

## Original Article

# Serum decoy receptor 3 is a diagnostic and prognostic biomarker in patients with colorectal cancer

Shun-Wu Chang<sup>1</sup>, Bao-Chun Wang<sup>1</sup>, Xiao-Guang Gong<sup>1</sup>, Xue-Li Zhang<sup>2</sup>

<sup>1</sup>Department of General Surgery, Hainan General Hospital, Haikou, China; <sup>2</sup>Department of General Surgery, Fengxian Hospital Affiliated to Southern Medical University, Shanghai, China

Received March 22, 2016; Accepted June 27, 2016; Epub July 1, 2016; Published July 15, 2016

**Abstract:** Background: Decoy receptor 3 (DcR3), a decoy receptor against Fas ligand belonging to the tumor necrosis factor receptor super-family, is over-expressed in types of tumor. The aim of this study was to explore the expression level of DcR3 and investigated its diagnostic value in colorectal cancer (CRC). Methods: Quantitative Real-time-PCR (qRT-PCR) and western blot were performed to determine the expression of serum DcR3 at mRNA and protein level in CRC samples and healthy controls, respectively. Chi-square test was used to analyze the relationship between DcR3 expression and clinical features. The receiver operating characteristics (ROC) curve was established to evaluate the diagnostic value of DcR3 in CRC. Results: The relative expression of serum DcR3 was increased in CRC patients compared with the matched healthy controls both at mRNA and protein level. The high serum DcR3 expression was associated with lymph node metastasis, distant metastasis, and TNM stage. ROC curve showed that DcR3 had a high diagnostic value with an AUC of 0.912 corresponding with a sensitivity of 78.8% and a specificity of 91.2%. Conclusion: The detection of DcR3 levels in the serum might serve as a new tumor biomarker in the diagnosis and assessment of prognosis of CRC.

**Keywords:** Colorectal cancer, decoy receptor 3, biomarker, prognosis

## Introduction

Colorectal cancer (CRC) is currently the third most common malignancy and the second leading cause of cancer mortality worldwide [1]. Approximately 60% of CRC patients present with resectable disease when diagnosed, and for these patients, curative surgical resection followed by adjuvant chemotherapy is considered the standard treatment strategy. However, 20-30% of patients will ultimately develop recurrent disease, and the prognosis after recurrence is poor [2, 3]. Therefore, novel and reliable biomarkers for CRC diagnosis and prognosis are in urgent needed in clinical practice.

Decoy receptor 3 (DcR3), also named TR6, is a member of the tumor necrosis factor (TNF) receptor superfamily, is a soluble decoy receptor with a pleiotropic immunomodulatory effect via “decoy” and “nondecoy” functions [4, 5]. DcR3 could recognize three TNF-super-family members, such as Fas ligand (FasL/CD95L/

TNFSF6), LIGHT (TNFSF14), and TNF-like molecule 1A (TL1A/VEGI-L/TNFSF15) [6-8]. Thus, inhibits the combination of the ligand and death receptors competitively, and further blocking the ligands induced apoptosis [9]. The overexpression of DcR3 has been observed in various malignant tumors. For example, Zhou et al showed that DcR3 was overexpression and predicted the prognosis and pN2 in pancreatic head carcinoma [10]. Wang et al indicated that serum DcR3 was increased in bladder transitional cell carcinoma and correlated with TNM stage and pathological classification of the tumor [11]. Yang et al reported that serum DcR3 was up-regulated in patients with hepatocellular carcinoma and related to the prognosis [12]. However, the diagnostic and prognostic values of serum DcR3 in CRC have not been fully evaluated to date.

