The expression and clinical significance of PDCD4 and Her-2 in human gastric cancer

Yuelou Yang,1,4 Xiangjun Jiang,2 Dong Li,3 Feiyan Wang,4 Qun Yang5 and Bin Li4

Abstract
To investigate the correlation and clinical significance between programmed cell death factor 4 (PDCD4) and epidermal growth factor receptor 2 (Her-2) expressions and clinicopathological parameters in patients with gastric cancer, a total of 65 cases of gastric cancer and the corresponding normal mucosa with PDCD4 and Her-2 protein expressions were detected by SP immunohistochemical staining, and 50 cases of gastric cancer and the corresponding normal mucosa with PDCD4 and Her-2 protein expression quantities were detected by Western blot, in order to analyze the relationship between the positive expressions of PDCD4 and Her-2 protein and the clinicopathological features of patients with gastric cancer. The results showed that the positive rate of PDCD4 protein expression in gastric cancer tissues was 7.7%, which was significantly lower than that in the corresponding normal tissues, that is, 77.5% (P < 0.05); the positive rate of Her-2 expression was 41.5%, which was significantly higher than that of the corresponding normal tissues, which is 2.5% (P < 0.05). The Western blot test showed that the expression of PDCD4 protein in gastric cancer was 0.3105 ± 0.0073, which was significantly lower than that in the corresponding normal tissues, that is, 0.9428 ± 0.0127 (P < 0.05); the expression level of Her-2 protein in gastric cancer tissues was 0.9428 ± 0.0127, which was significantly higher than that of the corresponding normal mucosa, which is 0.2054 ± 0.0264 (P < 0.05). The positive expressions of PDCD4 (5/65) and Her-2 (27/65) were significantly correlated with the differentiation degrees and TNM stages of gastric cancer (P < 0.05). However, no significant correlation can be observed from Table 2 (P > 0.05), regarding sex, age, tumor size, and lymph node metastasis. Our research claimed that PDCD4 and Her-2 may play an important role in the invasion and metastasis of gastric cancer, which has a negative correlation with biological behaviors of gastric cancer. The low expression of PDCD4 and the high expression of Her-2 in gastric cancer may promote the occurrence and progression of cancer. The PDCD4 and Her-2 test can be used as an index to evaluate the malignant biological behaviors of gastric cancer and prognosis, and provide a theoretical basis for targeted therapy.

Keywords
clinicopathological parameter, gastric cancer, Her-2, immunohistochemistry, PDCD4

Date received: 18 July 2018; accepted: 11 January 2019

1Department of Gastroenterology, Qingdao University, Qingdao, China
2Department of Gastroenterology, Qingdao Municipal Hospital, Affiliated Hospital of Qingdao University, Qingdao, China
3Department of Pathology, Qingdao Huangdao District Central Hospital, Qingdao, China
4Department of Gastroenterology, Qingdao Huangdao District Central Hospital, Qingdao, China
5Department of Maternity, Qingdao Huangdao District Central Hospital, Qingdao, China

Corresponding author:
Xiangjun Jiang, Department of Gastroenterology, Qingdao University, Affiliated Hospital of Qingdao University, Qingdao 266011, China. Email: yrngfan252@163.com
Introduction

Gastric cancer is one of the most common malignant tumors, has the second highest morbidity and mortality in the world, after lung cancer, and features hidden onset, easily of transmission, early misdiagnosis, a high recurrence rate, and so on. In China, the newly diagnosed morbidity of gastric cancer accounts for more than 17% of all malignant tumors, and the death rate accounts for more than 20%. In recent years, although the incidence of gastric cancer has declined in China, the mortality and recurrence rate have remained high. With the development of clinical diagnosis and treatment technology, the long-term overall survival (OS) of early gastric cancer was significantly improved, but the prognosis of patients in progressive stage is still at a low level. Because of lacking molecular markers with high sensitivity and strong specificity and simple and practicable domestic investigation system, most patients with gastric cancer have been in advanced or late stage, greatly affecting the clinical efficacy and quality of life. Surgery-based combined therapy is still the primary choice for the clinical therapy of gastric cancer currently, but the 5-year survival rate of patients after radical treatment was only 20%–50%, while the mean survival of metastatic patients (who cannot be cured radically) was only 3–24 months. Although the current treatment of gastric cancer has some effect, the long-term effect is unsatisfactory. Therefore, to elucidate the molecular mechanism of gastric cancer, determine a reasonable therapeutic target, and to formulate a more comprehensive therapeutic strategy has become a hot issue.

