

Original Article

Programmed cell death 4 (PDCD4) repression is involved with tumor cell differentiation and lymph node metastasis in patients with colon cancer

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Abstract: Programmed cell death 4 (PDCD4), a novel tumor suppressor, inhibits neoplastic transformation and tumor progression, and is downregulated or even lost in various types of carcinomas. However, the expression and roles of the molecule in colon cancer remains uncertain. In this study, we systematically investigated the expression patterns of PDCD4 in 25 benign colon tissue specimens and 42 colon cancer tissue specimens by immunohistochemical staining. The results showed that PDCD4 protein was predominantly located in cytoplasm around the nucleus of the normal glandular cells and colon cancer cells. Compared with the normal controls, expression level of PDCD4 in colon cancer tissues statistically significantly decreased. Interestingly, low level of PDCD4 expression was significantly associated with poor differentiation and lymph node metastasis in clinicopathologic characteristics review. Thus, our data suggested that repression of PDCD4 was closely involved with the progression and prognosis of cervical cancer and it might serve as a potential diagnosis marker.

Keywords: Programmed cell death 4, colon cancer, immunohistochemistry, cell differentiation, metastasis

Introduction

Colon cancer is one of the most common human malignancies worldwide [1], and the second leading cause of cancer-related deaths in Western countries [2]. Effective screening and early diagnosis are important for the curative effect and prognosis of the patients. Colonoscopy, currently widely used in screening of patients, shows poor sensitivity in recognition of the minimal lesions [3]. Biomarkers for colon cancer are urgently required to be developed, because of their highly sensitivity and the application advantages in guiding treatment.

Programmed cell death 4 (PDCD4) is previously considered a general apoptosis marker [4]. Currently, it is found to be downregulated or even lost in various types of carcinomas, including melanoma [5], hepatocarcinoma [6] and

non-small cell lung cancer [7]. The protein has been determined as a novel tumor suppressor [5-7]. However, the expression and roles of PDCD4 in colon cancer remain uncertain. In addition, the intracellular localization of PDCD4 may be critical for regulating its function [8]. Different patterns of localization are observed in a wide range of cancer cells types [9, 10]. The distribution of PDCD4 in colon cancer cells remains unknown.

In this study, expression level and distribution of PDCD4 in colon specimens from patients were detected. And the associations of PDCD4 expression level with various clinical pathological parameters in colon cancer patients were analyzed, so as to assess the potential of PDCD4 as a diagnostic biomarker in colon cancer.

Table 1. Association between Clinicopathologic variables and PDCD4 expression in the 42 patients with colon cancer

| Variables | n (%) | PDCD4 expression | | P value |
|------------------------------|-------|------------------|------------|----------------------|
| | | Low level | High level | |
| Age -yr | | | | |
| <50 years old | 19 | 14 (42.4) | 5 (55.6) | 0.746 ^a |
| ≥50 years old | 23 | 19 (57.6) | 4 (44.4) | |
| Gender | | | | |
| Male | 20 | 15 (45.5) | 3 (33.3) | 0.786 ^a |
| Female | 22 | 18 (54.5) | 6 (66.7) | |
| Tumor size | | | | |
| <5 cm | 25 | 19 (57.6) | 6 (66.7) | 0.913 ^a |
| ≥5 cm | 17 | 14 (42.4) | 3 (33.3) | |
| Differentiation | | | | |
| High or Moderate | 18 | 11 (33.3) | 7 (77.8) | 0.045 ^{a,*} |
| Poor | 24 | 22 (66.7) | 2 (22.2) | |
| Tumor stage (Duke's) | | | | |
| A+B | 25 | 18 (54.5) | 7 (77.8) | 0.381 ^a |
| C+D | 17 | 15 (45.5) | 2 (22.2) | |
| Lymph node metastasis | | | | |
| Yes | 21 | 20 (60.4) | 1 (11.1) | 0.024 ^{a,*} |
| No | 21 | 13 (39.4) | 8 (88.9) | |
| Distant metastasis | | | | |
| Yes | 10 | 9 (27.3) | 1 (11.1) | 0.570 ^{a,*} |
| No | 32 | 24 (72.7) | 8 (88.9) | |
| Ki-67 status | | | | |
| Negative | 9 | 5 (15.1) | 4 (44.4) | 0.150 ^a |
| Positive | 33 | 28 (84.9) | 5 (55.6) | |

^aContinuity correction test. *Statistically significant.