The aim of the present study was to evaluate the clinical significance of serum DcR3 expression in CRC. The correlation between the serum

## Serum DcR3 expression in CRC

**Table 1.** Correlation between the serum level of DcR3 and clinicopathological features in 78 CRC patients

Clinicopathological features	Total	Serum DcR3 expression		P value
		Low	High	
Age (years)				0.488
<60	31	17	14	
≥60	47	22	25	
Gender				0.255
Male	43	19	24	
Female	35	20	15	
Tumor size (cm)				0.496
<5	37	20	17	
≥5	41	19	22	
Differentiation				0.157
Well	29	17	11	
Moderate + Poor	50	22	28	
Tumor location				0.645
Colon	32	15	17	
Rectum	46	24	22	
Lymph node metastasis				0.000
No	51	33	18	
Yes	27	6	21	
Distant metastasis				0.018
No	59	34	25	
Yes	19	5	14	
TNM stage				0.013
I + II	37	24	13	
III + IV	41	15	26	

level of DcR3 and clinicopathological features was analyzed. In addition, the influence of serum DcR3 on the diagnosis and prognosis of CRC patients was also estimated.

### Methods and materials

#### Patients and specimens

Serum samples were obtained from the 78 patients who underwent surgery in the Department of General Surgery, Hainan General Hospital between January 2012 and November 2014. Control serum samples were collected from 60 healthy volunteers. Blood samples were drawn from all patients and healthy control subjects at the beginning of treatment. A 5 ml sample of peripheral venous blood was drawn from all study participants after an overnight fast and placed at room temperature for 60 min. Then the blood samples were centri-

fuged at 1000 g for 10 min at 4°C to spin down the blood cells. All serum samples were stored at -80°C prior to use. Clinicopathological information of each patient was obtained from the medical records and surgical pathological reports, and the characteristics of the patients were summarized in **Table 1**.

The selection criteria for patients with CRC were as follows: (1) pathologically confirmed patients with CRC; (2) the patients had no history of other cancers. None of the patients received preoperative treatment, such as radiotherapy or chemotherapy. The study protocols were approved by the Ethics Committee of Southern Medical University, and written informed consent was obtained from each patient.

#### RNA extraction and qRT-PCR

Total RNA was severally extracted from the serum of patients with CRC and healthy controls using TRIzol (Invitrogen) according to the manufacturer's protocol. Reverse transcription was conducted to synthesize the first-strand of cDNA using TaqMan Reverse Transcription Kit (Applied Bio-systems). RT-PCR reaction was performed in an ABI 7900HT real-time PCR system (Life Technologies). GAPDH was taken as an internal control. The relative mRNA expression of serum DcR3 was calculated using the comparative threshold cycle ( $2^{-\Delta\Delta Ct}$ ) method. Each sample was in triplicate.

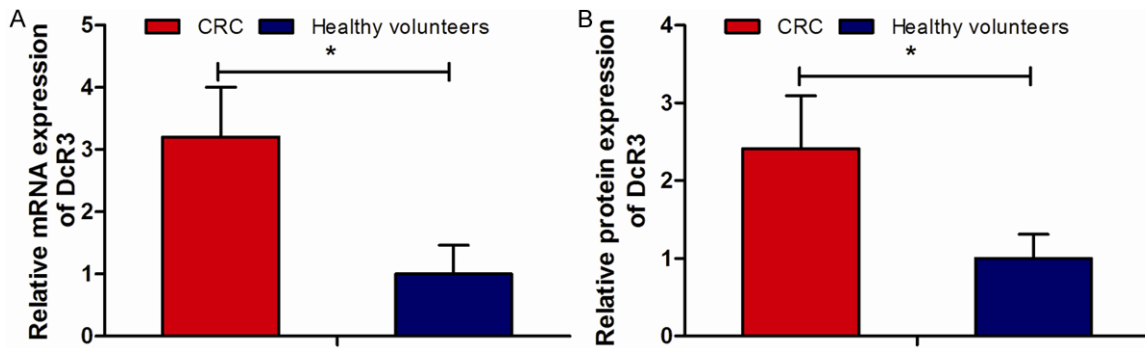
#### Western blot analysis

Total protein was isolated from the serum of patients with CRC and healthy controls, respectively. Then the protein was separated by SDS-PAGE and the brands were transferred onto nitrocellulose membranes. The membranes were incubated with primary antibody blocked after being blocked by 5% non-fat milk for overnight at 4°C. Subsequently, the secondary antibody anti-DcR3 mAb was added and incubated with the membranes at room temperature. Target protein was detected by an enhanced chemiluminescence substrate kit (Pierce Biotechnology).

