

Over-expression of Decoy Receptor 3 in Gastric Precancerous Lesions and Carcinoma

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Abstract

Introduction: Decoy receptor 3 (DcR3), a newly discovered member of the tumor necrosis factor receptor (TNFR) superfamily, is not only upregulated in cancer cells derived from various cell lineages, but also correlates with the overall survival of certain cancer patients. The objective of the present study was to investigate the expression of DcR3 protein in tissue of gastric precancerous lesions and carcinoma.

Material and methods: The expression of DcR3 protein in tissue of gastric carcinoma (GC, n=79), dysplasia (n=45), intestinal metaplasia (IM, n=37) and chronic superficial gastritis (CSG, n=42) was investigated by immunohistochemistry.

Results: Expression of DcR3 in GC was significantly higher than that in dysplasia ($P<.05$); IM ($P<.05$) and CSG tissue ($P<.001$), respectively. It was also found that DcR3 expression in well differentiated GC was significantly lower than that in poorly differentiated specimens ($P<.05$). Moreover, patients in tumor-node-metastasis (TNM) stages I and II showed significantly lower DcR3 expression compared with that in stages III and IV ($P<.05$). In addition, DcR3 expression in both lymph node metastasis-negative patients and patients without systemic metastasis was significantly decreased in comparison with that in lymph node metastasis-positive patients ($P<.05$) and patients with systemic metastasis ($P<.001$).

Conclusions: DcR3 is over-expressed in human GC and positively correlated with development and metastases of gastric lesions. The DcR3 gene might serve as an important molecular biological indicator in diagnosing and predicating the clinical outcome in GC patients.

Introduction

Decoy receptor 3 (DcR3)/TR6/M68 is a soluble decoy receptor in the tumor necrosis factor receptor (TNFR) superfamily, which involves 4 members: DcR1, DcR2, DcR3 and osteoprotegerin (1,2,3). Gene amplification as well as over-expression of DcR3 messenger RNA (mRNA) and protein have been demonstrated in lymphoma, glioblastoma and also in cancer of the lung, brain, pancreas, liver, esophagus, colon and gastrointestinal tract (2,4,5,6,7,8,9,10). More importantly, the DcR3 expression level was associated with lymph node metastasis and pathological sections in gastric carcinomas (9). The serum level of DcR3 was also significantly elevated in a variety of tumors, including cancers of the digestive system, thyroid, lung, breast and ovary (11, 12). All the evidence suggests that DcR3 can not only become a factor responsible for the progression and immune suppression of tumor cells, but also serve as an effector molecule to modulate pathological and physiological functions.

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Gastric carcinoma (GC) is one of the most common malignant diseases all over the whole world. Gastric carcinogenesis is considered as a multistage progressive process. The early indicator for the subject predisposed to GC is abnormal proliferation of gastric epithelial cells, so chronic atrophic gastritis (CAG), dysplasia and intestinal metaplasia (IM) are considered as key precancerous lesions with strong facilitation to the development of GC (13,14,15,16). However, the pathogenic mechanisms of this process remain unclear. Takahama et al (9) evaluated DcR3 expression in gastric cancer by Northern blot and in situ hybridization, but, to date no studies have been carried out of its associations with precancerous gastric lesions, namely, in IM and gastric dysplasia by immunohistochemistry (IHC). There has been little information available about the protein status of DcR3 in stomach mucosa collected by gastroscopy or gastrectomy. No detailed study comparing the expression patterns of DcR3 in gastric carcinoma with that in precancerous tissue has been available. Furthermore, whether over-expression of DcR3 is involved in the initiation or promotion of GC has not been established. Therefore, the aim of the present study was to investigate the expression of DcR3 in chronic gastritis, precancerous and cancerous gastric lesions by IHC analysis.

Materials and methods

From our surgical pathology files, CSG (n=42), IM (n=37), dysplasia (n=45) and GC (n=79) gastrectomy or gastroscopy specimens were selected in this retrospective study. All cases were randomly chosen in the First Affiliated Hospital, Guangxi Medical University, People's Republic of China between May 2001 and December 2005. Written informed consent to use the samples for research was obtained from the patients and clinicians. All the guidelines for experimental investigation with human subjects required by the institution were followed.

