PARP6 acts as an oncogene and positively regulates Survivin in gastric cancer

Xiying Sun1*, Yun Zhang1*, Mingliang Chu1-2, Lixin Wang1, Hede Shen1, Zhuxue Zhang1, Jianjun Hu1, Wei Yi1, Wenxiu Yang3, Xiaobo Ma4

1Department of Pathology, The Affiliated People’s Hospital of Guizhou Medical University, Guiyang, China; 2Central Laboratory, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, China; 3Department of Pathology, The Affiliated Hospital of Guizhou Medical University, Guiyang, China; 4Department of Medicine, George Washington University School of Medicine and Health Sciences, Washington, DC, USA. *Equal contributors.

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Abstract: Poly (ADP-ribose) polymerases 6 (PARP6) is a novel member of the PARP family. Previous studies focused mostly on the role of PARP6 in colorectal cancer; however, the role of PARP6 in gastric cancer is currently unclear. In the present study, we found a high-level expression of PARP6 in gastric cancer cells and PARP6 promoted cell proliferation, migration and invasion. Moreover, we found a positive correlation exists between PARP6 and Survivin, which contributes to tumor ongoing survival. In sum, our data suggest that PARP6 may contribute to gastric cancer progression by activating the Survivin pathway.

Keywords: PARP6, Survivin, gastric cancer

Introduction

Gastric cancer is widely diagnosed as one of the leading causes of cancer death [1]. The most common cause is infection by the bacterium Helicobacter pylori, which accounts for more than 60% of cases [2]. Smoking, dietary factors and obesity are other risk factors [3, 4]. However, the pathogenesis has not been fully elucidated yet.

Poly (ADP-ribose) polymerases (PARPs) are multifunctional protein translation enzymes that exist in most eukaryotic cells [5]. PARPs have been reported to be activated by damaging DNA fragments and were thought to have played an important role in DNA damage repair and apoptosis [6-8]. The PARP family has multiple members, including PARP1-4, PARP5α, PARP5β and PARP6-16 [9]. PARP1 was thought to act as an oncogenic gene in breast cancer [10], prostate cancer [11] and pancreatic cancer [12]. However, PARP6, a new member of the PARP family, has not been elucidated in cancer progression. Tuncel et al. [13] reported that PARP6 was mainly distributed in well-differentiated adenocarcinoma cells, and high expression of PARP6 could inhibit the proliferation of HeLa cells in cervical cancer cells. Honda et al. [14] reported that PARP6 was a poor prognostic molecular marker in hepatoblastoma patients. Recent studies revealed that PARP6 could inhibit the proliferation of colorectal adenocarcinoma cells, and was negatively correlated with Survivin in the tissues of colorectal adenocarcinoma [13, 15]. But other researchers showed the opposite result that PARP6 was a carcinogenic factor and there was a positive correlation between PARP6 and Survivin [16]. Survivin is a new member of the inhibitor of apoptosis (IAP) protein family [17]. It has been proven to be closely related to the occurrence, development and prognosis of various tumors [18], including gastric cancer [19, 20].

To date, the relationship between PARP6 and Survivin, and the role of PARP6 in gastric cancer is not yet known. That is the subject of the present study.

Materials and methods

Tissue samples

65 postoperative paraffin samples of gastric cancer were obtained from Guizhou Provincial
People’s Hospital during September 2016 - September 2017. 10 samples of normal gastric mucosa were examined as controls. All samples were obtained after approval by the Ethics Committees of Guizhou Provincial People’s Hospital. All cases were confirmed by surgery and routine pathology, and had no preoperative chemotherapy or radiotherapy. Patient characteristics are summarized in Table 1.

Cell lines

Human gastric cancer cells (BGC823 cells) and human normal gastric mucosal epithelial cells (GES-1 cells) were cultured in RPMI 1640 (Hyclone, USA) with 10% fetal bovine serum (FBS) and cultured at 37°C in 5% CO₂.

Plasmids, lentiviral production and infection

A whole-length PARP6 cDNA was subcloned into a vector (PLenti CMV Puro Empty) to get an overexpression vector (PLenti CMV-PARP6). A 21mer sequence (5-GGCGATGCCAACATTAATA-CT-3) was designed to generate the lentiviral vector of PARP6 short hairpin RNA (shRNA). Lentivirus production and transduction were done according to a previous method [21].

