• *BRIEF REPORTS* •

Relationship between the downregulation of HLA class I antigen and clinicopathological significance in gastric cancer

Yu-Qing Shen, Jian-Qiong Zhang, Feng-Qing Miao, Jian-Min Zhang, Qin Jiang, Hao Chen, Xiang-Nian Shan, Wei Xie

Yu-Qing Shen, Jian-Qiong Zhang, Feng-Qing Miao, Xiang-Nian Shan, Wei Xie, State Education Ministry Laboratory of Development Genes and Human Diseases, International "HLA Expressiom in China" Reference Laboratory, Southeast University Medical School, Nanjing 210009, Jiangsu Province, China
Jian-Min Zhang, Department of Pathology, The Affiliated Hospital of Southeast University Medical School, Nanjing 210009, Jiangsu Province, China
Qin Jiang, Hao Chen, Department of Pathology, No. 1 Hospital, Lianyungang 222002, Jiangsu Province, China
Correspondence to: Dr. Wei Xie, Department of Genetics and Developmental Biology, Southeast University Medical School, 87 Dingjiaqiao Road, Nanjing 210009, Jiangsu Province, China. wei.xie@seu.edu.cn
Telephone: +86-25-83272510   Fax: +86-25-83272510

Abstract

AIM: To discuss the expression of human leukocyte antigen (HLA) class I antigens in gastric cancer and correlate these with pathologic type and TNM stage.

METHODS: The expression of HLA class I antigen was detected by immunohistochemistry in 185 specimens of gastric cancer, 20 gastric cancer specimens with lymphatic metastasis and 22 controls of normal gastric mucosa using four monoclonal antibodies.

RESULTS: The expression of HLA class I antigen (B/C locus) was significantly downregulated in gastric cancer and in lymphatic metastasis than that in normal gastric mucosa ($\chi^2 = 7.712$, $P<0.05$). The expression of other HLA class I antigens was also downregulated, but the change was slight. There was no relationship between the downregulation of HLA class I antigen and that of β2m and LMP2. The expression of HLA class I (B/C locus) was statistically correlated with pathologic stage in gastric adenocarcinoma ($\chi^2 = 4.164$, $P<0.05$).

CONCLUSION: The expression of HLA class I antigen (B/C locus) was obviously downregulated in gastric cancer and in lymphatic metastasis. This abnormal expression would provide the tumor cells with a way to avoid immunological recognition.

Key words: Downregulation; Gastric cancer

INTRODUCTION

Gastric cancer is one of the most common forms of malignancy and is the major cause of mortality in human population of Jiangsu Province[1]. The development of gastric cancer is a multi-stage and multi-factor process and the host immune system plays an important role in defending tumor occurrence and metastasis. Human leukocyte antigen (HLA) is essential in anti-tumor immune response[2]. HLA molecules bind antigenic peptides generated by antigen processing machinery and present these peptides on the cell surface to T-cell receptor. The recognition of these peptides by CTLs triggers a series of events that can result in tumor cell lysis[3]. Downr-egulated expression of HLA class I molecules has been reported in many tumors of different origin including gastric cancer[4]. HLA class I antigen is a cell surface glycoprotein composed of heavy chain, β2m and a peptide. Any defect in the antigen processing progress such as the transporter proteins associated with antigen-processing TAP-1 and -2[5], the low molecular proteins LMP-2 and -7[6], and a TAP-associated protein Tapasin can result in the downregulation or loss of HLA class I antigen. In the present study, the expression of HLA class I antigen, β2m and LMP2 gene was detected in paraffin-embedded normal gastric mucosa, gastric cancer and lymphatic metastasis by the standard material and methods of international HLA work group[7]. The purpose was to explore the expression of HLA class I in gastric cancer and the relationship between the HLA class I expression and its clinical significance.

MATERIALS AND METHODS

Tissue specimens

A total of 185 gastric cancer specimens (148 males and 37 females; age range 27-80 years), 22 normal gastric mucosa samples and 20 lymphatic metastasis samples were obtained from the Affiliated Hospital of Southeast University Medical School and the No. 1 Hospital of Lianyungang. All the samples were routinely fixed in 40 g/L formaldehyde solution, embedded in paraffin, and cut into 4-μm-thick sections. Samples were selected according to the pathologic diagnosis

and reviewed by a pathologist to confirm the diagnosis.

