

## RESEARCH ARTICLE

# PDCD4 as a Predictor of Sensitivity to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Cancer Patients

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## Abstract

**Objective:** The purpose of this study was to examine the role of programmed cell death 4 (PDCD4) expression in predicting tumor response to neoadjuvant chemoradiotherapy and outcomes for patients with locally advanced rectal cancer. **Methods:** Clinicopathological factors and expression of PDCD4 were evaluated in 92 patients with LARC treated with nCRT. After the completion of therapy, 4 cases achieved clinical complete response (cCR), and thus the remaining 88 patients underwent a standardized total mesorectal excision procedure. There were 38 patients (41.3%) with a good response (TRG 3-4) and 54 (58.7%) with a poor one (TRG 0-2). **Results:** Immunohistochemical staining analyses showed that patients with high expression of PDCD4 were more sensitive to nCRT than those with low PDCD4 expression ( $P=0.02$ ). High PDCD4 expression before nCRT and good response (TRG3-4) were significantly associated with improved 5-year disease-free survival and 5-year overall survival ( $P<0.05$ ). Multivariate analysis demonstrated that the pretreatment PDCD4 expression was an independent prognostic factor. **Conclusion:** Our study demonstrated that high expression of PDCD4 protein is a useful predictive factor for good tumor response to nCRT and good outcomes in patients with LARC.

**Keywords:** Rectal cancer - programmed cell death 4 - neoadjuvant chemoradiotherapy - sensitivity - tumor regression

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## Introduction

Rectal cancer is one of the leading causes of cancer mortality worldwide. Currently, neoadjuvant chemoradiotherapy (nCRT) followed by total mesorectal excision (TME) is the standard treatment for patients with locally advanced rectal cancer (LARC). Several studies have demonstrated that nCRT significantly improved the local control of LARC when compared with preoperative radiotherapy alone or postoperative CRT (Sauer et al., 2004; Bosset et al., 2006; Gérard et al., 2006). However, the tumor responses to nCRT cover a wide spectrum, ranging from none to complete. Recent studies have demonstrated that good response to nCRT associated with good prognosis (Rödel et al., 2005; Vecchio et al., 2005; Suárez et al., 2008). Therefore, it is very important to identify the patients who show good pathological response to nCRT from the poor.

Programmed cell death 4 (PDCD4) was first identified as being differentially upregulated during apoptosis by Shibahara et al. (1995) in 1995. Nowadays, experimental evidence has demonstrated it is a novel tumor suppressor that localized in chromosome 10q24 (Soejima et al., 1999). It is known that PDCD4 plays an important role

in suppressing tumor genesis by regulating several other genes involved in multiple processes, including apoptosis, cell cycle and cell proliferation (Cmarik et al., 1999). Some research showed that when compared with the corresponding normal tissues, PDCD4 expression was lower in tumor tissues such as liver, lung, ovary, skin, brain and stomach (Mudduluru et al., 2007). Similarly, the lower PDCD4 expression in colorectal cancer was also reported by analysis of protein and/or mRNA levels (Chang et al., 2011; Lim and Hong, 2011; Horiuchi et al., 2012; Kheirleiseid et al., 2013). Until now, there have been few reports about the role of PDCD4 protein in predicting tumor response to nCRT in LARC. Thus, in the present study, we would explore the relationship between PDCD4 protein and tumor regression grade of patients with rectal cancer. In addition, the other clinicopathological factors were also determined at the same time.

## Materials and Methods

### Patients

Ninety-two rectal cancer patients with clinical T3-T4 stage were enrolled in our study. All patients were diagnosed with primary rectal adenocarcinoma and no

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evidence of metastasis was found. Before treatment, patients underwent a series of examination, including flexible endoscopy with biopsy, complete blood count, serum CEA and CA199 level. In order to exclude TNM stage I and IV tumors, chest X-ray, abdominal and pelvic computed tomography (CT), magnetic resonance imaging (MRI) and/or EUS were performed. If necessary, positron emission tomography (PET) was also used. Informed consent was obtained from all patients and the research protocols were approved by the Ethics Committee of Shandong Cancer Hospital and Institute.