Histopathological diagnosis for gastric epithelia was made according to the cellular morphological changes and tissue architecture using previously established criteria (17,18). In brief, CSG was characterized by an inflammation manifested by mild lymphocyte and plasma cell infiltration; IM, confirmed by the presence of goblet cells in gastric mucosa; dysplasia, characterized by nuclear atypia with or without architectural abnormalities in the gastric epithelium without invasion; ultimately, GC is characterized by invasion of neoplastic gastric cells through the basement membrane. Carcinomas were classified according to the histological classification of World Health Organization (WHO) criteria (19). Of 79 GCs, 51 and 28 were histologically graded as well differentiated and poorly differentiated, respectively. All of the 79 GCs in the present study were intestinal-type carcinomas, because in the multistep process of intestinal-type carcinogenesis, the genetic pathway can be divided into three subpathways: an intestinal metaplasia→adenoma→carcinoma sequence, an intestinal metaplasia→carcinoma sequence and de novo (12). The clinical tumor-node-metastasis (TNM) stage was processed according to the pathology TNM (pTNM) classification of WHO criteria (20). Of 79 GCs, 12 were

stage I, 15 stage II, 20 stage III and 23 stage IV. The patients were 123 men and 80 women whose ages ranged from 20 to 82 years (mean 47 ± 12 years). One or 2 of the most representative sections from each case were selected and stained with a polyclonal antibody against DcR3 (DcR3<H-130>:sc-25464) from Santa Cruz Biotechnology[®], inc.. DcR3<H-130>:sc-25464 is a rabbit polyclonal antibody raised against amino acids 171–300 of DcR3 of human origin and 1:300 dilution was used. The 79 GC patients had never received any radiation therapy or chemotherapy.

The procedure of IHC was done as described previously (7). One hundred cells from 5 representative areas in each lesion were counted. The staining results were evaluated according to the immunodetection of stain intensity and numbers of positive cells by two pathologists (G.C. and DZ.L.), who discussed each case until they reached a consensus. Stain intensity was up to the standard of the relative stain intensity of most cells. The degree of staining was subdivided as follows: the stain intensity could be from 0 to 3 (0, no staining; 1, focal or fine granular, weak staining; 2, linear or cluster, strong staining; and 3, diffuse, intense staining); and the positive cells in the observed gastric mucosa ranged from 0 to 3 in percentage (0, no staining; 1, < 30%; 2, 30%–70%; and 3 > 70%). The samples were scored by their summation: 0–1 (-); 2–3 (+); 4 (++) ; 5–6 (+++). Any staining score ≥ 2 (+) was considered as positive expression (21).

The Fisher exact test was used to compare the expression of DcR3 among different groups with SPSS 13.0 software for Windows (Munich, Germany). A *P* value of less than .05 was considered statistically significant. Multivariate analysis was used for identifying correlation of DcR3 over-expression with patients' age, gender, clinical and pathologic parameters. A *P* value of less than .05 (for 1 side) or less than .025 (for 2 sides) was considered statistically significant.

Results

DcR3 expression in different gastric tissue

Positive immunostaining for DcR3 was observed in the cytoplasm of gastric epithelial cells and cancer cells with different rates in different types of lesions (Image 1A, Image 1B, Image 1C, Image 1D). Focal DcR3 over-expression was observed in 2 of 42 cases of CSG mucosa. It was slightly increased in the IM and dysplasia, and significantly increased in GC. Over-expression of DcR3 was more often observed in GC than in dysplasia ($P < .05$), IM ($P < .05$) and CSG ($P < .001$, respectively). However, there was no difference in DcR3 expression when comparing dysplasia, IM and CSG (Table 1).

Correlation of DcR3 over-expression with GC differentiation

DcR3 over-expression was more often observed in poorly differentiated GC than in well differentiated GC ($P < .05$, Table 2).

