silencing inhibits the growth of colorectal cancer cells by inhibiting cancer cell proliferation and by inducing cancer cell apoptosis.[16] The inhibition of PRDX2 significantly sensitizes gastric cancer cells to cisplatin treatment, therapeutic approaches targeting PRDX2 may be useful in the treatment of gastric cancer.[17]Prdx2 expression is upregulated in gastric cancer tissue, but the biological mechanism is not clear.[18]

Until now, the biological functions of Prdx2 in gastric cancer have not been completely understood, and the underlying mechanisms remain elusive. In our study, Prdx2

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expression was analysed in gastric cancer tissues and cells, and the effects of Prdx2 on the growth of gastric cancer cells were investigated to identify its role in tumourigenesis.

Materials and Methods

Tissue Specimens

The resected specimens of 52 cases of gastric adenocarcinoma tissues and their corresponding adjacent normal mucosal tissues were randomly obtained from the Department of Gastrointestinal Surgery at the Cancer Hospital of Chongqing University between January 2015 and December 2017. All patients received no chemotherapy or radiotherapy before surgery. The histological types of the resected specimens were independently confirmed by two pathologists. Tumour staging was defined according to the 2016 International Union against Cancer (UICC) guidelines. The protocol was given approval by the Medical Ethics Review Committee for the Chongqing University Cancer Hospital.

Immunohistochemistry (IHC) Analysis

IHC staining was performed by the use of the immunohistochemical SP-9000 kit according to the manufacturer's instructions (Zhongshan Chemical, Beijing, China). The samples were paraffin embedded and serially sectioned, and the slides were deparaffinized; then, antigen retrieval was performed by using the microwave method. The slides were incubated in 3% hydrogen peroxide for 10 min and were then blocked with goat serum albumin for 15 min at room temperature. Next, the slides were incubated with a rabbit anti-Prdx2 primary antibody (1:100, Abcam, San Francisco, CA, USA) for 3 h at 37 °C with PBS as the negative control and were then washed with PBS repeatedly. The slides were incubated with the secondary antibodies for 20 min at 37 °C and were washed with PBS repeatedly. Next, the slides were incubated with streptavidin-HRP for 20 min at 37 °C, and the immunoreactivity was visualized using a DAB kit according to the manufacturer's instructions (Zhongshan Chemical, Beijing, China). Finally, the slides were counterstained with haematoxylin; then, the slides were dehydrated and mounted. Image acquisition was performed under the transmission light microscope, and the positive rates were counted using ImageJ software. The ratio of positive cancer cells was calculated. The scores of the positive cells were as follows: more than 50% is 3 points, 26–50% is 2 points, 5–25% is 1 point, no expression or < 5% is 0 points;. Then, the positive staining rate of the cancer cells was scored as follows: 3 points for the yellow-brown cells, 2 points for the yellow cells, 2 points for the light yellow cells, 0 points for cells with no colouring. The final score was multiplied by two numbers. The positive criterion was 2–9 points, and the negative criterion was 0–1 points.

Cell Culture and Antibodies

The human normal stomach mucosal cell GES-1 and the gastric cancer cells SGC-7901 and BGC-823 were purchased from the Shanghai Cell Bank at the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA). Then, 10% foetal bovine serum (TBD, Tianjin, China) and 2% penicillin/streptomycin (Beyotime, Jiangsu, China) were added into the medium, and all cells were cultured at 37 °C and 5% CO_2. The following antibodies were purchased: rabbit anti-Prdx2 (Abcam, San Francisco, CA, USA), rabbit anti-cleaved Caspase-3 (Abcam, San Francisco, CA, USA), rabbit anti-Caspase-7 (Abcam, San Francisco, CA, USA), rabbit anti-Caspase-9 (Abcam, San Francisco, CA, USA), rabbit anti-Bad (Abcam, San Francisco, CA, USA), and rabbit anti-GAPDH (Beyotime, Jiangsu, China).

Transfection Analysis Using siRNA

The Prdx2 siRNA vector sequences (forward, 5'-TCC TCT TTA TCA TCG ATG GCA ACT CGA GTT GCC ATC GAT GAT AAA GAG GTT TTT TC-3′; and reverse, 3'-TCG AGA AAA AAC CTC TTT ATC ATC GAT GGC AAC TCG AGT TGC CAT CGA TGA TAA AGA GGA-5′) were synthesized to suppress Prdx2 expression in the human gastric cancer cells SGC-7901 and BGC-823 according to the manufacturer's instructions (Genechem, Shanghai, China). SGC-7901 and BGC-823 cells were seeded into 6-well plates at a concentration of 0.5 X 10^5 cells per well on the day before transfection. Prdx2-siRNA-LV was transfected into SGC-7901 and BGC-823 cells at multiplicities of infection (MOIs) of 20 and 40 by using polybrene (10 μg/ml), respectively. The negative control viral victors were transfected into SGC-7901 and BGC-823 cells at same MOIs, respectively. The medium was replaced after incubation for 12 h. The expression of Prdx2 was detected to identify the effect of RNA interference after 72 h of incubation. Each experiment was repeated three times.

Quantitative RT-PCR Analysis

vThe total RNA from the cell lines GES-1, SGC-7901 and BGC-823 was isolated using the TRIzol Reagent Kit

(TaKaRa, Dalian, China) according to the manufacturer's instructions. Then, the total RNA was reverse transcribed using the PrimeScript RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. Quantitative PCR was carried out with the CFX96™ Real-Time System (Bio-Rad, USA) by using the SYBR Premix Ex Taq II Kit (TaKaRa, Dalian, China) as recommended by the manufacturer. The following primers were used: Prdx2 (forward 5'-CAC CTG GCT TGG ATC AAC ACC-3′ and reverse 5'-CAG CAC GCC GTA ATC CTC AG-3′), GAPDH was used as an internal control (forward 5'-ACC ACA GTC CAT GCC ATC CAC-3 and reverse 5'-TCC ACC ACC CTG TTG CTG TA-3′). The $2^{-(\Delta Ct\ sample-\Delta Ct\ control)}$) method was used to calculate the relative mRNA expression levels. Each experiment was repeated three times.