Materials and methods

Patients and specimens

The tissues samples from 42 patients with colon cancer and 25 patients with benign colon diseases as normal controls were obtained and pathologically conformed at the First Affiliated Hospital of Sun Yat-sen University. These 67 patients underwent curative resection between January 2012 and June 2014. Colon cancer cases were classified according to the Duke's staging system. The clinical characteristics of all these patients were collected and summarized in **Table 1**. All samples were anonymously coded in accordance with local ethical guidelines, and written informed consent was obtained.

Immunohistochemistry

Paraffin-embedded tissue specimens were cut in 4 μM thick sections, deparaffinized in xylene,

and rehydrated through an alcohol gradient. Then, the slides were immersed in distilled water containing 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity. Antigen retrieval was performed by microwave treatment in 0.01 M citrate buffer (pH=6.0) for 16 minutes. Sections were successively incubated with anti-PDCD4 primary antibody (1:100; Santa Cruz, USA; overnight at 4°C) and secondary antibody (ChemMate™ EnVision; room temperature for 1 hour). Detection was carried out with DAB (Dako) as the chromogen, and slides were counterstained with hematoxylin. The negative control was treated the same way, with the primary antibody replaced by PBS (phosphate buffer saline). The stained slides were scored by two independent pathologists blinded to patient data, according to the percentage of positively stained cells (0-100%) and staining intensity (0-3: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining). A histological score (percentage of positively stained cells × staining intensity) of ≤100 was defined as low expression, and a score of >100 was defined as high expression.

Statistical analysis

Data were analyzed with the SPSS software (version 20; SPSS Inc., Chicago, Ill). Chi-squared (χ²) test was used to assess the association of PDCD4 protein expression with the clinical parameters. *P*<0.05 was considered statistically significant.

Results

PDCD4 expression in colon cancer samples

We investigate the expression of PDCD4 in 42 colon cancer samples and 25 benign lesion samples by immunohistochemical staining. PDCD4 staining was predominantly located in cytoplasm around the nucleus of the normal glandular cells and colon cancer cells, while little staining was observed in nucleus. The benign lesion tissues samples showed strong staining, 88.0% (22 out of 25) of which showed high PDCD4 expression (**Figure 1A, 1C**). In contrast, weak staining (**Figure 1B, 1D**) was ubiquitously observed in colon cancer samples, only 21.4% (9 out of 42) of which showed high

