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BRIEF ARTICLE

Relationship between RGS5 expression and differentiation and angiogenesis of gastric carcinoma

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Abstract

AIM: To explore the regulator of G-protein signaling 5 (RGS5) expression in gastric carcinoma and its association with differentiation and microvascular density (MVD).

METHODS: Expression of RGS5 and CD34 were examined in 76 cases of gastric carcinoma, including 22 cases with lymph node metastasis and 54 cases without lymph node metastasis determined by immunohistochemistry (IHC). MVD was assessed using CD34 monoclonal antibody. The presence of RGS5 and CD34 was analyzed by IHC using the Envision technique.

RESULTS: The RGS5 expression in gastric carcinoma was positively correlated with the differentiation of the tumor (r = 0.345, P < 0.001), but not related with age, gender, tumor size, clinical stage and lymph node metastasis (P > 0.05). The average MVD in the group with lymph node metastasis was significantly higher than that in the group without lymph node metastasis (P < 0.05). RGS5 expression was negatively correlated with the average MVD (P < 0.05).

CONCLUSION: RGS5 expression level in gastric carcinoma is associated with the differentiation and MVD of the tumor, and may be used as an important parameter for determining the prognosis of gastric carcinoma patients.

Key words: RGS5 expression; Gastric carcinoma; Differentiation; Microvascular density

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INTRODUCTION

Gastric carcinoma is one of the most common malignant tumors, and lymph node metastasis is the most common and the earliest transfer channels. Regulator of G-protein signaling 5 (RGS5) is a member of the RGS superfamily and acts as a negative regulator of heterotrimeric G-protein-mediated signaling through G-protein-coupled recep-

tors (GPCRs)[1,2]. Recently, RGS5 has been found involved in tumor angiogenesis and metastasis[3-6]. We used immunohistochemical technique to examine the expression of RGS5 and CD34 in 76 cases of gastric carcinoma in order to explore the relationship between RGS5 expression and differentiation and angiogenesis of gastric carcinoma.

MATERIALS AND METHODS

Samples, histological examination and reagents

We studied 76 patients with gastric adenocarcinoma who underwent operation without radiotherapy and chemotherapy in the Nanjing General Hospital of Nanjing Military Command, from March 2005 to October 2009, including 46 men and 30 women, with an average age of 55.95 years. There were 42 stage Ⅰ, 12 stage Ⅱ and 22 stage Ⅲ cases; the tumors were highly differentiated in 22, moderately differentiated in 30 and poorly differentiated in 24 cases. Two senior pathologist reviewed the morphologic classification of the tumors according to the WHO specifications and evaluated the adequacy of biopsy specimens for further tests. Specimens were promptly fixed and embedded, and the slices were made with a thickness of 2-4 μm.

The antibody used for RGS5 was rabbit polyclonal (Sigma Inc., USA) at 1:80 dilution. The antibody used for CD34 was mouse monoclonal (Neo Markers Inc., USA) at 1:200 dilution. The diaminobenzidine tetrahydrochloride was obtained from DAKO Company.

Immunohistochemistry

The presence of RGS5 and CD34 was analyzed by Immunohistochemistry (IHC) using the Envision technique. Antigen retrieval was carried out by high temperature and pressure cooking of the slices in 15 mL EDTA. The slices were rinsed in phosphate buffered solution (PBS) (0.01 mol/L, pH 7.4) for three times, incubated with the primary antibody overnight at 4℃ and washed again in PBS for three times. They were then incubated with the anti-rabbit and mouse horse radish peroxidase polymer reagent for 12 min at room temperature, and washed in PBS three times as above. The reaction product was developed using diaminobenzidine tetrahydrochloride. Finally, the slices were counterstained with hematoxylin, dehydrated and mounted in resinous mountant. Negative controls with PBS (0.01 mol/L, pH 7.4) replacing the primary antibody were also included.