#### Statistical analysis

The mean serum DcR3 levels in the different groups were compared using students' t-test. The relationship between the serum DcR3 expression and clinicopathological features of

## Serum DcR3 expression in CRC



**Figure 1.** Relative expression of DcR3 in CRC patients (n=78) and healthy volunteers (n=60). DcR3 expression was significantly higher in CRC patients than that in healthy controls both at mRNA (A) and protein (B) level. \*P<0.05.

CRC patients were using  $\chi^2$  test. ROC curve was established to determine the diagnostic value of serum DcR3 in distinguishing patients with CRC from healthy controls. All *P* values are two-tailed and were regarded as significant when less than 0.05. All statistical analyses were conducted using SPSS software.

### Results

#### *DcR3 expression in the serum of CRC patients versus control*

The expression of serum DcR3 at mRNA and protein level were detected by qRT-PCR and western blot assays in 78 CRC patients and 60 healthy controls, respectively. **Figure 1** showed that serum DcR3 expression level on average was upregulated in CRC patients compared with the healthy controls both at mRNA and protein levels (P<0.05).

#### *Relationship between DcR3 expression and clinicopathological features of CRC patients*

To explore the function of DcR3 in the development of CRC, the patients were divided into two groups according to the median expression of DcR3: CRC patients who express DcR3 at levels less than the cut-off value were assigned to the low expression group (n=39), and those with expression above the cut-off value were assigned to the high expression group (n=39). We then investigated the associations between DcR3 expression level and the clinicopathologic features of the CRC patients, and we found that high serum DcR3 expression level was correlated with lymph node metastasis, distant metastasis, and TNM stage (P<0.05, **Table 1**). However, high serum DcR3 expression was not

associated with other clinicopathological factors of patients, including age, gender, tumor size, differentiation, as well as tumor location (P>0.05, **Table 1**).

#### *Diagnostic value of DcR3 in GC*

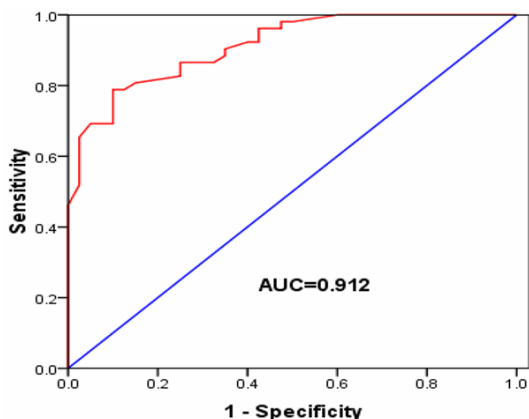
Receiver operating characteristic (ROC) curves was built to investigate the diagnostic value of DcR3 in CRC. The outcome suggested DcR3 had a high diagnostic value with a areas under the ROC curve (AUC) of 0.912 (95% confidence interval 0.857 to 0.963, P<0.05, **Figure 2**). At the cut-off value of 1.398, the optimal sensitivity and specificity were 78.8 and 91.2%, respectively. These indicated that DcR3 could play a key role in the distinction of the healthy people from the CRC patients.

### Discussion

Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide, and is the fifth most common cause of cancer related deaths in China [13]. Survival rates of patients with CRC have increased in the past few years, possibly as a result of earlier diagnosis and improved treatment regimens, nonetheless, approximately 30% of patients who undergo curative resection subsequently experience local tumor recurrence or metastasis [14]. Therefore, it is necessary to look for some useful accuracy biomarkers that will facilitate the identification of patients with a poor prognosis, and permit personalized treatment strategies for patients with high risk of CRC recurrence.

