Expression and Significance of nm23 mRNA, CD44s and CD44v6 in Human Gastric Adenocarcinoma

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Abstract **Objective** To investigate the relationship between the expression level of nm23 mRNA, CD44s, CD44v6 and the pathology grade, invasion, lymph node metastasis of human gastric adenocarcinoma. **Methods** The expressions of nm23 mRNA, CD44s and CD44v6 in 40 human gastric adenocarcinoma and 22 normal gastric mucosa were detected by immunohistochemical (IHC) staining and in situ hybridization (ISH). **Results** The expression of nm23 mRNA in gastric adenocarcinoma had no significant difference from that in normal gastric mucosa (P>0.05), and did not correlate with the invasion and pathology grade of the tumor (P>0.05). However, it was associated with the lymph node metastasis of gastric adenocarcinoma negatively (r=-4.93). The expression of CD44s in the tissues of gastric adenocarcinoma and normal gastric mucosa were 48% (19/40) and 13.6% (3/22), respectively, and had significant difference (P<0.01). But CD44v6 was expressed in the tissues of gastric adenocarcinoma only, and the positive rate was 63.3% (25/40), which was not only significantly associated with the lymph node metastasis and invasion of the tumor, but also associated with pathology grade (r=5.04). **Conclusion** The patients who had overexpression of CD44v6 and low expression of nm23 mRNA has a higher metastatic rate of lymph node. Combined detection of the expression of nm23 mRNA and CD44v6 is a reliable index to evaluate the metastatic capacity of gastric adenocarcinoma. Overexpression of CD44v6 is correlated with invasion of the tumor and pathology grade of gastric adenocarcinoma.

Key Words nm23 mRNA; CD44s; CD44v6; gastric adenocarcinoma; in situ hybridization; immunohistochemistry

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INTRODUCTION

Of all malignant neoplasm, human gastric adenocarcinoma is among the tumors with the poorest prognosis. This undesirable prognosis in the patients with gastric adenocarcinoma has been attributed to invasion and metastasis, which leads to the failure of the treatment of gastric adenocarcinomas. Metastasis is a complex progression of a series of sequential events, which is positively or negatively regulated by various genes[1]. During the several past years, the metastasis-associated genes and the ones inhibiting the metastasis process have been cloned in succession [2]. CD44 and its v6 spliced variant and nm23 genes expression in the intestinal, breast and lung carcinomas have been reported[3-6]. Therefore, the aim of this study was to study the relationship between the expression level of nm23 mRNA, CD44s and CD44v6 and the pathology grade, invasion, lymph node metastasis of human gastric adenocarcinoma by using immunohistochemical (IHC) staining and in situ hybridization (ISH).

MATERIALS AND METHODS

Materials

40 cases of gastric adenocarcinoma and 22 cases of normal gastric tissues were enrolled in this study. Among the 40 cases of gastric adenocarcinoma, 20 cases were mid-differentiated, 20 cases were poorly differentiated, 15 cases had lymph node metastasis, and 23 cases were invasive carcinomas. All patients underwent operation at Xijing Hospital of the Fourth Military University, China in 2001. The resected specimens were fixed in 10% formaldehyde and sliced into 5um sections.

Immunohistochemical analysis

Histological sections (5um) were mounted on poly-L-lysine-coated slides and dried for 12 to 24 hour at 37oC. Immunohistochemistry was performed with the SABC kit (Sina-America Biotechnology Company), and the primary antibodies were monoclonal anti-CD44s and -CD44v6 (MBI production). The method for staining was performed as described documents of SABC kit.

Detection of mRNA expression of nm23 by in situ hybridization

Pre-hybridization solution, nm23 oligonucleotide probe hybridization solution and the CSA Test Kit were bought from Boster Biotechnology Company. All reagents and containers were treated by DEPC. The sec-

tions were dewaxed to water by normal technique, and then immersed into 3% H_2O_2 in room temperature for 10 min and washed twice by DW. Fresh diluted protease K 20μg/ml was added and digested for 20 min in 37℃ to expose mRNA nucleic acid segments. 20% glycerine 20ml was added in dry bottom of the Test Kits to keep humidity. Pre-hybridization solution 20μl was added onto every section and keep for 4 h in 370C, then the supernumerary liquid was absorbed without washing. Hybridization solution was added as mentioned above. The section was covered by protective membrane then put it in homeothermia 40℃ overnight. After removing the cover glass, the slice was washed in 37℃ as follows: 2× SSC 5min twice; 0.5×SSC 15min once; 0.2×SSC 15min once. It was kept in 3% BSA for 30min and in seal solution for 30min. Then biotin labeled anti-digitoxin antibody was added and lasted for 60 min in 37℃, after washing by 0.5M PBS. Biotin-peroxidase was added and lasted for 20 min in 37℃ then washed by PBS. Color was showed by DAB. The last treatment as follows: redyeing, dehydrating, pellucidum and sealing the slice.