More and more evidence suggested that the incidence of gastric cancer is the result of multiple factors, such as programmed cell death factor 4 (PDCD4) and tumor suppressor gene related to cell circle and apoptosis; many studies have shown that most of the malignant tumors have a phenomenon of decreased expression, and its increased expression can promote tumor cell apoptosis and suppress tumor growth. At the same time, we also found that the high expression of epidermal growth factor receptor 2 (Her-2) gene in gastric cancer provides a target for our treatment of gastric cancer. However, there is only little research about the relationship between PDCD4 and Her-2 genes and gastric cancer. In this study, we aim to further evaluate the correlation and clinical significance between PDCD4 and Her-2 expressions and clinicopathological parameters in patients with gastric cancer.

Materials and methods

Materials

A total of 50 cases tested by the Western blot method with gastric cancer patients who had undergone surgical resection in our hospital from February 2013 to April 2017 were selected as the samples, including 58 males and 32 females, with age ranging from 51 to 76 years (mean = 58.2 ± 1.5 years); fresh gastric cancer tissues and the corresponding normal non-tumor tissues were obtained from the 50 patients (>5 cm away from the edge of the cancer tissue), and then subsequently placed in liquid nitrogen for the analysis.

In total, 65 cases of immunohistochemistry with gastric cancer patients who has surgical resection in our hospital from May 2012 to February 2017 were selected as the samples, including 39 males and 26 females, with age ranging from 52 to 75 years (mean = 57.7 ± 1.6 years); TNM stage is as follows: I + II stage in 40 cases and III + IV stage in 25 cases; 53 cases were moderate to poorly differentiated and 12 cases were well differentiated; the lymph node metastasis details are as follows: 45 cases had metastasis and 20 cases had no metastasis; the diameter of the tumor was less than 5 cm in 41 cases and greater than or equal to 5 cm in 24 cases; all cases had no preoperative neoadjuvant chemotherapy to intervention therapy, after surgery was diagnosed by pathology. Otherwise, 40 cases of peritumoral normal colorectal tissues, with more than 5 cm of gastric cancer and confirmed by pathology, were selected as the control group, including 21 males and 19 females, with age ranging from 51 to 74 years (mean = 57.3 ± 1.9 years).

The tissues and clinical information was obtained under the consent of patients, and the study was approved by the ethics committee of Qingdao Municipal Hospital affiliated to Qingdao University (QDMH 2017045).

Primary reagent

Anti-APS PDCD4 (A2570) and Her-2 (A2071) polyclonal antibodies (Wuhan ABclonal, Co., Ltd), immunohistochemical testing reagents (Beijing Solarbio Technology Co., Ltd), DAB HRP-OPD
(Shanghai Sanger Biotechnology Co., Ltd), Trizol RNA kit (Beijing Protein Innovation Co., Ltd); reverse transcription polymerase chain reaction (RT-PCR) kit (Wuhan Khayal Bio-Technology Co., Ltd), quantitative real-time reverse transcription polymerase chain reaction kit (Shanghai Li Chen Biotechnology Co., Ltd), specific primers (Shanghai Sangon Bio-Technology Co., Ltd), polyvinylidene difluoride (PVDF) membrane (Shanghai Xinfan Biotechnology Co., Ltd), and radio immunoprecipitation assay (RIPA) lysate (Shanghai Tocan Biotechnology Co., Ltd) were used in this study.

**Methods**

**Western blot method.** The tissues were ground and 400 μL of tissue lysate was added to it, crushed at low temperature, and centrifuged with ice, and a small amount of supernatant was added; the standard curve and protein level tested cases was prepared according to the bicinchoninic acid assay (BCA) protein level; protein was prepared by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for 1.5 h; the samples made from electrophoretic separation were transferred from the gel into the PVDF membrane of the solid phase vector; the primary antibodies were incubated overnight at 4°C (1:1000), and the secondary antibodies were incubated at 1°C (1:3000) for 2 h; the enhanced chemiluminescent (ECL) agent was placed on the PVDF membrane for about 5 min to react and finally developed and fixed.