Western Blotting Analysis

The total protein contents from the cell lines GES-1, SGC-7901 and BGC-823 were isolated using the Total Protein Extraction Kit (KeyGEN, Nanjing, China) as recommended by the manufacturer. The protein concentrations were measured using the BCA Protein Assay Kit (Beyotime, Jiangsu, China). The proteins (50 μg) were loaded onto 6% to 15% SDS-PAGE gels (KeyGEN, Nanjing, China). The proteins were transferred onto PVDF membranes (Millipore, Bedford, MA, USA) after electrophoresis. The membranes were blocked with 5% BSA for 1 h and were then probed with the following primary antibodies at 4 °C overnight: anti-Prdx2 (1:1000), rabbit anti-cleaved caspase-3 (1:1000), rabbit anti-caspase-7 (1:1000), rabbit anti-caspase-9 (1:1000), rabbit anti-Bad (1:1000), and anti-GAPDH (1:3000). The membranes were probed with the secondary antibody (1:4000) at 37 °C for 1 h. The membranes were visualized using the Enhanced Chemiluminescence Kit (KeyGEN, Nanjing, China). The results were measured using Quantity One 4.6.2 software. The grey values of the target band and the internal reference protein were detected, and the relative value of the target protein was determined by dividing the grey value of the target protein by the grey value of the reference protein. Each experiment was repeated three times.

Cell Proliferation Assay and Flow Cytometry Analysis

The resuspended cells SGC-7901 and BGC-823 were seeded into 96-well plates in triplicate at densities o 3 X 10^3 cells per well after the indicated treatments. The cell proliferation was detected at 0, 1, 2, 3, 4, 5, and 6 days using the MTT assay (Sigma–Aldrich, USA) as recommended by the manufacturer. The apoptotic activities were detected by Annexin V-PE and 7-AAD (7-amino-actinomycin D) Double Staining Apoptosis Detection kit (KeyGEN, Nanjing, China) as recommended by the manufacturer. The cells were characterized with fluorescence-activated cell sorting (FACS) analysis with CellQuest software version 3.3.

Animal Studies

Thirty four-week-old BALB/c nude mice were divided into six groups and were bred in a specific, pathogen-free unit of the Laboratory Animal Center of Chongqing University. SGC-7901 and BGC-823 cells (3 X 106) that were transfected with Prdx2 siRNA, the negative control virus, and the blank control were suspended in 100 μl of serum-free medium. The resuspended cells were implanted subcutaneously into the right flanks of the nude mice. The tumour diameters were measured every week. The tumours were harvested after 8 weeks, and the procedures strictly complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The tumour volumes were measured using the following formula: $v\ (mm3) = 4/3\pi \times (length \times width2)$. The protocol was given approval by the Medical Ethics Review Committee for the Chongqing University Cancer Hospital.

Statistical Analysis

All data were analysed by SPSS 17.0 (SPSS, Inc., Chicago, USA). The quantitative data between the two groups were analysed using t-test, and the enumeration data were analysed using the chi-squared test. $P < 0.05$ was considered statistically significant.

Results

Prdx2 Is Highly Expressed in Gastric Cancer Tissues and Cell Lines

The tissue specimens in 52 cases of gastric cancer were detected by IHC for Prdx2 expression. Prdx2 was mainly expressed in the cytoplasm and nucleus of gastric cancer cells. The positive staining of Prdx2 in the gastric cancer tissues was 55.77% (29/52), but the positive staining of Prdx2 in the normal stomach mucosal tissues was only 34.62% (18/52), and the difference was statistically significant ($p < 0.05$, Fig. 1a). Furthermore, Prdx2 was detected by quantitative real-time PCR and western blotting at the RNA and protein levels, respectively, in the human gastric cancer cell lines SGC-7901 and BGC-823 and in the normal stomach mucosal cell line GES-1. The results show that Prdx2 was highly expressed at both the RNA