Immunohistochemical assays

Paraffin sections were deparaffinized with xylene and rehydrated by passage through decreased concentration of ethanol (from 100% to 70%). Endogenous peroxidase activity was blocked by a 20-min incubation at room temperature with 3% H_2O_2. Sections were then microwaved in citrate solution at 750 W for 10 min and preincubated with 2% normal horse serum for 30 min at room temperature followed by an overnight incubation at 4 ℃ with primary antibodies. Sections were incubated with biotinylated secondary antibody and then with ABC reagent at room temperature for 30 min. Diaminobenzidine solution was added to each section until desired stain intensity developed. Counterstain with hematoxylin, clear and mount. Primary antibodies HC-A2 (anti-HLA A locus; working dilution 1:100), HC-10 (anti-HLA B/C locus; working dilution 1:100), L368 (anti-β2m; working dilution 1:50) and SY-1 (anti-LMP2; working dilution 1:100) were a kind gift of Dr. Soldano Ferrone and Dr. Xin-Hui Wang (Department of Immunology, Roswell Part Cancer Institute, Buffalo, NY, USA).

Review and scoring of the section

The standard evaluation method was established at the 12th International Histocompatibility Conference[8]: The percentage and the intensity of stained cells were evaluated independently by at least two investigators: the percentage of stained cells in the whole section was scored as >75%, 25-75% and <25%; the staining intensity was scored as intense, weak and absent. The last scores combined by the precedent two factors as +, ±, -. Lymphocyte and vascular endothelial cell present within the section were considered as positive control, while staining with the isotype matched irrelevant monoclonal antibody MK2-23 was used as negative control.

Statistical analysis

χ^2 test was adopted to examine the relationship between the variables. A *P* value <0.05 was considered statistically significant.

RESULTS

The expression pattern of HLA class I molecules

The staining pattern was same in normal gastric mucosas, gastric cancer, and lymphatic metastasis: the positive staining of HLA class I antigens was located in membrane, while the positive staining of β2m and LMP2 was located in cytoplasm and membrane (Figure 1).

Downregulated expression of HLA class I antigens (B/C locus) in gastric cancer tissue

Of the 22 normal gastric mucosa samples, 16 (73%) were classified as HLA class I (B/C locus) positive. There were 55 cases (35%) with positive HLA class I (B/C locus) expression in gastric cancer, while 5 cases (25%) in lymphatic
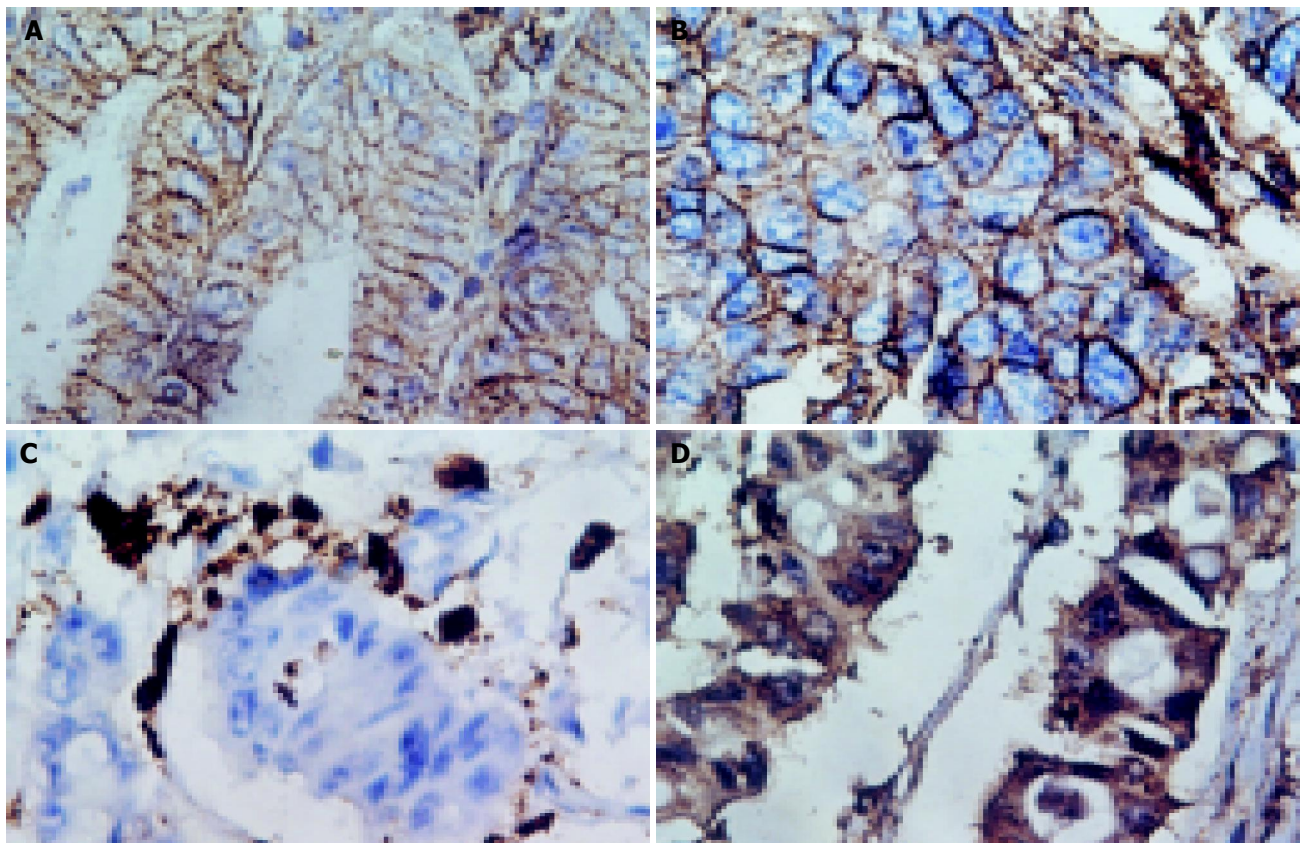