### *Treatment*

All patients received nCRT followed by total mesorectal excision (TME). In brief, patients received preoperative radiotherapy with a dose of 45 Gy in 25 fractions, and then, a boost of 5.4 Gy in 3 fractions to the primary tumor. Concurrent with radiotherapy, all patients received chemotherapy. The chemotherapeutic regimens as follows: 12 patients received 5-FU, oxaliplatin and leucovorin, 28 patients received continuous infusion of 5-FU, 17 patients received capecitabine and oxaliplatin, and 35 patients received capecitabine. After the completion of nCRT, 4 patients (4.3%) achieved clinical complete response (cCR). Therefore, all except these 4 cCR patients underwent the TME procedure after a long interval of 4–6 weeks.

### *Pathologic assessment*

We collected 92 patients' biopsies before nCRT, while after treatment, only 73 patients' biopsies were available. Postoperative tissues of the other 19 patients were not evaluable, for no or not enough tumor was left after the treatment of nCRT. Tumor response was evaluated using the tumor regression grade (TRG) system proposed by Dworak et al. (1997). Details as follows: grade 0, no regression; grade 1, minor regression (dominant tumor mass with obvious fibrosis in 25% or less of the tumor mass); grade 2, moderate regression (dominant tumor mass with obvious fibrosis in 26 to 50% of the tumor mass); grade 3, good regression (dominant fibrosis outgrowing the tumor mass; i.e., more than 50% tumor regression); and grade 4, total regression (no viable tumor cells, only fibrotic mass). In the present study, TRG 3 and 4 were defined as "good response" while TRG 0, 1 and 2 were defined as "poor response." The patients with cCR mentioned above were defined as "good response".

### *PDCD4 expression analysis*

A representative area that suitable for the study purpose was selected and biopsies were taken by two investigators, 1 week before and 4–6 weeks after nCRT. After the histopathological diagnosis, additional 4- $\mu$ m sections were taken from the paraffin blocks. The process of staining was performed according to the standard protocol. Briefly, all sections were deparaffinized in xylene and rehydrated with distilled water through a graded series of ethanol solutions. Antigen retrieval was performed by boiling sections under pressure for 2 min. The sections were stained with primary polyclonal rabbit anti-human PDCD4 antibody (ab51495) (Abcam, Cambridge, UK), with a diluted ratio

of 1:200. Secondary goat anti-rabbit antibody was brought from Beijing Zhongshan Golden Bridge Biotechnology Company (China) and the application was according to the manufacturer's instructions. PDCD4 expression was visualised using 3,3'-diaminobenzidine (DAB) and subsequently counterstained in haematoxylin. At last, all sections were dehydrated through a graded series of alcohols and xylene before being mounted under a cover slip.

The histological slides were coded and evaluated by two pathologists without knowledge of patients' identity or clinical status. For this evaluation, positive staining was defined as nuclear and cytoplasmic stained to be light yellow or pale brown. All sections were analyzed at a total magnification of  $\times 400$  and 10 microscopic fields were counted in each slice. The intensity of PDCD4 staining was divided into 4 levels: negative, weak, intermediate and strong, as described (Mudduluru et al., 2007). The negative and weak staining were defined as "low expression" while the intermediate and strong staining were defined as "high expression".

### *Follow-up*

All patients underwent a regularly follow-up every 3 months for the first year after the completion of the treatment, every 6 months for the second year, and yearly thereafter. The examinations include physical examination, serum CEA and CA199 levels, chest X-ray, abdominal and pelvic CT or MRI, and EUS. PET-CT was also used for the patients with suspected recurrence or metastasis. Recurrence and metastasis were defined as a combination of physical examination, radiological examinations or histological confirmation.

### *Statistical Analysis*

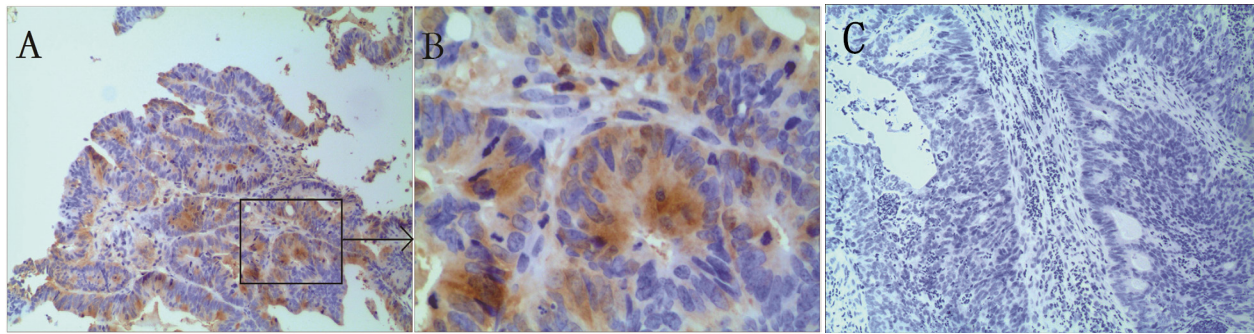
In the present study, with the aiming at identifying the potential predictors of response to nCRT, we explored the following parameters: age, gender, tumor size, histologic differentiation, cT classification, cN status, and distance from the anal verge.