Any brown cytoplasmic staining of cells was taken as positive expression for RGS5. The tissue sections were screened at a high power (× 200) and five areas with the most intense expression were selected. Briefly, a mean percentage of positive cells was determined in at least five areas (× 200) and assigned to one of the following five categories: < 5% (-); 5%-25% (+); 25%-50% (2+); 50%-75% (3+); and ≥ 75% (4+). A mean percentage of positive cells < 50% was considered as having low expression, and that ≥ 50% was considered as having high expression. Microvascular density (MVD) was assessed using CD34 monoclonal antibody. Any brown cytoplasmic or membranous staining of vascular endothelial cells was taken as positive expression for CD34. The tissue sections were screened at a low power (× 40) and three areas with the most intense neovascularization were selected. Microvessel counting was performed at a high power (× 400) in these areas.

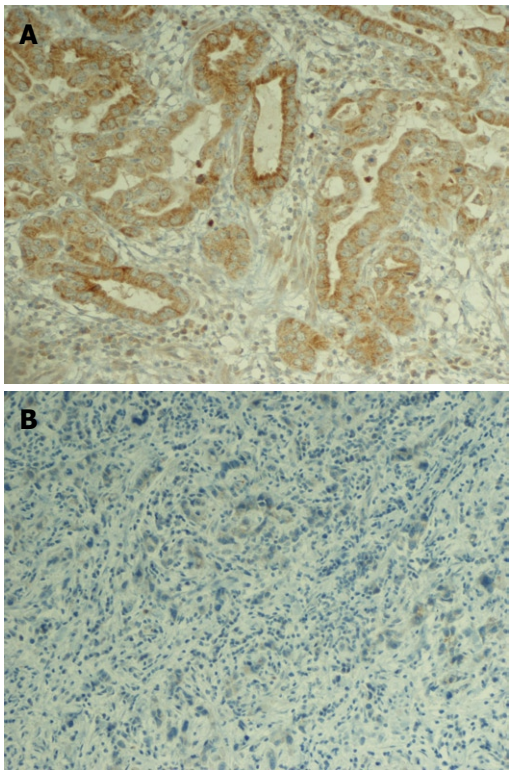

Figure 1 Expression of regulator of G-protein signaling 5 (× 200). A: High regulator of G-protein signaling 5 (RGS5) expression in highly differentiated gastric adenocarcinoma; B: Low RGS5 expression in poorly differentiated gastric adenocarcinoma.

Statistical analysis

Statistical analyses were performed using the Software Packages for Social Science 13.0 for Windows (SPSS, Inc, Chicago, IL, USA). Associations of RGS5 expression with clinical parameters of patients were described by the Chi-square test. Fisher's exact test was also used when necessary. Relationship between expression of RGS5 and clinical parameters of patients was analyzed using the Spearman rank correlation analysis. Average MVD was calculated and analyzed using the independent-samples *T* test. *P* values < 0.05 were considered significant.

RESULTS

Expression of RGS5

RGS5 was mainly expressed in the cytoplasm of gastric carcinoma cells, and the strong positions were in the regions infiltrated by the tumor cells (Figure 1). The RGS5 expression level in gastric carcinoma was positively correlated with the differentiation ($r = 0.345$, $P < 0.001$, Table 1),

Table 1 Relationship between clinical parameters of patients and expression of regulator of G-protein signaling 5 in gastric carcinoma

Variables	*n*	+	+ +	+ + +	+ + + +	Spearman correlation	*P*
Differentiation						0.345	0.000
Low	24	16	4	4	0		
Moderate	30	4	8	12	6		
High	22	6	8	0	8		
Gender						-0.219	0.059
Male	46	12	14	8	12		
Female	30	14	6	8	2		
Lymph node metastasis						0.082	0.343
Positive	22	8	8	2	4		
Negative	54	18	12	14	10		
Tumor size (cm)						-0.053	0.766
≤ 4	58	20	14	12	12		
> 4	18	6	6	4	2		
Age (yr)						0.122	0.197
≤ 55	36	16	6	8	6		
> 55	40	10	14	8	8		
Clinical stages						-0.192	0.184
Ⅰ	42	12	8	12	10		
Ⅱ	12	6	4	2	0		
Ⅲ	22	8	8	2	4		

Table 2 Relationship between microvascular density and clinical parameters of patients and expression of regulator of G-protein signaling 5 in gastric carcinoma

