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위암환자에서 GAGE 유전자의 발현과 임상적 의미

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The expression and clinical implication of GAGE gene in Stomach Cancer

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Abstract

Background: There is still little information on the function of GAGE gene in the stomach cancer except for cancer-specific gene expression recognized by autologous T lymphocytes. This study attempted to evaluate GAGE mRNA expression to elucidate its functional role in the carcinohistogenesis and clinical implication in gastric cancer. Material and Method: Tumor and non-neoplastic paired samples from 60 patients with gastric cancer were studied by using reverse transcription - nested polymerase chain reaction (RT-PCR) with common primer. Results: No expression of GAGE was observed in non-neoplastic tissues. Fifteen out of 60 tumor tissues expressed GAGE (25.0%) mRNA , of which 13 cases (86.7%) were intestinal type and only 2 cases (13.3%) were diffuse type. GAGE expressions in cancer tissues have a significant tendency to be higher by stage and lymph node metatastasis ($p<0.05$). However, they did not show significant relationship with tumor cell differentiation and vascular and perineural invasions. Conclusions: This results suggest that GAGE gene might have an important role in the development and progression of intestinal type of stomach cancer and GAGE gene may be a useful molecule for target of cancer-specific immunotherapy.

Key words : Stomach cancer, GAGE gene, histogenesis, immunotherapy

Introduction

In recent years, several tumor-specific shared antigen families, such as MAGE, GAGE BAGE, and LAGE/NY-ESO-1 which are recognized by autologous CTL on human tumors have been characterized at the molecular level[1]. These usually consist of peptides derived from intracellular proteins and presented to CTL by HLA class I molecules[2,3]. These antigens are of particular interest in tumor immunology, because their expression, with exception of testis and fetal tissues, seems to be restricted to tumor cells[3]. Therefore, these gene products have been called as "Cancer/testis antigen (CTAs)".

GAGE are located in the p11.2-p11.4 region of chromosome X and has more than 8 isotypes (GAGE 1-8)[4,5]. Various types of malignancies, such as melanoma, lung cancer, urinary bladder cancer were reported to express GAGE genes[4,5]. However, little information is presently known about the function or clinical implication of GAGE genes in addition in stomach cancer.

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Stomach cancer is the foremost cancer in Korean. In spite that various ways to fight the stomach cancer have been tried, advanced stomach cancer in Korea is still problematic. Immunotherapy can be considered as promising adjuvant or recurrent-preventive therapy by using GAGE gene products[6,7]. Basic knowledge about GAGE genes such as the expression pattern and functional role in stomach cancer will be required, before clinical trial of this immunotherapy. Hence, this study attempted to evaluate the expression of GAGE gene in surgical samples of gastric cancer and gastric juice and to analyse the relationship between GAGE expression and the clinico-pathological parameters of gastric carcinoma.

Materials and Method

1. Materials : Patients and Sample Collection

Sixty primary gastric cancer underwent gastrectomy with lymph node dissection were selected. In each patient, 60 fresh and paired non-neoplastic gastric tissue adjacent tumor were collected. In 18 out of 60 patients, gastric juices were collected.

All surgical specimens were processed for standard histologic analysis; a portion of the tumor was frozen in liquid nitrogen immediately after resection and stored at 80oC until RNA extraction. The clinicopathologic data from these patients are summarized in Table 1.

Table 1. Clinicopathological features of 60 patients with stomach cancer

Variable	Diffuse type	Intestinal type
Number of cases	30	30
Differentiation		
Well-	10	10
Moderate-	10	10
Poorly-	10	10
Depth of invasion		
Mucosa	7	7
Submucosa	7	7
Muscle	7	7
Serosa	9	9
Metastasis		
Positive	15	15
Negative	15	15
Vessel invasion		
Positive	15	15
Negative	15	15
Perineural invasion		
Positive	15	15
Negative	15	15

Tumors were staged and graded histologically, according to the 5th edition of TNM classification of the International Union against Cancer (UICC).

2. Method : Oligonucleotide primers for GAGE

Pairs of inner primers are designed to amplify sections of coding sequence appropriate for the screening GAGE1-8 subtypes in one or two runs, as all 8 subtypes contain the same segment of sequences(Table 2).

Table 2. Common Primer sets of GAGE genes for nested PCR

Sbtypes	Sequence	No. of base	GC%
for first round PCR			
(sense)	CC TCTACTGAGATTCATCTGTGTG	24	46%
(antisense)	GCAGCCTGCATCATTTCAACGTG	23	52%
for second round PCR			
(sense)	AGTTGGCGAGGAAGATCGAC	20	55%
(antisense)	TCTTCTTTTAACACTGTGATTGC	23	35%

GAGE primer pairs are designed in such way that the 5' (sense) and 3' (antisense) primer span at least one intron in the genomic DNA.