Assessing of immunohistochemical and in situ hybridization staining

① IHC Staining: Membrane staining and positive signals were brown-yellow particles. Specimen was considered to be positive when >50% of the tissue component was stained in the appropriate cellular compartment. ② ISH Staining: Cytoplasmic staining and positive signals were blue particles. Specimen was considered to be positive when >50% of the tissue component was stained in the appropriate cellular compartment.

Statistical analysis

Statistical analysis was performed with Chi-square test and correlation analysis. P<0.05 was considered as statistically significant.

RESULTS

The relationship between the expression of CD44s and the clinical pathology of human gastric adenocarcinoma

The expression of CD44s in the tissues of gastric adenocarcinoma and normal gastric mucosa were 48% (19/40) and 13.6% (3/22) respectively (Fig 1,2), and had significant difference (χ^2=10.29, P<0.01). However, the expression of CD44s did not significantly associated with the lymph node metastasis. In addition, there was no statistical significance found between the middle- and poor- differentiation or with and without invasion, P >0.05, respectively (Table 1).

The relationship between the expression of CD44v6 and the clinical pathology of human gastric adenocarcinoma

The expression of CD44v6 in the tissues of gastric adenocarcinoma and normal gastric mucosa were 63.3% (25/40) and 0 (0/22) respectively (Fig 3). There was statistical significance found between the middle- and poor-differentiation of gastric adenocarcinoma (χ^2=9.19, P<0.01), with and without invasion (χ^2=22.22, P<0.001), as well as with and without the lymph node metastasis (χ^2=10.36, P<0.01). (Table 1).

The relationship between the expression of nm23mRNA and the clinical pathology of human gastric adenocarcinoma

The expression of nm23mRNA in the tissues of gastric adenocarcinoma and normal gastric mucosa were

Table 1 The relationship between the expression of CD44s, CD44v6, nm23 mRNA and pathologic features

Pathologic features	case	CD44s		CD44v6		nm23mRNA	
		+	%	+	%	+	%
Pathologic grade							
Ⅱ	20	8	40.0	10	50.0	9	45.0
Ⅲ	20	10	50.0	15	75.0	10	50.0
Invasion							
+	23	11	47.8	18	78.2	11	47.8
-	17	7	41.1	7	41.1	8	47.1
Lymph node metastasis							
+	15	6	40.0	12	80.0	4	26.7
-	25	12	48.0	13	52.0	15	60.0
Age(Yr)							
>60	21	10	47.2	13	61.9	9	42.9
<60	19	8	42.1	12	63.1	1 0	52.6
normal gastric mucosa	22	3	13.6	0	0	9	43.0

47% (19/40) and 43% (9/22) respectively (Fig 4) and there was no statistical significance difference (P>0.05). However, the expression of nm23mRNA in gastric adenocarcinoma with/without the lymph node metastasis was 26.7%(4/15) and 60%(15/25), respectively. The positive rate of nm23mRNA had statistical significant difference (χ^2=18.47, P<0.001) (Table 1).

The relationship between the expression of CD44s, CD44v6 and nm23mRNA and the age of patients

The expression level of nm23 mRNA, CD44s and CD44v6 is not associated with the age of patients (P>0.05) (Table 1).

The relationship between the expression of CD44v6 and nm23mRNA in the human gastric adenocarcinoma

The expression of CD44v6 was associated with the lymph node metastasis of gastric adenocarcinoma positively (r=5.04). However, the expression of nm23 mRNA was negatively associated with that (r=-4.93). (Table1).

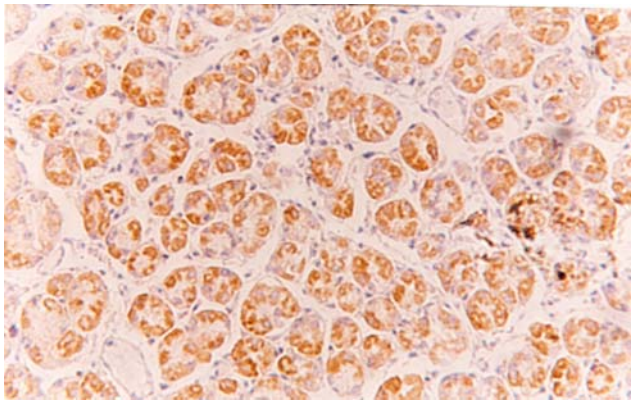

Fig.1 The expression of CD44s in the tissues of normal gastric mucosa SABC ×200

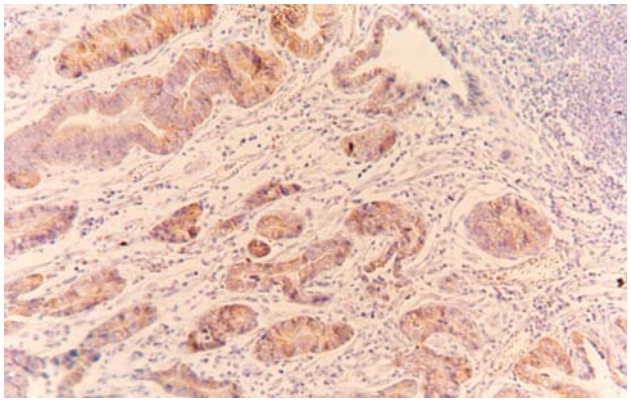

Fig. 2 The expression of CD44s in the tissues of gastric adenocarcinoma SABC ×200