**SP immunohistochemistry.** The expressions of PDCD4 and Her-2 protein in gastric cancer tissues and the corresponding normal non-tumor tissues were detected by SP immunohistochemistry. The procedure was based on the instructions. The positive sections of Her-2 in gastric cancer tissues were used as positive controls for Her-2, and the positive sections of PDCD4 were used as the positive controls for itself, and phosphate-buffered saline (PBS) was used as the negative control instead of the primary antibody.

**Results of evaluation**

PDCD4 is mainly located in the cytoplasm of gastric cancer cells, and a few of them in the nucleus. Her-2 is mainly located in the cytoplasm and cytoplasmic membrane. There were at least 100 positive cells randomly in the 10× high-magnification view; first, marks were assigned to color intensity, that is, 0 meant colorless, 1 meant light yellow, 2 meant claybank, and 3 meant brown; then the ratio of positive cells was marked: 0 meant negative, 1 meant the positive cells ≤ 10%, 2 meant they accounted for 11%–50%, 3 meant they accounted for 51%–75%, and 4 meant the ratio was ≥75%. The results were the products of the two scores, that is, the product of the color intensity and the ratio of positive cells; if the product is >3, it was positive, and if the product was between 0 and 3, it was negative.

**Statistical analysis**

SPSS 20.1 software was used for data processing and analysis; the measurement data were described as (X ± s) and was analyzed by χ² test; the enumeration data were analyzed by paired-sample t-test, and correlation analysis was performed by Pearson correlation test. P < 0.05 suggested the statistically significant difference.

**Results**

**Analysis results of immunohistochemistry**

As shown in Figures 1 and 2, the positive rate of PDCD4 protein in tumorous tissues was 7.7%, which was significantly lower than that in the corresponding normal non-tumor tissues, which was 77.5% (P < 0.05); the positive expression rate of Her-2 in tumorous tissues was 41.5%, which was significantly higher than that of the corresponding normal non-tumor tissues, which was only 2.5% (P < 0.05), as shown in Table 1.

**Analysis results of Western blot**

The results of Western blot showed that the protein expression level of PDCD4 in gastric cancerous tissues was 0.3105 ± 0.0073, which was significantly lower than that in the corresponding normal non-tumor tissues, it was 0.6891 ± 0.0725 (P < 0.05). The protein expression level of Her-2 in gastric cancerous tissues was 0.9428 ± 0.0127, which significantly higher than that in the corresponding normal non-tumor tissues, which was 0.2054 ± 0.0264 (P < 0.05). As shown in Figure 3, β-actin was used as the internal reference.
Relationship between the PDCD4 and Her-2 proteins and the clinicopathological features of patients with gastric cancer

The positive expressions of PDCD4 (5/65) and Her-2 (27/65) were significantly correlated with the differentiation degrees and TNM stages of gastric cancer \((P < 0.05)\). However, no significant correlation can be observed from Table 2 \((P > 0.05)\), regarding sex, age, tumor size, and lymph node metastasis.

Discussion

Despite the gradual decline in the incidence and mortality of gastric cancer in recent years, its prognosis has not improved significantly.\(^3,^4\) To find the biological markers that can predict the prognosis of gastric cancer does not only help clinicians to determine the prognosis and make individualized treatment programs, but also help identify the mechanism of gastric cancer development and in addition has important significance for formulating the treatment strategies for gastric cancer.\(^1^1\)

PDCD4 is a kind of tumor suppressor gene newly discovered which is associated with cell cycle and apoptosis, located in human chromosome 10q24. Its coding protein has multiple phosphorylation sites that can be combined with protein kinase C, proline kinase, and tyrosine kinase. The PDCD4 is regulated by protein kinase S6K1 and ubiquitin ligase SCF-TRCP. With the in-depth study of
PDCD4 gene, PDCD4 mRNA and protein expressions are detected to be reduced or even deleted in various malignant tumors such as prostate cancer and breast cancer, and are closely related to tumor staging and prognosis, which plays an important role in cell growth, apoptosis, and inhibition of tumor.7–9 Our results indicated that the expression of PDCD4 protein in tumorous tissues was significantly lower than that in the corresponding normal non-tumor tissues, which means that PDCD4 is a tumor suppressor gene which plays a role as the tumor suppressor gene in gastric cancer.