3. Reverse transcripted-nested polymerase chain reaction (RT-nested PCR) Assay

Total RNA was extracted by homogenizing the sample in RNAZOL B reagent. CDNA was synthesized from 2g of total RNA in a reaction volume of 20L with 4L of 5X reverse transcriptase buffer(GIBCO BRL, Catbersbug,

MD), 1L each of 2.5 mM deoxynucleotide(dNTPs), 1L of a 500g/mL solution of oligo(dT)12-18 primers, 20 units of RNaseOUT(GIBCO BRL), 2L of 0.1M 1,4-dithiothreitol, and 200 units of Moloney murine leukemis virus reverse transcriptase (GIBCO BRL); the reaction was incubated at 42oC for 60 minutes and then diluted to 40L with water.

Two microliters of the cDNA mixture were used for each PCR amplification in a 50L reaction Volume containing 1L of each primer40(M), 1L each of 2.5mMdNTP, 1.5mM MgCl2, and 2 units Taq DNA polymerase(promega;Madison:WZ) in buffer A, which was supplied by manufacturer. The primers used in this study was described in Table 2. Thirty-two amplification cycles were run: 1 minute at 94oC and 2 minutes at 55oC and 2 minutes at 72oC. Cycling was concluded with a final extension step of 15 minutes at 72oC. To verify RNA integrity in each sample, 23 cycles(for 1 minute at 94oC, 2 minutes at 72oC) were run with primers specific for beta-actin. After amplification, PCR products were analyzed by agarose gel electrophoresis.

4. Statistical Analysis

Statistical analyses were performed using ASA statistical package(SAS, INC., Cary,NC). Differences between groups were assessed by chi-square analysis, Fisher exact test, a student t-test, as indicated. All p values<0.05 were considered significant.

Results

1. Expression of GAGE genes in gastric cancer; The expression rates of GAGE gene were 25.0% (15/ 60 cases) in gastric cancer tissue (Fig.1),

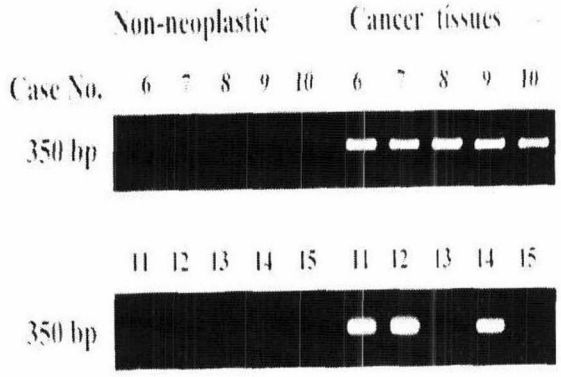

Fig. 1. Representative cases of GAGE expression in paired non-neoplastic and carcinoma tissues of stomach

whereas GAGE gene was not expressed in non- neoplastic gastric tissues and juices.

2. The correlation between GAGE expressions and Lauren classification of gastric cancer ; GAGE gene was largely expressed in intestinal type of gastric carcinoma with 86.7% (13/15 cases) and only 2 cases (13.3%) were diffuse type.

3. The relationship between GAGE expression and clinicopathological parameters; GAGE was expressed 7 cases of poorly-differentiated carcinoma and 8 cases of well- and moderately-differentiated carcinoma. There was no significant correlation between GAGE mRNA expression and tumor cell grades by AJCC (Table 3).

Table 3. Prevalence of GAGE expression in relation to differentiation of tumor cells

Differentiation	Diffuse type		Intestinal type	
	+	-	+	-
Total of cases	2	28	13	17
Well-	0	10	5	5
Moderate-	0	10	3	7
Poorly-	2	8	5	5

P>0.05

In diffuse type carcinoma, all two GAGE-expressed cases were serosa- involved cases. In intrestinal type carcinoma, the GAGE mRNA expression showed 14.3% in mucosal carcinoma and 77.8% in serosa-involved cases. The GAGE expression have a significant tendency to be higher by depth of invasion($p<0.05$) (Table 4).

Table 4. Prevalence of GAGE expression in relation to depth of invasion

Depth of Invastion	Diffuse type		Intestinal type	
	+	-	+	-
Total cases	2	28	13	17
Mucosa	0	7	1	6
Submucosa	0	7	2	5
Muscularis	0	7	3	4
Serosa	2	7	7	2

$P<0.05$

The GAGE expression was significantly correlated with the presence of lymph node involvement($p<0.05$) (Table 5)

Table 5. Prevalence of GAGE expression in relation to metastasis

Metastasis	Diffuse type		Intestinal type	
	+	-	+	-
Total of cases	2	28	13	17
Positive	2	13	9	6
Negative	0	15	4	11

$P<0.05$

The incidence of vessel invasion and peri-or-neural invasion were 8 cases(13.7%) in each. There are no significant relationships between GAGE expression and vascular and perineural invasions (Table 6).