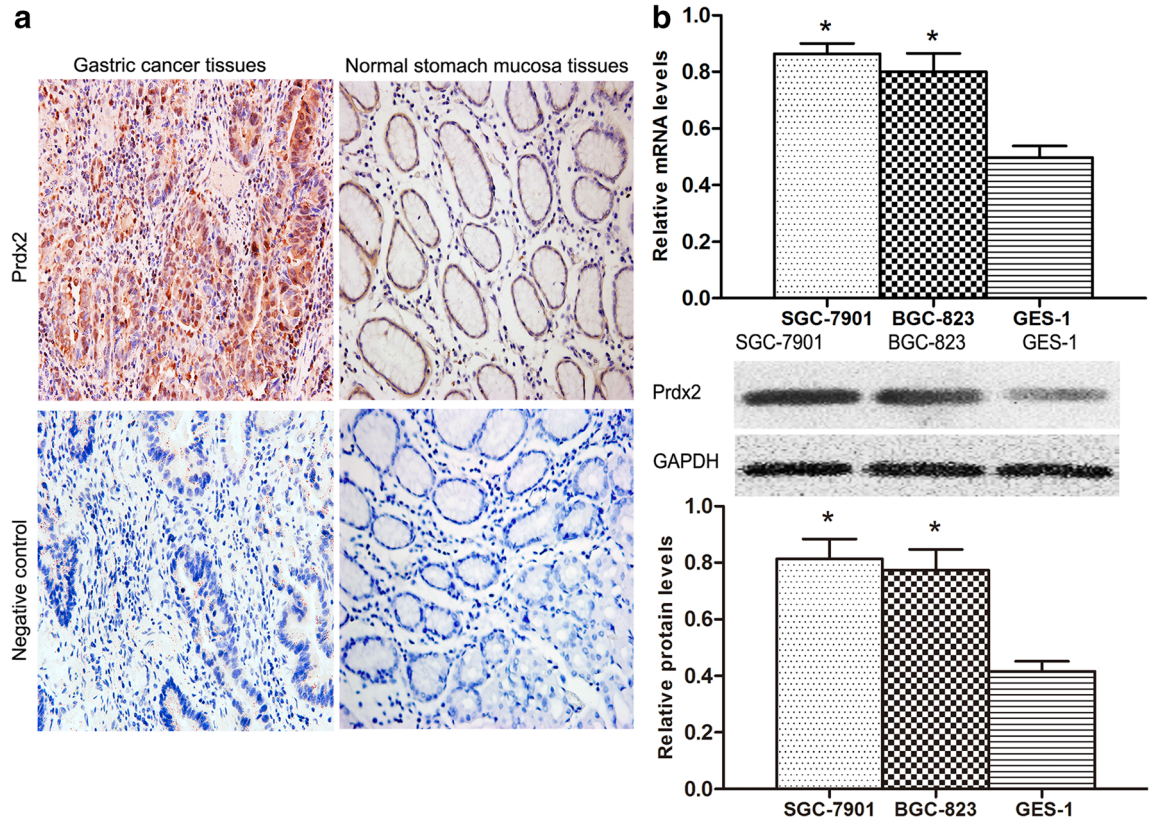

Fig. 1 Prdx2 expression in human gastric tissues and cell lines. a. The IHC staining of Prdx2 in gastric cancer and normal stomach mucosal tissues. The strong staining of Prdx2 is observed in the gastric cancer tissues, and the weak staining of Prdx2 is observed in the normal stomach mucosal tissues (400X). b. The expressions of Prdx2 in human gastric cancer cells and normal stomach mucosal cells were determined by qPCR and western blotting analyses. Prdx2 is highly expressed at both the mRNA and protein levels in the gastric cancer cell lines SGC-7901 and BGC-823 compared with that in the normal stomach mucosal cell line GES-1 (*$p < 0.05$ vs. GES-1)

and protein levels in gastric cancer cells (SGC-7901 and BGC-823) compared with that in GES-1 cells ($p < 0.05$, Fig. 1b). We hypothesize that Prdx2 may play an important role in the progression of gastric cancer.

Over-Expression of Prdx2 Is Correlated with the Progression of Gastric Cancer

To investigate the clinical significance of Prdx2 expression in gastric cancer, the association between Prdx2 expression and the clinicopathologic parameters of 52 cases of gastric cancer patients was analysed. The various clinicopathologic features are shown in Table 1. We observed that Prdx2 expression was not significantly different between the following parameters: age, gender, tumour size, and tumour differentiation ($p > 0.05$). However, Prdx2 expression was significantly associated with TNM stage and tumour metastasis, and Prdx2 was more highly expressed in gastric cancer tissues at TNM stages IV and in gastric cancer tissues with lymphatic node metastasis than its expression in gastric cancer tissues at TNM stages I-III or in gastric cancer tissues without lymphatic node

Table 1 Clinical correlation of Prdx2 expression in gastric cancer

Paremeters	Total	Prdx2		*P* value
		positive	negative	
Age(Y)				
<60	35	17	18	0.134
≥60	17	12	5	
Gender				
Male	28	16	12	0.829
Female	24	13	11	
Tumor size				
<3 cm	18	10	8	0.982
≥3 cm	34	19	15	
Differentiation				
Well and moderately	33	16	17	0.163
Poorly and undifferentiated	19	13	6	
TNM stage				
I + II + III	38	18	20	**0.044**
IV	14	11	3	
Lymph node metastasis				
No	12	3	9	**0.014**
Yes	40	26	14	

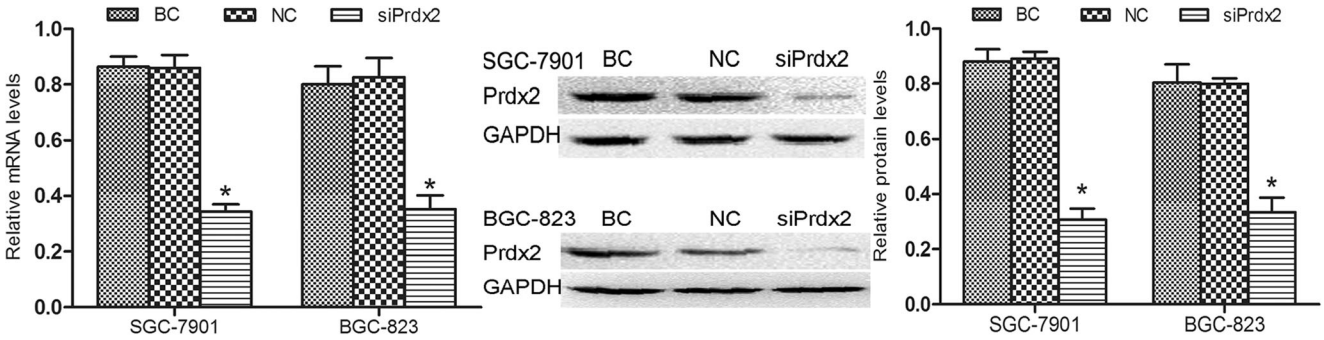

Fig. 2 Effects of Prdx2 siRNA on Prdx2 expression in gastric cancer cells. a. Prdx2 mRNA expression in gastric cancer cells SGC-7901 and BGC-823 transfected with Prdx2 siRNA was significantly reduced compared to that of the blank control and the negative control, as determined by qPCR analysis ($p < 0.05$). b,c. Prdx2 protein expression was suppressed in the gastric cancer cell lines SGC-7901 and BGC-823 transfected with Prdx2 siRNA compared with those of the blank control and the negative control, as shown by western blotting analysis (*$p < 0.05$ vs. controls)

metastasis ($p < 0.05$). Our results indicate that Prdx2 might be an important prognostic factor for gastric cancer.