Her-2 gene is an oncogene, located in human chromosome 17q21, which encodes a cell membrane glycoprotein with tyrosine kinase activity, belonging to the tyrosine kinase type I receptor family, and participates in cell division, growth, reproductive regulation, and relates with the cell motility, enhancement of cell viability, and metastasis of cancer cells.12–15 It is inactive under normal circumstances; when it is affected by carcinogenic factors, its structure or expression is out of control and active, and, in turn, features with tumor transformation activity which induces malignant transformation of cells. The high expression of Her-2 has been found in a variety of tumors, such as colorectal cancer, bladder cancer, and ovarian cancer. Studies have shown that more than 30% of human tumor tissues were associated with Her-2 gene overexpression, especially breast cancer. The expression of Her-2 protein was positively correlated with the degree of differentiation and clinical stage of breast cancer; the higher the expressions are, the worse the prognosis is.13 The expression rate of Her-2 protein was 9%–38% as reported previously. The positive expression rate of this gene in this study was 41.5%, which may be related to the number of samples.

This study shows that the positive expressions of PDCD4 (5/65) and Her-2 (27/65) were significantly correlated with the differentiation degrees and TNM stages of gastric cancer ($P < 0.05$). However, no significant correlation was observed regarding sex, age, tumor size, and lymph node metastasis. These results suggest that PDCD4 and Her-2 are closely related to differentiation and clinical classification of gastric cancer, while the molecular mechanisms need further study in the future.

In summary, PDCD4 and Her-2 play important roles in the invasion and metastasis of gastric cancer, which has a negative correlation with biological behaviors of gastric cancer. The low expression of PDCD4 and the high expression of Her-2 in gastric cancer may promote the occurrence and progression of cancer. The PDCD4 and Her-2 tests can be used as an index to evaluate the malignant biological behaviors of gastric cancer and prognosis, and provide a theoretical basis for targeted therapy. The shortcoming of this study is the limitation of sample size, which needs to be expanded in the future; in addition, this is a retrospective study, and it is suggested that prospective studies should be carried out in the future to explore the relationship

### Table 1. The expressions of PDCD4 and Her-2 proteins in gastric cancerous tissues and the corresponding normal non-tumor tissues.

| Item                  | n   | PDCD4 protein | Her-2 protein |
|-----------------------|-----|---------------|---------------|
|                       |     | Positive cases (case) | Positive rate (%) | Positive cases (case) | Positive rate (%) |
| Tumorous tissues      | 65  | 5             | 7.7           | 27             | 41.5           |
| Corresponding normal  | 40  | 31            | 77.5          | 1              | 2.5            |
| non-tumor tissues     |     |               |               |                |                |
| $\chi^2$              | 8.051 |               |               | 9.442        |
| $P$                   | <0.05 |               |               | <0.05        |

PDCD4: programmed cell death factor 4; Her-2: epidermal growth factor receptor 2.

![Figure 3. The results of PDCD4 and Her-2 proteins in gastric tissues tested by Western blot. 1 and 2 represent the corresponding normal non-tumor tissues; 3 and 4 represent the tumorous tissues.]
between PDCD4 and Her-2 and gastric cancer comprehensively.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD
Xiangjun Jiang https://orcid.org/0000-0003-1875-6802