To explore the significant univariate predictors of tumor response to nCRT, chi-squared test or Fisher's exact test was used, which depended on the nature of the data. A multivariate stepwise logistic regression analysis was performed in order to determine the independence of all variables that were significant in the univariate analysis. In our study, we defined the disease-free survival (DFS) and overall survival (OS) of all patients from the time of diagnosis. Survival analysis was carried out by the Kaplan–Meier method and the comparisons between potential prognostic factors were performed with the log-rank test. Finally, a Cox proportional hazards model was used for the multivariate survival analysis. For all the statistical analysis, Statistics 17.0 was used. The *P* values less than 0.05 or 95% was considered statistically significant differences.

## **Results**

### *Patient characteristics*

The clinical or pathological data of the 92 patients



**Figure 1. Different Levels of PDCD4 expression in Pretreatment Rectal Cancer Biopsies** (A and B: positive expression; C: negative expression)

**Table 1. Clinical and Pathological Factors in 92 Patients with Locally Advanced Rectal Cancer**

Variables	TRG0-2 (n=54)	TRG3-4 (n=38)	P value
Age			
<50	32	21	0.70
≥50	22	17	
Gender			
Male	35	23	0.68
Female	19	15	
Tumor size(cm)			
<5	34	21	0.46
≥5	20	17	
Histology			
Differentiated	48	35	0.73
Undifferentiated	6	3	
cT stage			
3	23	20	0.34
4	31	18	
cN stage			
Negative	17	25	0.01
Positive	37	13	
Distance from anal verge (cm)			
<4	28	23	0.41
≥4	26	15	
PDCD4			
Low	37	15	0.02
High	17	23	

**Table 2. Multivariate Analysis for Good Tumor Response**

Variables	Odds ratio	95% CI	P value
cN stage	1.18	0.32-4.99	0.01
PDCD4	3.38	1.79-7.02	<0.01

was detailed in Table 1. There were 38 patients (41.30%) with good response (TRG 3-4) and 54 patients (58.70%) with poor response (TRG 0-2). PDCD4 was expressed in 77 patients (83.70%) and the remaining 15 patients had no staining. Patients without staining were considered as negative ones (Figure 1). As listed in Table 1, the lymph node status and PDCD4 expression were significantly different in two groups ( $P=0.01$  and  $P=0.02$ , respectively) while no association between the other factors and the tumor regression was observed ( $P<0.05$ ).

*High expression of PDCD4 showed correlation with good tumor regression and good outcomes*

To determine the independent pretreatment clinicopathological factors of tumor response to nCRT,

**Table 3. Analysis of Prognostic Factors for 5-year Disease-free Survival and 5-year Overall Survival**

Variables	Cases	5-year disease-free Survival (%)	P value	5-year overall Survival (%)	P value
Age					
<50	53	0.62	0.35	0.72	0.72
≥50	39	0.65		0.80	
Gender					
Male	58	0.68	0.69	0.75	0.58
Female	34	0.65		0.71	
Tumor size(cm)					
<5	55	0.74	0.60	0.86	0.65
≥5	37	0.59		0.66	
Histology					
Differentiated	83	0.61	0.71	0.69	0.42
Undifferentiated	9	0.45		0.47	
cT stage					
3	43	0.56	0.41	0.70	0.86
4	49	0.43		0.55	
cN stage					
Negative	42	0.73	0.04	0.80	0.03
Positive	50	0.51		0.60	
Distance from anal verge (cm)					
<4	51	0.67	0.65	0.68	0.53
≥4	41	0.79		0.83	
Tumor response					
TRG 0-2	54	0.63	<0.01	0.75	0.02
TGR 3-4	38	0.79		0.85	
PDCD4					
Low	52	0.58	0.03	0.74	0.02
High	40	0.72		0.84	