Prdx2 Expression Is Suppressed by Prdx2 siRNA in Gastric Cancer Cells

Prdx2 is highly expressed in gastric cancer cells, so the lentiviral vector harbouring the Prdx2 siRNA sequence was synthesized to suppress Prdx2 expression in the human gastric cancer cell lines SGC-7901 and BGC-823. The effectiveness of Prdx2 interference was measured by quantitative real-time PCR and western blotting, which showed that both the mRNA and protein levels of Prdx2 in the Prdx2 siRNA group significantly decreased compared to the levels in the negative control group and the blank control group ($P < 0.05$) (Fig. 2). Our results indicate that the transfection of the Prdx2 siRNA vector can effectively suppress Prdx2 expression in gastric cancer cells.

Effects of Prdx2 RNAi on the Viability of Gastric Cancer Cells

To determine the effects of Prdx2 siRNA on cell proliferation, the proliferation of gastric cancer cells was measured using the MTT assay. The viability of the SGC-7901 and BGC-823 cells transfected with the Prdx2 siRNA vector was significantly lower than those of negative control group and the blank control group ($P < 0.05$) (Fig. 3). Our results indicate that Prdx2 siRNA can effectively suppress the proliferation of gastric cancer cells.

Effects of Prdx2 siRNA on the Apoptosis of Gastric Cancer Cells

To elucidate whether Prdx2 siRNA led to growth inhibition due to increased apoptosis, the amount of apoptotic cells was measured using the flow cytometry. The apoptosis ratios in SGC-7901 and BGC-823 cells transfected with the Prdx2 siRNA vector were increased compared to those of the negative control group and the blank control group ($P < 0.05$) (Fig. 4). Our results indicate that Prdx2 siRNA can effectively promote the apoptosis of gastric cancer cells.

Effects of Prdx2 siRNA on pro-Apoptotic Pathways in Gastric Cancer Cells

To elucidate the molecular mechanisms of Prdx2 siRNA in gastric cancer, the effects of Prdx2 siRNA on the pro-

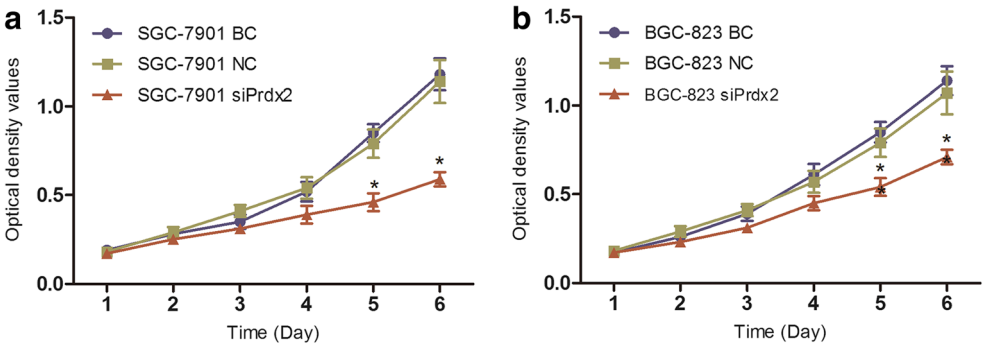

Fig. 3 Effects of Prdx2 siRNA on the proliferation of gastric cancer cells. a,b. Cell proliferation was markedly reduced in the gastric cancer cell lines SGC-7901 and BGC-823 transfected with Prdx2 siRNA than those of the blank control and the negative control, as determined by the MTT assay (*$p < 0.05$ vs. controls)