References
1. Qiu JF, Yang B, Fang L et al. (2014) Safety and efficacy of laparoscopy-assisted gastrectomy for advanced gastric cancer in the elderly. International Journal of Clinical and Experimental Medicine 7(10): 3562–3567.
2. Eachkoti R, Hussain I, Afroze D et al. (2007) BRCA1, and TP53, mutation spectrum of breast carcinoma in an ethnic population of Kashmir, an emerging high-risk area. Cancer Letters 248(2): 308–320.
3. Xue YW and Wei Y (2010) The relationship of prognosis to surgery and pathologic characteristics of stage IV (M0) gastric cancer patients. Chinese Journal of Cancer 29(4): 355–358.
4. Wyatt Sikorskii A, Rahbar MH, Victorson D et al. (2012) Health-related quality-of-life outcomes: A reflexology trial with patients with advanced-stage breast cancer. Oncology Nursing Forum; 39(6): 568–577.
5. Baek JH, Kim JG, Sohn SK et al. (2005) Biweekly irinotecan and cisplatin as second-line chemotherapy in pretreated patients with advanced gastric cancer: A multicenter phase II study. Journal of Korean Medical Science 20(6): 966–970.
6. Jiang Y, Zhang SH, Han GQ et al. (2009) Association of programmed cell death factor 4 (PDCD4) with hepatocellular carcinoma and smoking in a Chinese male population. Journal of International Medical Research 37(4): 1179–1183.
7. Jin Kim TH, Hwang SK, Chang SH et al. (2006) Aerosol delivery of urocanic acid-modified chitosan/programmed cell death 4 complex regulated apoptosis, cell cycle, and angiogenesis in lungs of K-ras null mice. Molecular Cancer Therapeutics 5(4): 1041–1049.
8. Zhang Ozaki I, Mizuta T, Hamajima H et al. (2006) Involvement of programmed cell death factor 4 in transforming growth factor-beta1-induced apoptosis in human hepatocellular carcinoma. Oncogene 25(45): 6101–6112.
9. Veeriah S, Brennan C, Meng S et al. (2009) The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. Proceedings of the National Academy of Sciences of the United States of America 106(23): 9435–9440.
10. Ko HH, Lee JJ, Chen HM et al. (2015) Upregulation of vascular endothelial growth factor mRNA level

Table 2. The relationship between the PDCD4 and Her-2 proteins and the clinicopathological features of patients with gastric cancer (n = 65).

| Clinicopathological features | n  | Positive cases of PDCD4 protein (n = 5) | χ² (P) | Positive cases of Her-2 (n = 27) | χ² (P) |
|-----------------------------|----|--------------------------------------|--------|---------------------------------|--------|
| Sex                         |    |                                       |        |                                 |        |
| Male                        | 39 | 3 (7.7%)                              | 1.542 (>0.05) | 14 (36.9%)                     | 0.509 (>0.05) |
| Female                      | 26 | 2 (7.7%)                              |        |                                 |        |
| Tumor size (cm)             |    |                                       |        |                                 |        |
| <5                          | 41 | 3 (7.3%)                              | 1.067 (>0.05) | 15 (36.6%)                     | 0.472 (>0.05) |
| ≥5                          | 24 | 2 (8.3%)                              |        |                                 |        |
| TNM stage                   |    |                                       |        |                                 |        |
| I + II                      | 40 | 4 (10%)                               | 8.952 (<0.05) | 9 (22.5%)                      | 7.613 (<0.05) |
| III + IV                    | 25 | 1 (4%)                                |        |                                 |        |
| Differentiation             |    |                                       |        |                                 |        |
| Poor + moderate             | 53 | 5 (9.4%)                              | 7.024 (<0.05) | 15 (28.3%)                     | 6.895 (<0.05) |
| High                        | 12 | 0 (0%)                                |        |                                 |        |
| Lymph node metastasis       |    |                                       |        |                                 |        |
| Yes                         | 45 | 3 (6.7%)                              | 0.205 (>0.05) | 14 (31.1%)                     | 0.318 (>0.05) |
| No                          | 20 | 2 (10%)                               |        |                                 |        |

PDCD4: programmed cell death factor 4; Her-2: epidermal growth factor receptor 2.
is significantly related to progression and prognosis of oral squamous cell carcinomas. *Journal of the Formosan Medical Association* 114(7): 605–611.

11. Xu XY, Gou WF, Yang X et al. (2012) Aberrant SERCA3 expression is closely linked to pathogenesis, invasion, metastasis, and prognosis of gastric carcinomas. *Tumour Biology: The Journal of the International Society for Oncodevelopmental* 33(6): 1845–1854.

12. Maitra A, Wanzer D, Weinberg AG et al. (2015) Amplification of the HER-2/neu oncogene is uncommon in pediatric osteosarcomas. *Cancer* 92(3): 677–683.

13. Slamon DJ, Clark GM, Wong SG et al. (1987) Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235(4785): 177–182.

14. Jung JE and Ioshii SO (2013) Immunohistochemical assessment of HER2 expression in gastric cancer in a cohort of 118 Brazilian patients. *Jornal Brasileiro de Patologia e Medicina Laboratorial* 49(5): 361–367.

15. Xie SD, Xu CY, Shen JG et al. (2009) HER 2/neu protein expression in gastric cancer is associated with poor survival. *Molecular Medicine Reports* 2(6): 943–946.