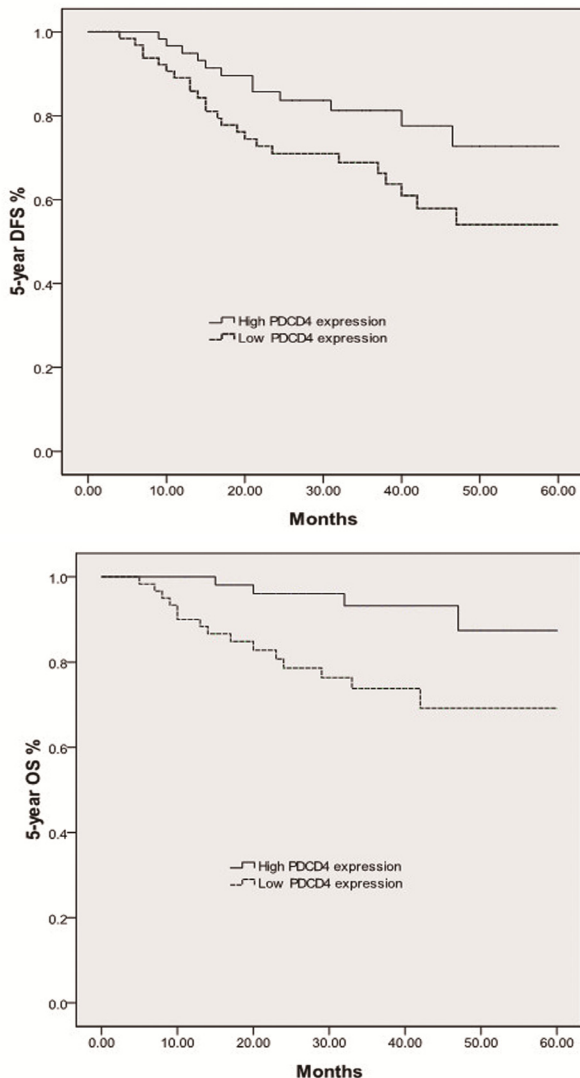
a logistic regression model was used. By multivariate analysis, we found high expression of PDCD4 showed significant correlation with good tumor regression (OR=3.38; 95% CI 1.79-7.02,  $P<0.01$ ). It means tumors with high expression of PDCD4 are more sensitive to nCRT than those with low PDCD4 expression. Moreover, the lymph node status was also kept in the model as a predictive factor for tumor response (OR=1.18; 95% CI 0.32-4.99,  $P=0.01$ ) (Table 2).

By univariate analysis, lymph node status, PDCD4 expression and tumor response were found to be significantly correlated with 5-year DFS and OS ( $P=0.04$  and  $P=0.03$ ,  $P=0.03$  and  $P=0.02$ ,  $P<0.01$  and  $P=0.02$ , respectively). As listed in Table 3, the 5-year DFS and OS in patients with high expression of PDCD4 were 72.40% and 83.80% while the data in patients with low expression of PDCD4 were 57.70% and 73.50%, respectively (Figure 2). When kept above factors into the multivariate analysis



**Table 4. Multivariate Analysis for 5-year Disease-free Survival and 5-year Overall Survival**

Variables	5-year disease-free survival			5-year overall survival		
	Hazard ratio	95% CI for hazard ratio	P value	Hazard ratio	95% CI for hazard ratio	P value
cN stage	2.94	0.88-6.53	<0.01	1.39	0.93-3.14	0.03
PDCD4	4.80	0.42-9.92	0.04	1.82	0.25-4.34	0.01
Tumor response	3.89	1.29-7.82	0.03	2.03	1.19-4.95	0.23

**Figure 2. The Impact of Pretherapeutic PDCD4 Expression Level on 5-year DFS and OS**

model, we found the PDCD4 expression and lymph node status were two important prognostic factors for two end points while the tumor response was only the prognostic factor of 5-year DFS (Table 4).

## Discussion

Although large numbers of patients with LARC have benefited from the current multi-modal treatment, the individual therapy hasn't been achieved. The variety of tumor response to nCRT increased the need to find a useful predictive model to identify the good response patients from poor. Our data of the present study indicated that pretreatment expression of PDCD4 protein could be used as a supplemental tool in predicting tumor response to nCRT and oncologic outcomes.