Several studies have shown that various genetic and epigenetic alterations including oncogenes and tumor suppressor genes are involved

## Serum DcR3 expression in CRC



**Figure 2.** Receiver operating characteristics (ROC) curve analysis for the diagnostic value of DcR3 in CRC. The AUC was 0.912 (95% confidence interval 0.857 to 0.963,  $P < 0.05$ ).

in the course of carcinogenesis and progression of CRC [15]. For example, Wu et al investigated the role of CXCL9 and found it could act as a potential tumor oncogene as well as an independent prognostic factor in CRC patients [16]. Al-Maghrabi et al indicated that overexpression of PAK-1 was an independent predictor of disease recurrence in CRC [17]. Mokutani et al suggested that downregulation of miR-132 was associated with poor prognosis of CRC patients [18]. Thus, identification of CRC-specific biomarkers involved in these procedures is very important for diagnosis, therapy and prognosis prediction in clinical.

DcR3 is located on chromosome position 20q-13, and have demonstrated to be a regulator of cell proliferation, apoptosis and immune response by antagonizing FasL-, LIG- and TLIA-mediated signals [19]. The over-expression of DcR3 in tumor cells can protect them from apoptosis and has been detected in various types of body fluid of cancers which make it shows great potential to become a biomarker for the early non-invasive diagnosis for malignant tumors [20, 21]. DcR3 protein is closely related to cell differentiation, tumor occurrence, progression and prognosis [22]. However, the diagnostic and prognostic values of DcR3 in serum have not been fully evaluated in CRC patients.

In the present study, we performed independent validation experiments using a cohort of samples from 78 patients with CRC and 60

control subjects. Our results suggested that serum DcR3 was over-expression in CRC which indicated that it might be an oncogene in CRC progression. Furthermore, we explored its association with clinical factors. Our data indicated that high serum DcR3 expression level was correlated with lymph node metastasis, distant metastasis, and TNM stage, indicating that DcR3 might play a vital role in the development of CRC by influencing the invasion and metastasis. ROC curve analysis showed that DcR3 was useful marker for discriminating cases from healthy controls, with an AUC of 0.912. At the cut-off value of 1.398, the optimal sensitivity and specificity were 78.8 and 91.2%, respectively. The results revealed that the serum DcR3 could be used as an efficient diagnostic biomarker for the detection of CRC patients.

In conclusions, our studies indicated that the increased expression of DcR3 might serve as a new tumor biomarker in the diagnosis and assessment of prognosis of CRC. However, as the limitations of samples scales and other unfavorable factors in the study, some further researches are still need in the future.

### Acknowledgements

This work was supported by the National Natural Science Foundation of Hainan Province (Grant No. 20158353).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Xue-Li Zhang, Department of General Surgery, Fengxian Hospital Affiliated to Southern Medical University, 6600 Nanfeng Road, Shanghai 201499, China. E-mail: happy\_apple76@sina.com

### References

- [1] Siegel R, Desantis C and Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 104-117.
- [2] Sadanandam A, Lyssiotis CA, Homicsko K, Collisson EA, Gibb WJ, Wullschlegler S, Ostos LCG, Lannon WA, Grotzinger C and Del Rio M. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med* 2013; 19: 619-625.