Variables	*n*	MVD (mean ± SD)	*P*
Gender			
Male	46	11.08 ± 7.87	0.221
Female	30	16.07 ± 13.99	
Lymph node metastasis			
Positive	22	19.17 ± 12.03	0.014
Negative	54	10.29 ± 9.04	
Tumor size (cm)			
≤ 4	58	13.59 ± 12.06	0.548
> 4	18	11.27 ± 5.87	
Age (yr)			
≤ 55	36	11.61 ± 10.23	0.481
> 55	40	14.05 ± 11.17	
Expression of RGS5			
High	30	9.15 ± 7.16	0.023
Low	46	16.75 ± 12.37	

MVD: Microvascular density; RGS5: Regulator of G-protein signaling 5.

but not correlated with age, gender, tumor size, clinical stages and lymph node metastasis ($P > 0.05$, Table 1).

CD34 was expressed in the cytoplasm or membrane of vascular endothelial cells. The staining of cells was uniform. And the areas with intense microvascularization were the regions infiltrated by the tumor cells (Figure 2). The average MVD in the group with lymph node metastasis group was significantly higher than that in the group without lymph node metastasis ($P < 0.05$, Table 2), but not correlated with age, gender and tumor size ($P > 0.05$, Table 2).

Relationship between RGS5 expression and MVD in gastric carcinoma

The average MVD in high RGS5 expression group was significantly lower than that in low RGS5 expression group ($P < 0.05$, Table 2, Figure 2).

DISCUSSION

G protein-coupled biological processes are important for an ever-increasing number of human diseases[7]. RGS5 is a member of the RGS superfamily, and is involved in a number of processes of diseases, such as atherosclerosis[8]. In normal tissues, RGS5 was found to be highly up-regulated in PDGFR-β^+ pericytes and played an important role in developmental processes of blood vessels[9-12]. Recently, it has been indicated that RGS5 expresses in the early stages of blood vessel maturation and regulates the development of vascular pericytes[13-23]. However, there have been fewer studies about RGS5 expression in tumors. Chen *et al*[3] and Furuya *et al*[4] discovered that RGS5 highly expressed in vascular pericytes of both hepatocellular carcinoma and renal cell carcinoma. In addition, tumor metastasis depends on the newborn blood vessels, and RGS5 was found involved in tumor angiogenesis[24]. However, the function of RGS5 in development or angiogenesis of gastric carcinoma is still unclear.

We used IHC method for the first time to examine the expression of RGS5 protein in gastric carcinoma. The results showed that expression of RGS5 protein varies in different gastric carcinoma patients, indicating that the function of RGS5 protein in occurrence of gastric carcinoma is manifold. Forty-six (61%) cases had low RGS5 expression, and most of them had poorly differentiated gastric carcinoma ($P < 0.001$). Tumor occurrence and development are associated with multiple genes, and RGS5 protein is only a part of the signal transduction pathway. RGS5 acts as a negative regulator of heterotrimeric G-protein-mediated signaling through GPCRs[1]. RGS5