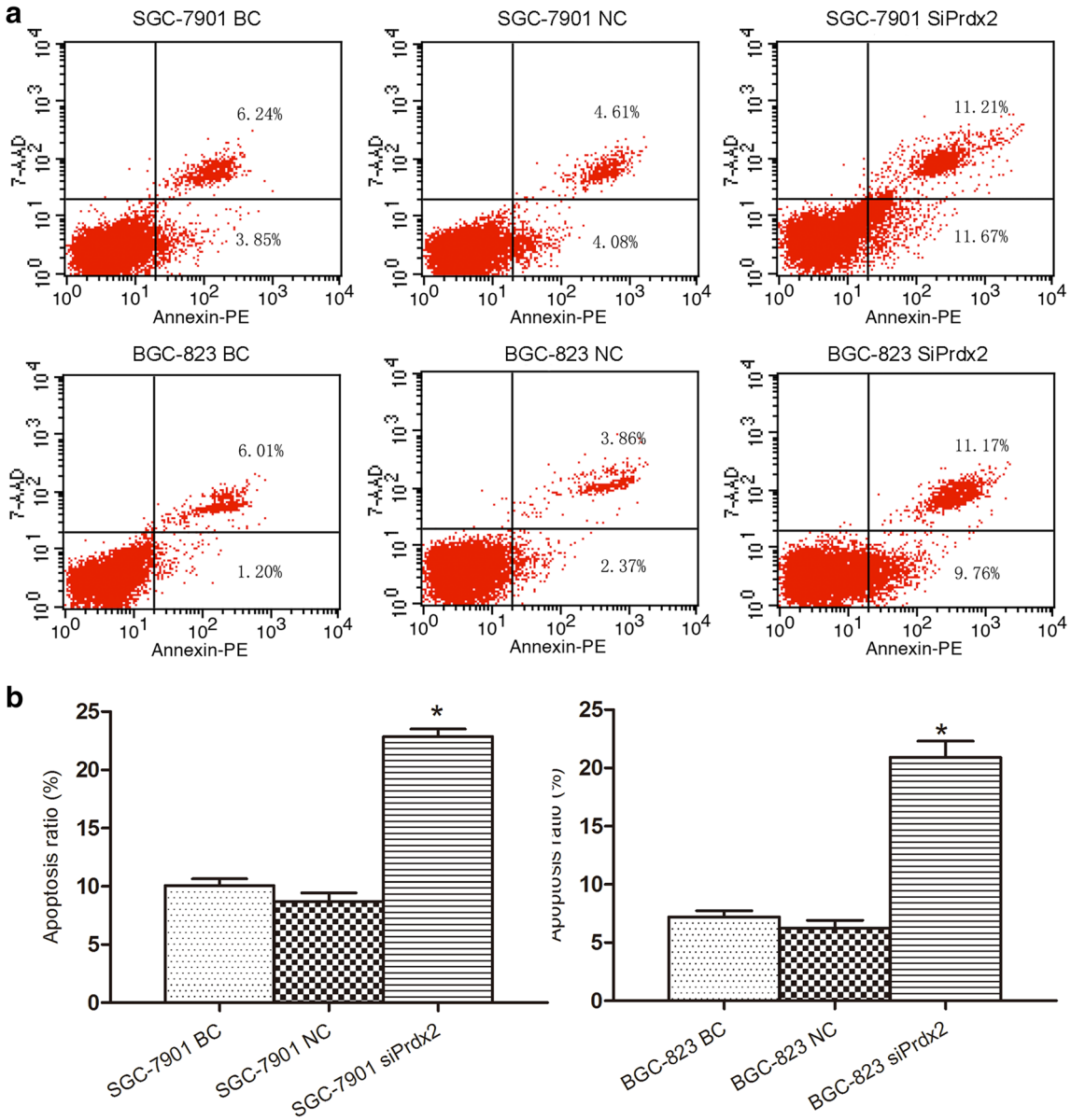

Fig. 4 Effects of Prdx2 siRNA on the apoptosis of gastric cancer cells. a,b. The cell apoptosis ratio was markedly increased in the gastric cancer cell lines SGC-7901 and BGC-823 transfected with Prdx2 siRNA than those of the blank control and the negative control, as determined by the flow cytometry (*$p < 0.05$ vs. controls)

apoptotic pathways were detected in gastric cancer cells. The protein expression levels of the pro-apoptotic markers, such as cleaved caspase-3, caspase-7, caspase-9, and Bad were detected by western blotting. The protein expression levels of cleaved caspase-3, caspase-7, caspase-9, and Bad in SGC-7901 and BGC-823 cells transfected with the Prdx2 siRNA vector were significantly higher than those of the negative control group and the blank control group ($p < 0.05$) (Fig. 5). Our results indicated that Prdx2 siRNA can inhibit the growth of gastric cancer cells by upregulating the apoptosis-related genes in vitro.

Silencing of Prdx2 Inhibited Tumour Growth In Vivo

To elucidate whether Prdx2 siRNA influenced the growth of tumours in vivo, the xenograft models of gastric cancer cells were established by inoculating nude mice with SGC-7901 and BGC-823 cells that were transfected with the Prdx2 siRNA vector, the negative control vector and the blank control. The tumours were harvested after 8 weeks, and the average tumour volumes in the SGC-7901 and BGC-823 cells that were transfected with the Prdx2 siRNA vector were significantly smaller than those in the negative control group and the blank control group ($p < 0.05$) (Fig. 6). The average tumour