## Serum DcR3 expression in CRC

- [3] Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y and Shima K. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Eng J Med* 2012; 367: 1596-1606.
- [4] Hsieh SL and Lin WW. Decoy Receptor 3. *Encyclopedia of Cancer*. Springer; 2011. pp. 1071-1072.
- [5] Lin WW and Hsieh SL. Decoy receptor 3: a pleiotropic immunomodulator and biomarker for inflammatory diseases, autoimmune diseases and cancer. *Biochem Pharmacol* 2011; 81: 838-847.
- [6] Zhang Y, Li D, Zhao X, Song S, Zhang L, Zhu D, Wang Z, Chen X and Zhou J. Decoy receptor 3 suppresses FasL-induced apoptosis via ERK1/2 activation in pancreatic cancer cells. *Biochem Biophys Res Commun* 2015; 463: 1144-1151.
- [7] Bamias G, Siakavellas SI, Stamatelopoulos KS, Chrysoschoou E, Papamichae IC and Sfrikakis PP. Circulating levels of TNF-like cytokine 1A (TL1A) and its decoy receptor 3 (DcR3) in rheumatoid arthritis. *Clin Immunol* 2008; 129: 249-255.
- [8] Chen J, Zhang L and Kim S. Quantification and detection of DcR3, a decoy receptor in TNFR family. *J Immunol Methods* 2004; 285: 63-70.
- [9] Roth W, Isenmann S, Nakamura M, Platten M, Wick W, Kleihues P, Bähr M, Ohgaki H, Ashkenazi A and Weller M. Soluble decoy receptor 3 is expressed by malignant gliomas and suppresses CD95 ligand-induced apoptosis and chemotaxis. *Cancer Res* 2001; 61: 2759-2765.
- [10] Zhou J, Song S, Li D, He S, Zhang B, Wang Z and Zhu X. Decoy receptor 3 (DcR3) overexpression predicts the prognosis and pN2 in pancreatic head carcinoma. *World J Surg Oncol* 2014; 12: 52.
- [11] Wang D, Wang J and Chen G. [Association of serum decoy receptor 3 protein level with the clinicopathologic features of bladder transitional cell carcinoma]. *Nan Fang Yi Ke Da Xue Xue Bao* 2013; 33: 1831-1832.
- [12] Yang M, Chen G, Dang Y and Luo D. Significance of decoy receptor 3 in sera of hepatocellular carcinoma patients. *Ups J Med Sci* 2010; 115: 232-237.
- [13] Chen W, Zheng R, Zhang S, Zhao P, Zeng H, Zou X and He J. Annual report on status of cancer in China, 2010. *Chin J Cancer Res* 2014; 26: 48-58.
- [14] Mouradov D, Domingo E, Gibbs P, Jorissen RN, Li S, Soo PY, Lipton L, Desai J, Danielsen HE and Oukrif D. Survival in stage II/III colorectal cancer is independently predicted by chromosomal and microsatellite instability, but not by specific driver mutations. *Am J Gastroenterol* 2013; 108: 1785-1793.
- [15] Coppedè F, Lopomo A, Spisni R and Migliore L. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. *World J Gastroenterol* 2014; 20: 943-956.
- [16] Wu Z, Huang X, Han X, Li Z, Zhu Q, Yan J, Yu S, Jin Z, Wang Z and Zheng Q. The chemokine CXCL9 expression is associated with better prognosis for colorectal carcinoma patients. *Biomed Pharmacother* 2016; 78: 8-13.
- [17] Al-Maghrabi J, Emam E, Gomaa W, Al-Qaydy D, Al-Maghrabi B, Buhmeida A, Abuzenadah A, Al-Qahtani M and Al-Ahwal M. Overexpression of PAK-1 is an independent predictor of disease recurrence in colorectal carcinoma. *Int J Clin Exp Pathol* 2015; 8: 15895.
- [18] Mokutani Y, Uemura M, Munakata K, Okuzaki D, Haraguchi N, Takahashi H, Nishimura J, Hata T, Murata K and Takemasa I. Down-Regulation of microRNA-132 is associated with poor prognosis of colorectal cancer. *Ann Surg Oncol* 2016; [Epub ahead of print].
- [19] Arakawa Y, Tachibana O, Hasegawa M, Miyamori T, Yamashita J and Hayashi Y. Frequent gene amplification and overexpression of decoy receptor 3 in glioblastoma. *Acta Neuropathol* 2005; 109: 294-298.
- [20] Simon I, Liu Y, Krall KL, Urban N, Wolfert RL, Kim NW and McIntosh MW. Evaluation of the novel serum markers B7-H4, Spondin 2, and DcR3 for diagnosis and early detection of ovarian cancer. *Gynecol Oncol* 2007; 106: 112-118.
- [21] Macher-Goeppinger S, Aulmann S, Wagener N, Funke B, Tagscherer KE, Haferkamp A, Hohenfellner M, Kim S, Autschbach F and Schirmacher P. Decoy receptor 3 is a prognostic factor in renal cell cancer. *Neoplasia* 2008; 10: 1049-1056.
- [22] Wu Y, Guo E, Yu J and Xie Q. High DcR3 expression predicts stage pN2-3 in gastric cancer. *Am J Clin Oncol* 2008; 31: 79-83.