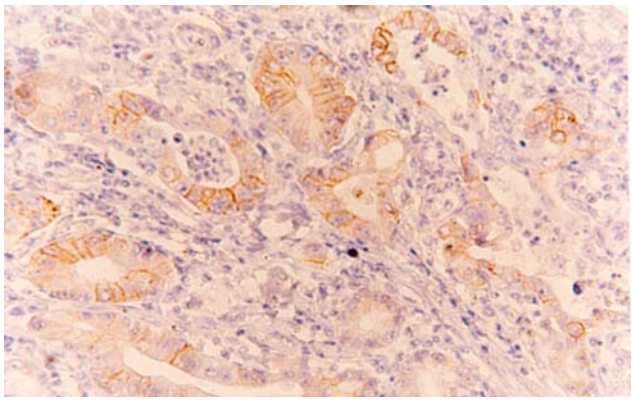

Fig. 3 The expression of CD44v6 in the tissues of gastric adenocarcinoma SABC ×400

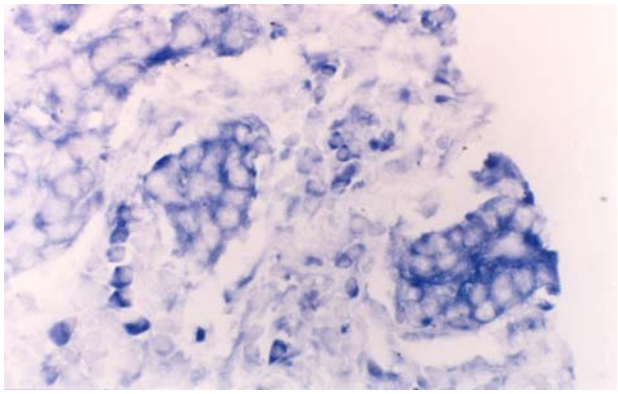

Fig. 4 The expression of nm23 mRNA in the tissues of gastric adenocarcinoma ISH ×400

DISCUSSION

As a cell adhesion molecule, CD44s, hyaluronic acid receptor, is considered important in regulating invasion and metastasis of tumor[7]. Previous studies have shown that the expression of CD44s was an important biological marker to predict metastatic potential [8,9]. Using reverse transcription polymerase chain reaction followed by Southern Blotting, Yamamichi[10] examined the expression of the standard and variant forms (V6 and V9) of CD44 mRNA in 73 cases of gastric cancer, the results showed that the expression status of the standard form of CD44 mRNA correlated with peritoneal dissemination only, and that of CD44v9 mRNA did not significantly correlate with any clinic-pathologic factor. Li[11] reported that 74% of gastric cancer and 80% of invasive carcinoma were positive for CD44v6 respectively, these data show that CD44v6 is also a useful marker of tumor invasion and metastasis.

In this study, the expression of CD44v6 significantly correlated with tumor differentiation, lymph node metastasis and invasion of tumor. The expression of CD44s and CD44v6 in gastric cancer was much higher

than normal stomach and was associated with genesis, metastasis and clinically aggressive behavior of gastric adenocarcinoma. Xin[12,13] thought that the expression of CD44v and its variants was associated with prognosis and five-year survival period. But some other researchers reported[14~16] that the expression of CD44v did not correlate with five-year survival period.

In generally, nm23 variants, such as allele deletion, site mutation and aberrant proliferation, are significantly associated with high metastatic potential of a malignant tumor. Our studies showed that the expression of nm23 mRNA did not correlate with tumor invasion and histological grades (p>0.05), but negatively correlated with lymph node metastasis (r=-4.93).

A gene synergic hypothesis that the aberrant activation of two or more genes plays different roles during different stages of genesis, progression and metastasis of malignant tumor is widely identified. They think that these genes synergically facilitate the cancerization. Our studies show that the expression of nm23 mRNA (lower expressed) in gastric adenocarcinoma negatively correlate with that of CD44v6 (higher expressed), which was closely associated with the metastasis of gastric adenocarcinoma. These results indicate that nm23 and CD44v6 synergically play positive and negative roles during the lymph node metastasis of gastric adenocarcinoma. Therefore, further studies should be continued to investigate the relationship between nm23 and CD44v6, which will be important for tumor metastasis.

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