Correlation of DcR3 over-expression with clinical stages

In addition to its correlation with the grade of differentiation, over-expression of DcR3 also tended to correlate with the clinical stage of GC. DcR3 expression in the clinical TNM stage I and II was significantly lower than that in stages III and IV ($P < .05$, Table 2).

Correlation of DcR3 over-expression with metastasis

DcR3 expression in the lymph node metastasis-negative patients was significantly lower than in patients with lymph node metastasis ($P < .05$). Moreover, the rate of DcR3 expression in patients without systemic metastasis was significantly lower than that in the group with systemic metastasis ($P < .05$, Table 2).

Correlation of DcR3 over-expression with other clinopathologic parameters

Multivariate analysis revealed no relationship between DcR3 expression in GC and patients' age, sex or the tumor invasive depth (data not shown).

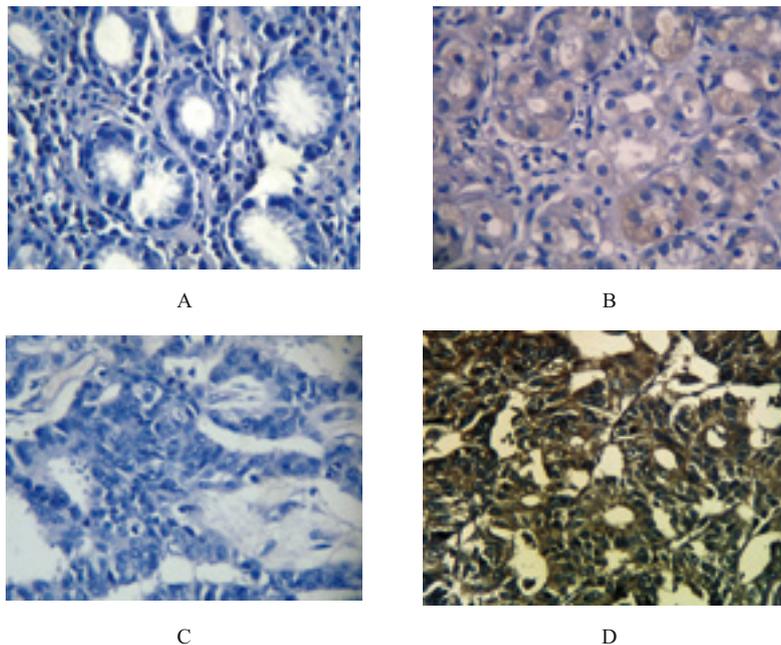


Figure 1. Over-expression of Decoy Receptor 3 (DcR3) in gastric carcinoma. A and B, chronic superficial gastritis (CSG) (A, DcR3 negative; B, DcR3 positive, $\times 400$). C and D, gastric carcinoma (GC) (C, DcR3 negative; D, DcR3 positive, $\times 400$).

Table 1. Over-expression of decoy receptor 3 (DcR3) in different types of gastric tissue*

	DcR3	P Compared With		
		Intestinal metaplasia	Dysplasia	Gastric carcinoma
CSG	2/42(4.86)	>.050	>.050	<.0001
IM	4/37(10.81)	–	>.050	<.050
Dysplasia	7/45(15.56)	–	–	<.050
GC	27/79(34.18)	–	–	–

* Data are given as number scored 2+/total tested (percentage); 2+ indicates linear or cluster, strong staining.

Table 2. Correlation between the expression of decoy receptor 3 (DcR3) and different clinicopathological parameters*

Clinicopathological Parameters	DcR3	P
Differentiation	Poorly-differentiated	14/28(50)
	Well-differentiated	13/51(25.49)
Clinical TNM Stages	I/II	8/37(21.62)
	III/IV	19/42(45.24)
Nodal status	Negative	4/29(13.79)
	Positive	23/50(46)
Metastatic status	Negative	9/55(16.36)
	Positive	18/24(75)

*Data are given as number scored 2+/total tested (percentage); 2+ indicates linear or cluster, strong staining.