Immunohistochemistry

Biopsy specimens were fixed in 10% formalin, embedded in paraffin and stained with Hema3-Eosin. Immunostaining was performed using the Leica Bond-Max automation system (Leica Biosystems, Bannockburn, IL). Primary antibody Survivin (1:100; ZA-0530, ZSGB-BIO, Beijing, China), or PARP6 (1:300, NBP1-8733, Novus Biologicals, LLC, Littleton, CO, USA) were used. The expression of PARP6 and Survivin was semi-quantitatively evaluated by using immunohistochemical scoring model: -, the majority of the cells were weak or negative; +, 1%-20% of the tumor cells showed moderate immunoreactivity; ++, over 20% of the tumor cells showed moderate or intense immunoreactivity. The high expression of PARP6 and Survivin was graded as ++, and low expression was graded as - or +. The assessment was made by a single senior pathologist (Y.Z).

Western blotting

Protein samples (Control, PARP6 knockdown, and PARP6 overexpression BGC823 cells) were separated on a 10% SDS gel. Proteins were transferred to PVDF membrane (Millipore, Billerica, Massachusetts). After being blocked for 2h at room temperature, the membranes were incubated with primary antibody PARP6 (1:1500, NBP1-52950, Novus Biologicals, LLC, Littleton, CO, USA), Survivin (1:1000; ZA-0530, ZSGB-BIO, Beijing, China), and GADPH (1:20000, 10491-1-AP, ProteintechTM) overnight at 4°C. The proteins were then incubated with the corresponding secondary antibody for 1 h at room temperature. After a thorough wash of the membrane the band was illuminated using Clarity Western ECL-Substrate (Bio-Rad Laboratories, Inc.: Hercules, CA, USA.).

Wound healing assay

For wound healing scratch assay, control cells, overexpressed PARP6 BGC823 cells and knockdown PARP6 BGC823 cells were seeded in 6-well plates. Scratch wounds were made in confluent cell monolayers using a pipette tip. Monolayers were washed twice with PBS and the cells were cultured in serum-free media. The same area of the gap was imaged by using a microscope equipped with a digital camera at 0, 24 and 48 h after scratching was performed.

CCK-8 assay

Cells were seeded in 96-well plates at an initial concentration of 1×10⁴ cells per well. The cells were incubated with 100 μl of complete medium and cultured for 24 h, 48 h and 72 h in a 5% CO₂ incubator at 37°C. Then 100 μl serum free RPMI 1640 containing 10% CCK8 (CCK-8; Dojindo, Kumamoto, Japan) reagent was added in each well, and the cells were cultured for 2 h at 37°C. Finally, the absorbance (D value) of each well at 450 nm was detected by enzyme-linked immunosorbent assay to reflect the cell proliferation ability.

Cell migration and invasion

The top chambers (Corning Incorporated, Corning, NY, USA) were seeded with cells in serum-free media and 20% FBS was used as the chemottractant in the bottom chamber. Cells were allowed to migrate for 24 h at 37°C. Then, migrated cells were fixed with 4% paraformaldehyde and stained using 0.1% Crystal violet solution. Invasion assay cells were seeded in
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200 μl serum-free media into the upper wells which were previously coated with Matrigel basement extract, and 500 μl of media into the bottom wells. After 24 h of CO₂ incubation at 37°C, the invasive cells on the bottom surface of the membrane were fixed and stained using 0.1% Crystal violet solution. For further quantification of the migration and invasion cells, five random regions of the filter were counted under microscope. Three filters were used and the experiments were carried out in triplicate.

Statistical analysis

Data were analyzed using the SPSS 13.0 softwares (SPSS Inc., Chicago, Ill., USA). One-way ANOVA and chi-square test were used to compare the data between the groups. Correlations between PARP6 and Survivin expression were determined by Spearman’s correlation coefficient. P<0.05 was considered significant.

Results

Expression of PARP6 and Survivin

PARP6 and Survivin expression was not detectable in normal gastric mucosa (0/10), while most gastric cancer samples had high-level protein expression of PARP6 (56/65) and Survivin (60/65) (Figure 1, Table 1). Furthermore, we detected the expression of PARP6 and Survivin in normal gastric mucosa cell lines (GES-1) and gastric cancer cell lines (BGC823). Results showed that the
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Results showed that the migration rate of knockdown of PARP6 cells was significantly lower than control cells at 24 and 48 hours. Meanwhile, overexpression of PARP6 cells was significantly higher than control cells (Figure 3).