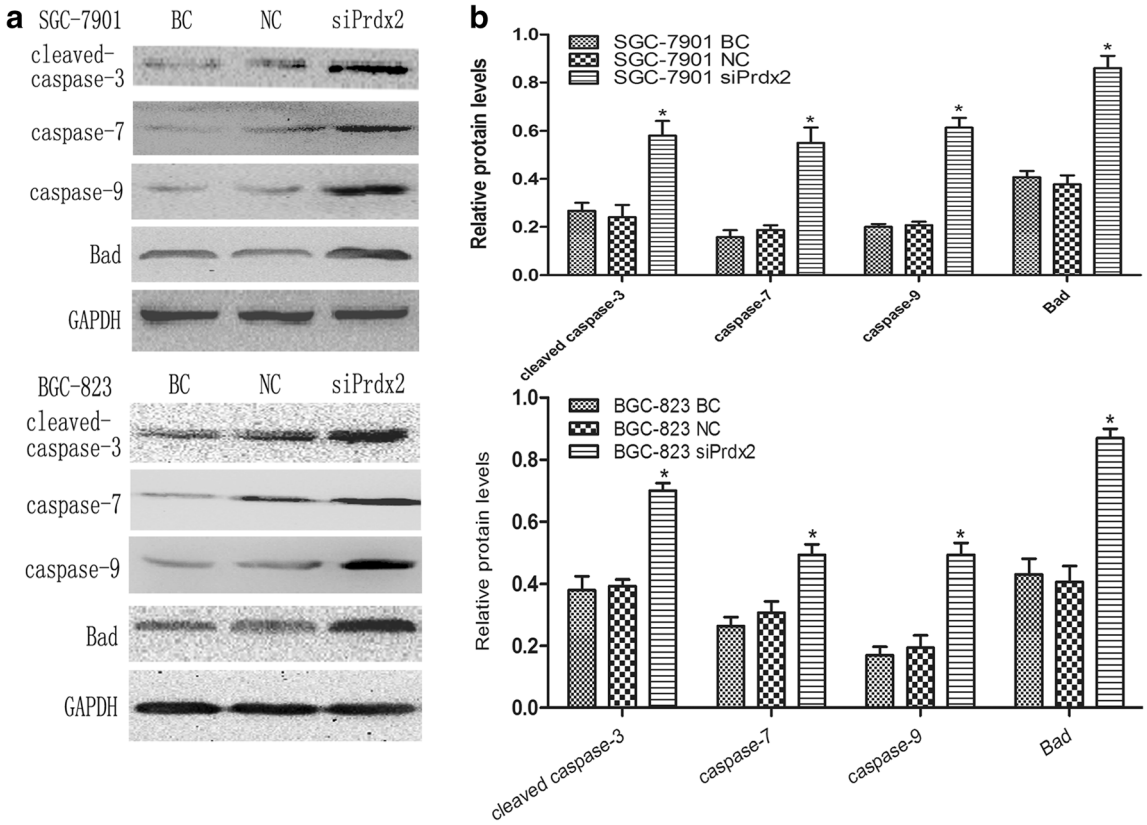

Fig. 5 Effects of Prdx2 siRNA on the protein expression levels of the pro-apoptotic markers in gastric cancer cells. a,b. The protein expression levels of cleaved caspase-3, caspase-7, caspase-9, and Bad in the gastric cancer cell lines SGC-7901 and BGC-823 transfected with Prdx2 siRNA were significantly higher than those of the blank control and the negative control, as determined by western blotting analysis (*$p < 0.05$ vs. controls)

weights in the SGC-7901 and BGC-823 cells that were transfected with the Prdx2 siRNA vector were significantly lighter than those in the negative control group and the blank control group ($p < 0.05$) (Fig. 6). These results were consistent with the results of the MTT assay and the flow cytometry assay shown above. We concluded that Prdx2 is closely related to tumour progression in gastric cancer.

Discussion

Prdx2 has been characterized as an important guard of the intracellular redox state, and the functions of Prdx2 are continuously being discovered.[19] It was shown that the down-regulation of Prdx2 by RNAi in cancer cells results in increased cell death, suggesting that Prdx2 protects tumour survival.[12] Many studies have shown that Prdx2 is highly expressed in cancer cells and in tumour tissues.[9, 20, 21] The overexpression of Prdx2 could assist cancer cell survival as Prdx2 functions as an important scavenger of the ROS produced by tumour cells.[12] Prdx2 has been shown to regulate the cellular processes of apoptosis, proliferation, and the activity of multiple signalling pathways.[22, 23] The high expression level of Prdx2 is associated with adverse clinicopathological features in colorectal cancer.[11] Prdx2 silencing could inhibit the growth of colorectal cancer cells by down-regulating the Wnt signalling pathway.[16] However, the function of Prdx2 remains controversial in some studies, wherein Prdx2 seems to suppress tumour progression.[24] To the best of our knowledge, there has only been one report on Prdx2 expression in gastric cancer tissues other than the current study; Prdx2 expression in gastric cancer tissues was reduced in the younger patients compared with that in the older patients by using IHC analysis, and the research was only limited to the poorly differentiated intramucosal gastric cancer tissues.[25] To a large extent, the biological functions of Prdx2 in gastric cancer and the underlying mechanisms remain elusive.

In this study, the Prdx2 expression levels were detected in gastric cancer tissues and the corresponding adjacent normal tissues by IHC. We found that the protein expression of Prdx2 was higher in the gastric cancer tissues than that in the corresponding adjacent normal tissues. Furthermore, both the mRNA and protein levels of Prdx2 were higher in the gastric cancer cells than that in normal cells as was seen by quantitative real-time PCR and western blotting. In addition, the increased expression of Prdx2 was positively correlated with the