Discussion

Gastric carcinogenesis is considered as a multistage and progressive process and an early indicator for a patient predisposed to GC is abnormal proliferation of gastric epithelial cells, such as dysplasia and IM, which have both been considered as pre-cancerous lesions for GC (13, 14, 15, 16). However, information about the mechanism of gastric carcinogenesis is very limited. Studies of DcR3 protein expression levels at different stages of gastric carcinogenesis may help answer why different stages of cancerous development occur.

Human DcR3, mostly being expressed in many different classes of tumor cells, can combine with the TNF family members FasL (2), LIGHT (4, 22) and TL1A (2),

thus to block their interaction with their respective receptors. A study of Takahama et al demonstrated that DcR3 mRNA was over-expressed in 22 (26%) primary GC in 84 gastric carcinomas compared with each noncancerous tissue by Northern blot analysis (9). In this study, we showed that DcR3 had higher expression rate (34.18% vs 26%) in GC compared with the CSG and precancerous lesions. Such a variation between previous studies and the present study may depend on different detecting methods (Northern blot vs IHC). However, we found that the positive immunostaining rate of DcR3 was abnormally higher in GC than that in dysplasia, IM and CSG, which is indicative of abnormally high cell proliferation activity in GC tissue. The sequential increase of DcR3 overexpression suggest that indeed DcR3 might be involved in gastric carcinogenesis. The results may also provide a better understanding of the pathogenesis and perhaps permit more accurate diagnosis of GC.

It is known that GC can result from the metaplasia→dysplasia→carcinoma sequence, i.e., CSG→IM→dysplasia→GC. However, the molecular mechanisms in such a process remain unclear. In the present study, the expression of DcR3 was positively correlated with the degree of malignancy of the gastric mucosa and the development of gastric lesions. With the likelihood of malignant lesions progressed from CSG→IM→dysplasia→GC, the positive immunostaining rates for DcR3 similarly increased, showing a good linear correlation between DcR3 expression and lesion progression. Though there was almost identical immunoreactivity for IM and dysplasia, the higher expression of DcR3 in GC can still indicate that DcR3 expression might be related to the hyperplastic status of gastric mucosa epithelial cells. These data were consistent with the views of Li et al, who suggested that the degree of dysplasia in esophagus tissue was strongly associated with increased levels of DcR3 activity (7). In our study on DcR3 in hepatocellular carcinoma (HCC) tissue, we also found a similar increase of the DcR3 expression. The present results indicate that an increased expression of DcR3 might be an important molecular event involved in the process of gastric carcinogenesis. This conclusion is consistent with that of Takahama et al. suggesting that measurement of DcR3 activity might be a valuable clinical marker in the progression of dysplasia and subsequent development of GC (9).

Another interesting observation was that the expression of DcR3 directly correlated with the differentiation level of GC. The DcR3 immunostaining rate in well differentiated GC was lower than in poorly differentiated GC. This conclusion differed from that of Takahama et al, who found no differences between the histological type, differentiation and the DcR3 expression levels (9). We believe that variations in technique, materials and methods may partly explain this difference. However, this result is consistent with the findings in human malignant gliomas, which showed that expression of DcR3 correlated with the grade of malignancy (8). The present results may be taken to indicate that over-expression of DcR3 reflects the differentiation level of the stomach mucosa and the positive correlation between DcR3 over-expression with degree of differentiation may aid in monitoring the progression of GC.

Our finding of a relationship between the DcR3 expression and the clinicopathological parameters is consistent with the study of Takahama et al. showing that the positive immunostaining rate of DcR3 was correlated well with GC clinical TNM stages, lymph node metastasis and systemic metastasis (9). When followed up for 63 months, Takahama et al (9) found DcR3 over-expression in cancerous tissue that was associated with a significantly shortened duration of overall survival compared with that in patients whose cancerous tissue expressed a normal level of DcR3. So, the high coincidental expression of DcR3 protein accumulation might be an important event to enhance GC and a useful biomarker to assess risk for the development and aggressiveness of GC.

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