The overexpression of PDCD4 was initially identified in cells during the process of apoptosis (Shibahara et al., 1995), suggesting PDCD4 may be a gene that associated with apoptosis. Afonja et al. (2004) demonstrated that the overexpression of PDCD4 in breast cancer cells is sufficient to induce apoptosis through a caspase-dependent mechanism. Zhang et al. (2006) concluded that the accumulation of PDCD4 in the nuclei was crucial for apoptosis. They reported PDCD4 protein downregulated in human hepatocellular carcinoma and involved in transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)-induced apoptosis via mitochondria events and caspase cascade. At the molecular level, PDCD4 was found to be a binding partner of eukaryotic translation initiation factor 4A (eIF4A), which lies downstream of the AKT/mTOR pathway and plays an important role in response to DNA damage (Dorrello et al., 2006; Bitomsky et al., 2008; Woodard et al., 2008). Existing studies have revealed that the PDCD4 expression increased during apoptosis in response to different inducers such as retinoic acid (Shibahara et al., 1995; Zhang and Dubois, 2001). In addition, topoisomerase inhibitors (Onishi and Kizaki, 1996), COX-2 inhibitors (Zhang and Dubois, 2001), Myb (Schlichter et al., 2001), and Akt (Palamarchuk et al., 2005) were also reported to regulate the expression of PDCD4.

In our study, the potential value of PDCD4 protein in predicting the response and outcome of tumors to nCRT was investigated. Some researchers have observed the relationship between radiotherapy/chemoradiotherapy and the expression of PDCD4 level. By using the technique of microarray analysis, Supiot et al. (2013) reported preoperative radiotherapy significantly up-regulated 31 genes and down-regulated 6 genes in patients with rectal cancer. According to their study, PDCD4 was observed up-regulated as the apoptosis gene, indicating the potential role of PDCD4 in rectal cancer patients that underwent radiotherapy. In another study, a gene set that differentiated PR from CR was obtained for predicting response to nCRT, including PDCD4 (Kim et al., 2007). Chao et al. (2013) reported the increased PDCD4 expression could enhance the radiation sensitivity of glioblastoma cells. All above findings indicated the potential role of PDCD4 in predicting tumor response to nCRT. In our study, we firstly explored the correlation between the expression of PDCD4 protein and TRG to nCRT in rectal cancer patients. We found that patients with high expression of PDCD4 were more sensitive to nCRT thus good tumor regression was achieved ( $P=0.02$ ). Besides, our data revealed that high PDCD4 expression indicated a long time of 5-year DFS and OS ( $P=0.04$  and  $P=0.01$ , respectively), suggesting PDCD4 could be considered an indicator of patients prognosis.

The chemotherapy/radiotherapy sensitivity is a complex phenomenon and regulated by a series of internal factors, including cell apoptosis, cell cycle arrest and DNA damage repair (Horsman et al., 2006). PDCD4 can enhance cisplatin-induced apoptosis by mainly activating the death receptor pathway in ovarian cancer cells (Zhang et al., 2010). In gastric cancer cells, Wang et al. (2010) demonstrated that PDCD4 regulated tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sensitivity by inhibiting the PI3K/Akt signaling pathway. For radiation, PDCD4 promotes the apoptosis of glioblastoma cells (Chen et al., 2008), which is more obvious after radiotherapy (Chao et al., 2013). It seems that PDCD4-mediated cancer cells apoptosis is the same basis of chemoradiotherapeutic sensitivity. At the same time, by analysis of cell cycle, researchers reveals that cycle G2/M arrest is the important molecular basis of apoptosis that mediated by miR-21 and PDCD4 (Anastasov et al., 2012; Chao et al., 2013). In addition, Bitomsky et al. (2008) found diminished PDCD4 expression could deregulate the normal DNA-damage response thereby favoring the survival of DNA-damaged cells and preventing them from undergoing apoptosis, revealing PDCD4 could regulate cell apoptosis by affecting the fate of DNA-damage cells. However, more studies are needed to further unveil the mechanism of radiotherapy/chemotherapy resistance of rectal cancer patients.

The inherent limitation of this study was that our observation was a retrospective study. Also, the number of patients in our study was small. In addition, Sanghera et al. have reported that a higher pathologic complete response rate was observed in studies using 2 drugs with infusional 5-FU, indicating the influence of variability of concurrent chemotherapy regimens to the tumor response to nCRT (Sanghera et al., 2008). Therefore, the larger, more homogeneous multicenter studies that applying unified measurement techniques should be performed to obtain a widely accepted and standardized value of PDCD4.

In conclusion, our study demonstrated that high expression of PDCD4 protein was useful predictive factor of good tumor response to nCRT and good outcomes in patients with LARC.

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