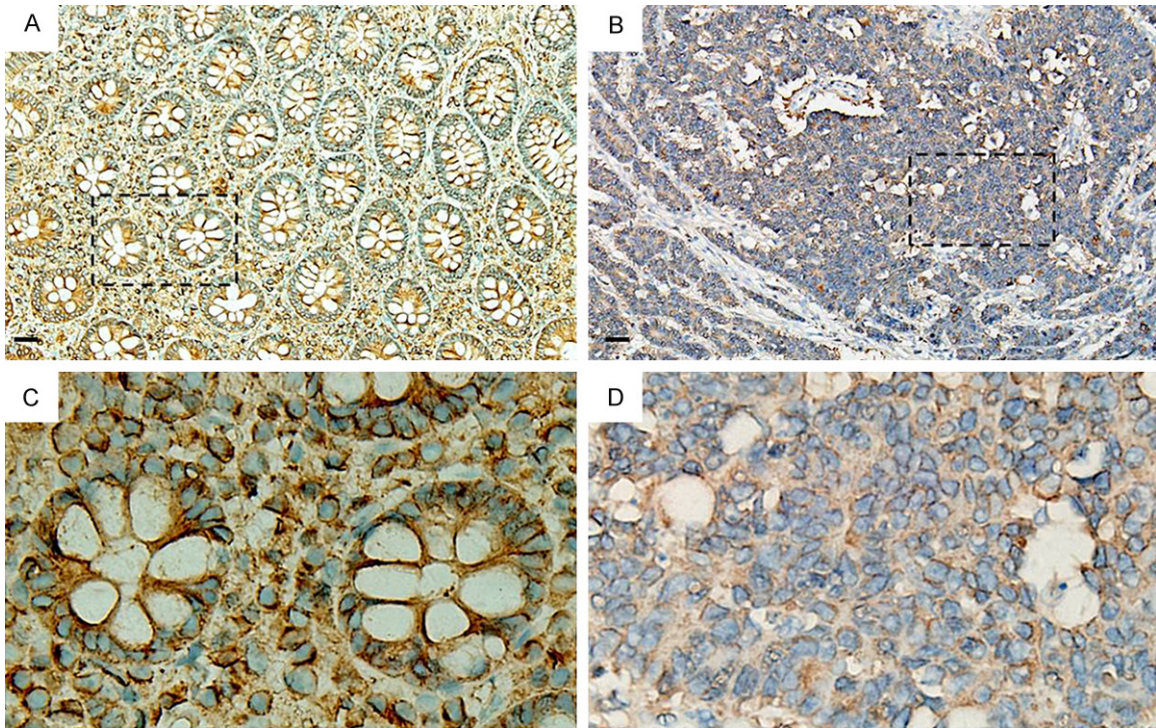


Figure 1. Representative examples of immunohistochemical staining of PDCD4 in colon tissues. A, B. High expression of PDCD4 in normal controlled samples. C, D. Low expression of PDCD4 in colon cancer samples. A, B. Magnification, x100; C, D. Magnification, x400, scale bar represents 30 μ m.

PDCD4 expression. Compared with the normal controls, expression level of PDCD4 in colon cancer tissues statistically significantly decreased ($P < 0.01$).

Correlation of PDCD4 expression with clinical parameters of patients with cervical cancer

To further characterize the role of PDCD4 in colon cancer progression, we assessed the associations of PDCD4 expression with various clinical parameters of colon cancer patients. Interestingly, low level of PDCD4 expression was significantly associated with poor differentiation ($P = 0.045$) and lymph node metastasis ($P = 0.024$). However, no significant correlation was found between PDCD4 expression and other clinical parameters, including age, gender, tumor size, tumor stage, distant metastasis and status of Ki-67 (**Table 1**).

Discussion

Previous studies demonstrated that suppression of PDCD4 expression plays important roles in neoplastic transformation and development of various cancers [11, 12]. This study com-

pared the expression of the protein in colon cancer specimens and normal controlled specimens. The results expectedly revealed a remarkably decreased expression of PDCD4 in colon cancer tissues. We also found that colon cancer patients with low PDCD4 expression displayed poor tumor differentiation and higher incidence of lymph node metastasis. It seems that downregulation of PDCD4 may be relevant with invasion, metastasis and poor prognosis of colon cancer. Recent studies suggested that miR-21 and miR183 influenced tumor invasion and metastatic potential by targeting PDCD4 [13-15]. The involvement of PDCD4 in colon cancer metastasis and progression should be clarified in further studies. In addition, A few mechanisms of PDCD4 in tumors have been proposed. reduced PDCD4 expression might promoted the cancer progression through the activation of IL-6/STAT3 [16], PI3K/Akt [7] and NF- κ B/TNF- α [17] pathways, together with the regulation of p21 [18], homeobox-interacting protein kinase-2 [19], E-cadherin, and MAP4K1 [20]. PDCD4 is a nuclear-cytoplasmic shuttling protein, and its intracellular localization has been proposed to obviously influencing its func-

tion. In the cytoplasm, PDCD4 suppresses protein translation by directly interacting with the eukaryotic initiation factor (eIF) 4A to inhibit the formation of translation-initiation complex [21]. While PDCD4 inhibits the transcriptional activity of Twist1 in the nucleus [22]. However, the subcellular localization of PDCD4 in cancer cells is conflicting. Our results showed that PDCD4 was accumulated in the cytoplasm around the nucleus in colon normal and cancer cells, which might suggests an important role of the molecule in the cytoplasm of colon cells.

In conclusion, this study indicated that PDCD4 expression is suppressed in colon cancer, and might be a potential indicator for the prognosis of patients. However, the roles and the regulatory mechanisms should be elucidated in the future studies.

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Disclosure of conflict of interest

None.

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