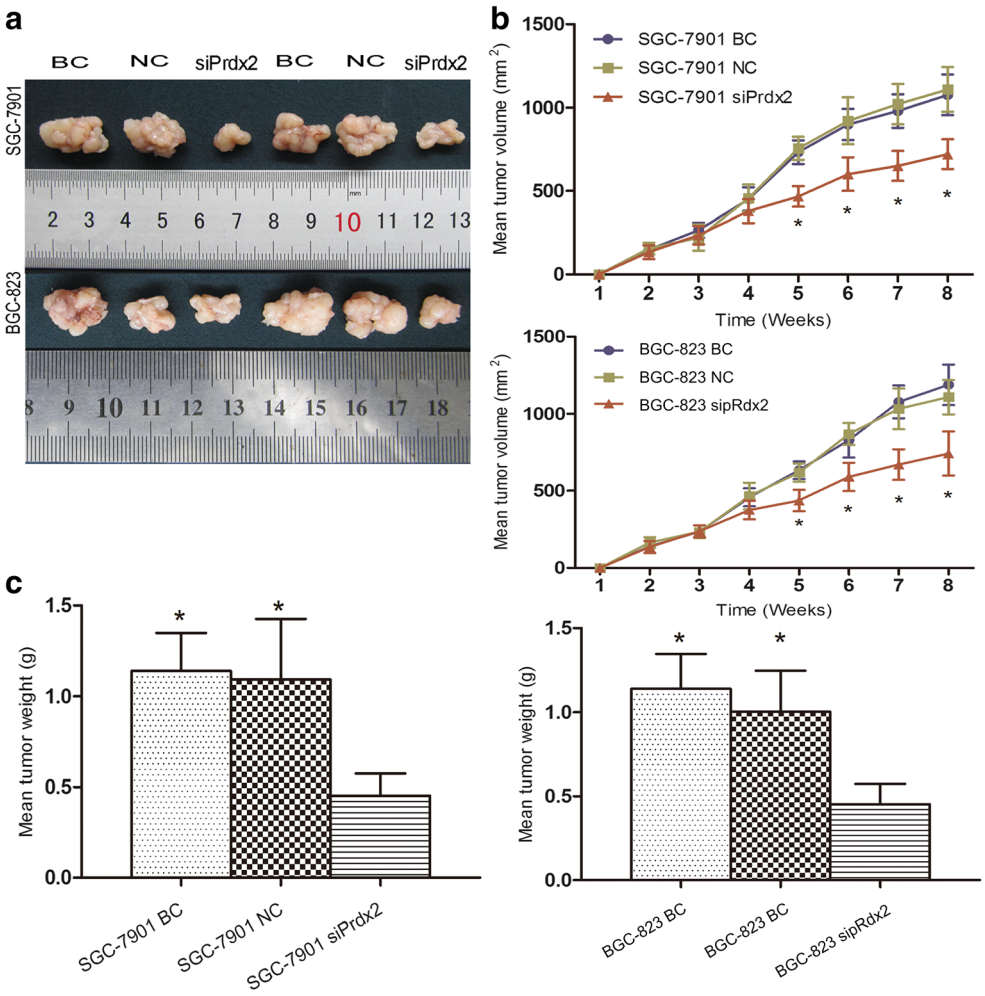

Fig. 6 Effects of Prdx2 siRNA on the growth of xenograft tumours in nude mice. a. The Representative xenograft tumours are shown. b. The tumour volumes were analysed by growth curves (*$p < 0.05$ vs. controls). c. The tumour weights were analysed by the statistical analysis of columns (*$p < 0.05$ vs. controls)

tumour size, TNM stage and lymph node metastasis, but was not correlated with other clinicopathological characteristics, such as age, gender, tumour size, or tumour differentiation; however, we also found that the Prdx2 expression level in the gastric cancer tissues was slightly less than that of the corresponding adjacent normal tissues of the young patients. These results indicate that the upregulated expression in gastric cancer may participate in the carcinogenesis and development of gastric cancer.

To further investigate the function of Prdx2 in gastric cancer cells, the lentiviral vector harbouring the Prdx2 siRNA sequence was used to silence Prdx2 expression in gastric cancer cells. The results show that both the mRNA and protein levels of Prdx2 were significantly decreased after the transfection of the lentiviral vector harbouring the Prdx2 siRNA sequence in gastric cancer cells. Previous studies have indicated that Prdx2 could increase cell proliferation and decrease cell apoptosis in colorectal cancer.[16] To elucidate the effects of Prdx2 siRNA on cell proliferation and apoptosis in gastric cancer cells, we detected the effects of Prdx2 siRNA on the viability and apoptosis of gastric cancer cells. Our results indicate that Prdx2 siRNA could effectively suppress the viability of gastric cancer cells and could increase the apoptosis ratios of gastric cancer cells.

Cancer cell apoptosis is regulated by multiple signal pathways, and Prdx2 has been shown to regulate the cellular processes of apoptosis via multiple signal pathways.[26, 27] The pro-apoptotic proteins have important roles in the cellular processes of apoptosis. There are many pro-apoptotic genes, such as caspase-7, caspase-9, activated-caspase-3, and Bad involved in the signalling cascade of apoptosis.[28] As the executor of apoptosis, caspase-3 can be activated by caspase-9. Cleaved caspase-3, or activated caspase-3, is very important in the cellular processes of apoptosis.[29] To elucidate the effect of Prdx2 siRNA on the expression levels of the pro-apoptotic proteins in gastric cancer cells, we detected the protein levels of cleaved caspase-3, caspase-7, caspase-9, and Bad by western blotting. Our results show that the protein levels of cleaved caspase-3, caspase-7, caspase-9, and Bad increased after the transfection of the lentiviral vector harbouring the Prdx2 siRNA sequence in gastric cancer cells. Finally, to elucidate the effects of Prdx2 siRNA on the growth of tumours in vivo, the subcutaneous transplanted tumour model was established. Our results show that the tumour

growth was much slower after Prdx2 siRNA transfection in gastric cancer cells, which was in accordance with our in vitro results.

In summary, we found that the protein expression levels of Prdx2 were higher in the gastric cancer tissues and that this expression correlated with tumour progression. Prdx2 siRNA could increase cell apoptosis and decrease cell proliferation in gastric cancer cells, and the growth reduction of the subcutaneous tumour after Prdx2 siRNA transfection also suggests that Prdx2 may participate in the carcinogenesis and development of gastric cancer. However, more research is still needed to explore the molecular mechanisms of Prdx2 in the carcinogenesis and progression of gastric cancer.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