**PARP6 affects BGC823 cell Proliferation**

Cell proliferation was measured by recording changes in absorbance (optical density, OD) at 450 nm wavelength. Results showed that knockdown of PARP6 significantly inhibited BGC823 cell proliferation at 24, 48 and 72 hours (Figure 4). While overexpression of PARP6 in BGC823 cells resulted in a greater percentage of cell proliferation (Figure 4).

**Effects of PARP6 on BGC823 cell migration and invasion**

Transwell assay showed that PARP6 knockdown decreased cell migration and invasion of BGC823 cells, while overexpression of PARP6 increased cell migration and invasion of BGC823 cells (Figures 5, 6).

**Discussion**

PARP6, a new member of the PARPs family, is rarely to be studied. Previous studies showed conflicting results regarding the role of PARP6 and the correlation with Survivin in colorectal cancer [13, 15, 16]. In this study, we explored them in gastric cancer. Our results showed high level expression of PARP6 and Survivin in gastric cancer samples, and there was not expression of them in normal gastric mucosa (Figure 1). Moreover, we detected the expression of PARP6 and Survivin in cell lines. Results showed high level expression of PARP6 and Survivin in BGC823 cancer cells compared with GES-1 normal gastric mucosa cells (Figure 2). These results suggested that PARP6 maybe act as oncogene and had a very deep relationship to Survivin. So, we firstly analyzed the correlation of them. Immunohistochemical results showed a positive correlation between PARP6 and Survivin in gastric cancer (γ=0.720,
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**Figure 3.** Wound healing of control, shRNA-PARP6 and overexpression-PARP6 cancer cells in vitro. (Magnification, 100×). The experiment was repeated independently three times.

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**Figure 4.** Proliferation of control, shRNA-PARP6 and overexpression-PARP6 cancer cells in vitro. Cell numbers were counted at the following time points: 24, 48, and 72 hours. Cell proliferation was measured using CCK8 assay. The experiment was repeated independently three times. *P<0.05, **P<0.01.

P<0.05) (**Table 2**). Furthermore, we found that the expression of Survivin was synchronized with the expression of PARP6 in knockdown or overexpression PARP6 cancer cells (**Figure 2**). Thus, we concluded that PARP6 and Survivin have a perfect rank correlation, and PARP6 maybe the upstream regulatory factor.

In view of that PARP6 was upregulated in gastric cancer cells, and a significant correlation exists between PARP6 and Survivin, we suspected that PARP6 may be carcinogenic in gastric cancer. To prove this idea, we further assessed the motility, proliferation, migration and invasion of PARP6 overexpression and knockdown gastric cancer cells (**Figures 3-6**). Our results supported the hypothesis that PARP6 plays a carcinogenic role in gastric cancer.

All of these results indicate that PARP6 plays a carcinogenic role in gastric cancer and there was a significant correlation between PARP6 and Survivin. Since Survivin is universally recognized as an oncogene [18-20, 22, 23], and PARP6 may be an upstream regulatory factor of Survivin, we provide new insight that PARP6 may play a carcinogenic role through the Survivin pathway in gastric cancer. Additional studies are needed to clarify this point.

Taken together, our results provide new insights into the regulation of PARP6 and Survivin in gastric cancer. They are promising targets for the development of new strategies for the diagnosis and treatment of gastric cancer.

**Acknowledgements**

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Figure 5. Migration of control, shRNA-PARP6 and overexpression-PARP6 cancer cells in vitro. (Magnification, 200×). The experiment was repeated independently three times. *P<0.05, **P<0.01.

Figure 6. Invasion of control, shRNA-PARP6 and overexpression-PARP6 cancer cells in vitro. (Magnification, 200×). The experiment was repeated independently three times. *P<0.05, **P<0.01.
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Disclosure of conflict of interest

None.

Address correspondence to: Mingliang Chu and Wei Yi, Department of Pathology, The Affiliated People’s Hospital of Guizhou Medical University, 83 Zhongshan Road, Guiyang 550002, China. Tel: +86-851-85936118; Fax: +86-851-85936118; E-mail: chumingliang@foxmail.com (MLC); yiwei6252@sina.com (WY)

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