Table 6. Prevalence of GAGE expression in relation to perineural and vessel invasion

Variables	Diffuse type		Intestinal type	
	+	-	+	-
Total of cases	2	28	13	17
Vessel invasion				
Positive	1	14	1	8
Negative	1	14	6	9
Perineural invasion				
Positive	1	14	7	8
Negative	1	14	6	9

Discussion

Human tumor antigens recognized by CTLs have been identified in recent years. An important category of these so called T-cell-defined tumor antigens consists of normal gene products that are not expressed in most body tissues, with the exception of male germ cells and placenta, while are activated in a number of different tumors[3,4]. These antigens are currently regarded promising targets for active, specific tumor immunotherapy. GAGE gene belongs to one of these tumor-shared antigens.

This report demonstrated the expression of GAGE genes by RT-PCR with originated common primers in gastric carcinoma samples were 25.0% in association with well-known poor prognosticators. Li J et al[8] reported the expression rate of gastric cancer cell lines and surgical samples were 36% and 16%. This means both results is approximately concordant.

In other studies, esophageal squamous cell carcinoma showed 17% and stomach cardiac adenocarcinoma showed 13%[9] of GAGE expression. Hence, the expression pattern of GAGE gene is heterogenous to histologic types of tumor ranging from 0% in colon cancer to 30% in melanoma[3,4]. This results showed most of positive cases were intestinal type (86.7% in tumor sample and 100% in gastric juices), which seems to be supported by the Li J et al[8] carcinoma.

Since only intestinal type carcinoma is associated with chronic atrophic gastritis, severe intestinal metaplasia and dysplasia, the duration of oncogensis is longer in intestinal type than in diffuse type. This means the intestinal type carcinoma may offer more opportunities to alter DNA methylation status.

In this study, the results suggest that stomach cancer tends to express GAGE mRNA in the course of the development and progression towards invasive cancer, as showing higher frequency in deeply invasive and metastatic tumors than in early invasive and non-metastatic tumors. There was a report similar to this result by Zambon[9] that BAGE or GAGE expression was related to significantly to a poor prognosis. This result also can be supported by Cheung (1999[10]) report that evaluated the prognostic significance of GAGE expression in the blood and bone marrow of a cohort 133 patients with malignant melanoma found a significant association between the molecular detection of GAGE in peripheral blood drown immediately after surgical resection and overall poor survival, suggesting that GAGE positivity marks tumor cells with greater malignant potential. Thus, the adverse implications of GAGE detection in gastric tumor cells for patient survival especially in intestinal type deserve further investigation, although the real mechanism on tumor progression is unclear at this moment.

GAGE gene belongs to a family of more than 8 subtypes, of which 1/2 subtype encodes tumor antigen presented by HLA-Cw6[11] and is recognized by autologous cytolytic T lymphocytes. And this gene is a promising target of tumor immunotherapy. Therefore, this results suggest that a subset of patients with gastric cancer may be eligible for active specific immunotherapy against GAGE antigens as well as GAGE gene expression might be feasible for the tumor marker with greater malignant potential in gastric carcinoma.

Conclusion

This results suggest that GAGE gene might have an important role in the development and progression of intestinal type of stomach cancer and GAGE gene may be a useful molecule for target of cancer-specific immunotherapy.

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국문초록

배경 : 자가 T 임파구에 의해 인식된 암-특정 유전자 발현을 제외하고 위암에서 GAGE 유전자의 기능에 대한 정보가 아직도 거의 없다. 이 연구는 위암에서 임상적 암시와 암조직발생에서의 기능적인 역할을 밝히기 위해 GAGE mRNA 발현을 평가하기 위한 시도이다. **방법** : 위암 환자 60명으로부터 종양과 비종양 조직을 둘씩 짝을 지어 추출하여 common primer를 가지고 reverse transcription-nested polymerase chain reaction(RT-PCR)을 이용하여 연구하였다. **결과** : 비종양조직에서는 GAGE 발현이 없었다. 60개의 종양 조직으로부터 15개는 GAGE mRNA 발현(25.0%), 13개는 intestinal type(86.7%), 2개만 diffuse type(13.3%)을 나타냈다. 암 조직에서 GAGE 발현은 높은 암의 단계와 림프절 전이에 중대한 영향을 가진다($p<0.05$). 그러나 그들은 종양 세포 분화와 혈관, 신경 주위 침범과는 중대한 관계를 보여 주지 않았다.
결 론 : GAGE 유전자가 위암의 intestinal type 발생과 진행에 중요한 역할을 하고 GAGE 유전자가 암-특정 면역요법의 유용한 목표 분자일지도 모른다는 것을 암시한다.

중심단어 : 위암, GAGE 유전자, 조직발생, 면역요법