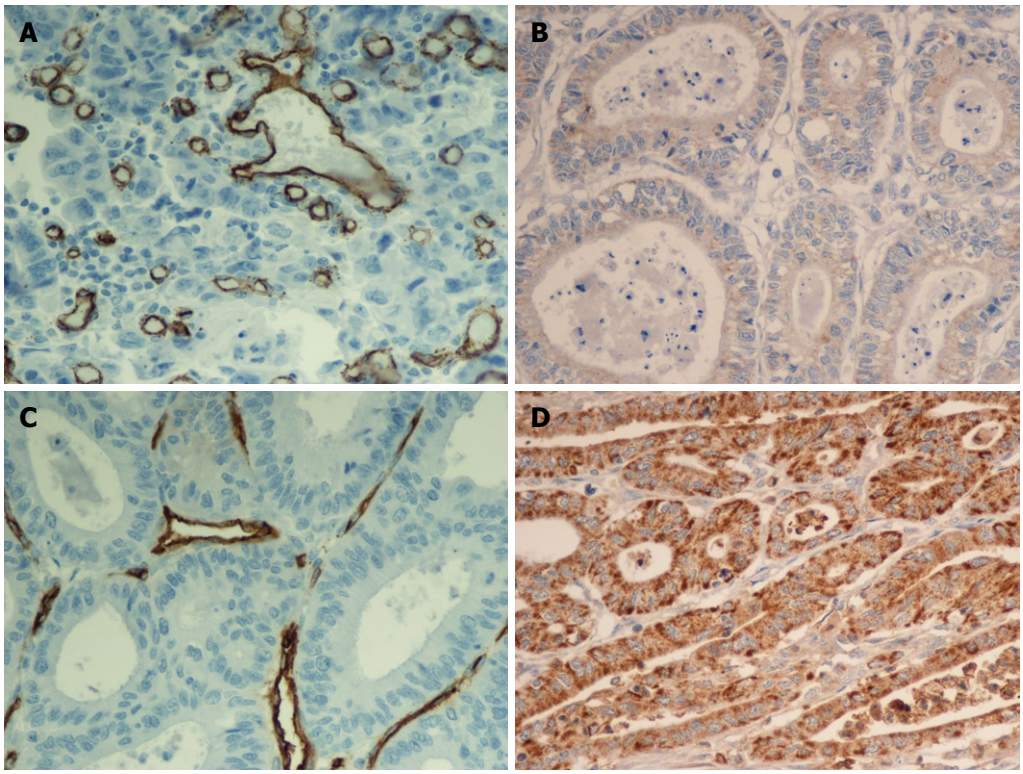

Figure 2 High microvascular density in gastric adenocarcinoma with low regulator of G-protein signaling 5 expression (A, B) and low microvascular density in gastric adenocarcinoma with high regulator of G-protein signaling 5 expression (C, D). Envision, × 400.

was found to be involved in tumor angiogenesis, and perhaps regulate tumor progress.

We found that the RGS5 expression level in gastric carcinoma was negatively correlated with the average MVD. The average MVD in the group with lymph node metastasis was significantly higher than that in the group without lymph node metastasis, but the RGS5 expression level in gastric carcinoma was not correlated with lymph node metastasis. This may be related to the number of samples and methods of detection. In addition, in the aspect of regulation of the tumor growth, we have found that the tumor blood vessels of RGS5 gene knockout mice tended to be mature and differentiated[25-28], indicating that expression of RGS5 is a factor of tumor blood vessel abnormalities and may play a certain role in regulating the invasion and metastasis of tumor cells.

In addition, RGS5 expression can be used to determine the prognosis of renal clear cell carcinoma together with a number of other indicators[8,29,30]. Our study suggested that RGS5 expression level in gastric carcinoma was significantly associated with the differentiation and MVD of the tumor, also indicating the trend of disease development. Many researches have confirmed that the differentiation, MVD and some other clinical pathological parameters are important factors for the prognosis of gastric carcinoma. Therefore, RGS5 protein may be an effective indicator for evaluating the malignant degree of gastric carcinoma.

However, there have been fewer studies about the relationship between RGS5 and tumors reported in literature. The function of RGS5 in tumor development or tumor angiogenesis needs to be further studied.

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COMMENTS

Background

G protein-coupled biological processes are important for an ever-increasing number of human diseases. Regulator of G-protein signaling 5 (RGS5) is a member of the RGS superfamily and acts as a negative regulator of heterotrimeric G-protein-mediated signaling through G-protein-coupled receptors. Recently, RGS5 has been found involved in tumor angiogenesis and metastasis.

Research frontiers

High RGS5 expression has been found in the vascular pericytes of hepatocellular carcinoma and renal cell carcinoma. In addition, tumor metastasis depends on the newborn blood vessels, and RGS5 is involved in tumor angiogenesis. The expression of RGS5 is a factor of tumor blood vessel abnormalities and may play a certain role in regulating invasion and metastasis of tumor cells.

Innovations and breakthroughs

The authors used immunohistochemical method for the first time to examine the expression of RGS5 protein in gastric carcinoma.

Applications

There have been fewer studies about the relationship between RGS5 and tumors. The function of RGS5 in tumor development or tumor angiogenesis needs to be further studied.

Peer review

In this manuscript, Wang *et al* examined the expression of RGS5 and its relationship with differentiation and microvascular density (MVD) in a panel of gastric tumors. They found that RGS5 expression is associated with differentiation,

and negatively correlated with average MVD. These findings have not been described and are potentially important. The quality of representative pictures is high.

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