References

1. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014 [J]. CA Cancer J Clin 64(1):9–29
2. Hu B, El Hajj N, Sittler S et al (2012) Gastric cancer: classification, histology and application of molecular pathology [J]. J Gastrointest Oncol 3(3):251–261
3. Gotoda T, Kusano C, Moriyasu F (2014) Future perspective of gastric cancer endotherapy [J]. Ann Transl Med 2(3):25
4. Roessner A, Kuester D, Malfertheiner P et al (2008) Oxidative stress in ulcerative colitis-associated carcinogenesis [J]. Pathol Res Pract 204(7):511–524
5. Devasagayam TP, Tilak JC, Boloor KK et al (2004) Free radicals and antioxidants in human health: current status and future prospects [J]. J Assoc Physicians India 52:794–804
6. Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress [J]. Curr Biol 24(10):R453–r462
7. Poole LB (2011) Overview of Peroxiredoxins in oxidant defense and redox regulation [J]. Chapter (Unit7.9)
8. Memon AA, Chang JW, Oh BR, Yoo YJ (2005) Identification of differentially expressed proteins during human urinary bladder cancer progression [J]. Cancer Detect Prev 29(3):249–255
9. Jarvela S, Rantala I, Rodriguez A et al (2010) Specific expression profile and prognostic significance of peroxiredoxins in grade II-IV astrocytic brain tumors [J]. BMC Cancer 10:104
10. Karihtala P, Mantyniemi A, Kang SW et al (2003) Peroxiredoxins in breast carcinoma [J]. Clin Cancer Res 9(9):3418–3424
11. Wu XY, Fu ZX, Wang XH (2010) Peroxiredoxins in colorectal neoplasms [J]. Histol Histopathol 25(10):1297–1303
12. Lee KW, Lee DJ, Lee JY, Kang DH, Kwon J, Kang SW (2011) Peroxiredoxin II restrains DNA damage-induced death in cancer cells by positively regulating JNK-dependent DNA repair [J]. J Biol Chem 286(10):8394–8404
13. Park SH, Chung YM, Lee YS, Kim HJ, Kim JS, Chae HZ, Yoo YD (2000) Antisense of human peroxiredoxin II enhances radiation-induced cell death [J]. Clin Cancer Res 6(12):4915–4920
14. Parmigiani RB, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P, Marks PA (2008) HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation [J]. Proc Natl Acad Sci U S A 105(28):9633–9638
15. Lu W, Fu Z, Wang H, Feng J, Wei J, Guo J (2014) Peroxiredoxin 2 is upregulated in colorectal cancer and contributes to colorectal cancer cells' survival by protecting cells from oxidative stress [J]. Mol Cell Biochem 387(1–2):261–270
16. Lu W, Fu Z, Wang H, Feng J, Wei J, Guo J (2014) Peroxiredoxin 2 knockdown by RNA interference inhibits the growth of colorectal cancer cells by downregulating Wnt/beta-catenin signaling [J]. Cancer Lett 343(2):190–199
17. Wang S, Chen Z, Zhu S et al (2019) PRDX2 protects against oxidative stress induced by *H. pylori* and promotes resistance to cisplatin in gastric cancer [J]. Redox Biol 28:101319
18. Yang SF, Ma R, Pan LL, Cao J, Sheng N (2018) RKIP and peroxiredoxin 2 expression predicts the proliferative potential of gastric cancer stem cells [J]. Oncol Lett 15(3):3173–3177
19. Hanschmann EM, Godoy Jé R, Berndt C et al (2013) Thioredoxins, Glutaredoxins, and Peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling [J]. Antioxid Redox Signal 19(13):1539–1605
20. Lomnytska MI, Becker S, Bodin I, Olsson A, Hellman K, Hellström AC, Mints M, Hellman U, Auer G, Andersson S (2011) Differential expression of ANXA6, HSP27, PRDX2, NCF2, and TPM4 during uterine cervix carcinogenesis: diagnostic and prognostic value [J]. Br J Cancer 104(1):110–119
21. Yue HY, Cao J, Cui JF, Dai Z, Su JJ, Duan XX, Yang C, Yue HF, Li Y, Liu YK (2007) The expression of peroxiredoxin II in hepatocellular carcinoma and its significance [J]. Zhonghua Gan Zang Bing Za Zhi 15(5):366–369
22. Rhee SG, Woo HA (2011) Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H(2)O(2), and protein chaperones [J]. Antioxid Redox Signal 15(3):781–794
23. Oláhová M, Veal EA (2015) A peroxiredoxin, PRDX-2, is required for insulin secretion and insulin/IIS-dependent regulation of stress resistance and longevity [J]. Aging Cell 14(4):558–568
24. Neumann CA, Fang Q (2007) Are peroxiredoxins tumor suppressors? [J]. Curr Opin Pharmacol 7(4):375–380
25. Hirahashi M, Koga Y, Kumagai R, Aishima S, Taguchi K, Oda Y (2014) Induced nitric oxide synthetase and peroxiredoxin expression in intramucosal poorly differentiated gastric cancer of young patients [J]. Pathol Int 64(4):155–163
26. Hu X, Weng Z, Chu CT, Zhang L, Cao G, Gao Y, Signore A, Zhu J, Hastings T, Greenamyre JT, Chen J (2011) Peroxiredoxin-2 protects against 6-hydroxydopamine-induced dopaminergic neurodegeneration via attenuation of the ASK1 signaling cascade [J]. J Neurosci 31(1):247–261
27. Ishii T, Warabi E, Yanagawa T (2012) Novel roles of peroxiredoxins in inflammation, cancer and innate immunity [J]. J Clin Biochem Nutr 50(2):91–105
28. Yang C, Fu Z (2014) Liposomal delivery and polyethylene glycol-liposomal oxaliplatin for the treatment of colorectal cancer (review) [J]. Biomed Rep 2(3):335–339
29. Walters J, Pop C, Scott FL et al (2009) A constitutively active and uninhibitable caspase-3 zymogen efficiently induces apoptosis [J]. Biochem J 424(Pt 3):335–345