Figure 1 Expression of HLA class I molecule detected by immunohistochemistry in normal gastric mucosa and gastric cancer (original magnification: ×400). **A:** In normal gastric mucosa, the positive expression of HLA class I antigen (B/C locus) was located in membrane; **B:** In gastric cancer, the positive expression of HLA class I antigen (A locus) was located in membrane; **C:** In gastric cancer, the expression of β2m gene was negative (in infiltrate lymphocyte, the expression was positive); **D:** In gastric cancer, the positive expression of LMP2 was located in cytoplasm and membrane.

Table 1 HLA class I antigen (B/C locus) expression in normal gastric mucosa, gastric cancer and lymphatic metastasis, n (%)

Tissue	HLA class I antigen (B/C locus) expression			
	+	±	-	Total
Normal gastric mucosa	16 (72.7)	2 (9.1)	4 (18.2)	22
Gastric cancer				
Histological grade				
I	8 (42.1)	6 (31.6)	5 (26.3)	19
II	13 (39.4)	15 (45.5)	5 (15.1)	33
III	18 (26.1)	23 (33.3)	28 (40.6)	69
Other type	16 (44.4)	13 (36.1)	7 (19.4)	36
Lymphatic metastasis	5 (25)	9 (45)	6 (30)	20

metastasis. These results are shown in Table 1. The positive ratio was significantly higher in normal gastric mucosa than that in gastric cancer and in lymphatic metastasis ($\chi^2 = 7.712$, $P<0.05$). The expression of HLA class I (A locus), $\beta 2m$ and antigen processing molecular LMP2 was also downregulated, but the change was slight (data not shown). There was no relationship between the downregulation of HLA class I antigen and that of $\beta 2m$ and LMP2.

Downregulation of HLA class I antigen (B/C locus) is correlated with pathologic type

To further investigate the relationship between the expression of HLA class I antigen and the clinical pathology, we sorted the gastric carcinomas based on histological grades. Histological grade I means well-differentiated adenocarcinoma, II means moderately differentiated adenocarcinoma and III means poorly differentiated. The results indicated that expression of HLA class I (B/C locus) was statistically correlated with pathologic stage in gastric adenocarcinoma ($\chi^2 = 4.164$, $P<0.05$). We could not find any relationship between the expression of HLA class I antigen (B/C locus) and clinical TNM stage.

DISCUSSION

Recognition of tumor cells by cytolytic T lymphocytes depends on cell surface MHC class I expression. As a mechanism to evade T cell recognition, many malignant cancer cells, including gastric cancer, downregulate MHC class I. Ferron[9], Lopez-Nevot[10], Teh[11] have reported the expression of HLA antigen in gastric cancer in 1980s. But the results were not consistent because of different reagents and methods used by many laboratories. The "HLA expression in cancer" group established in the 12th International Histocompatibility Conference provided a series of standard reagents and methods to several labs that focus on HLA expression in cancer and its correlation with disease progression. Using the same criteria, the research teams could compare their data with others. At the International "HLA Expression in Cancer" reference laboratory, we investigated HLA molecule expression in gastric cancer, which is one of the most common forms of malignancy in Jiangsu Province by using the standard materials and methods of international HLA work group, and correlated these with pathologic type and TNM stage.

In this study, we first investigated the expression of HLA class I antigen in normal gastric mucosa, gastric cancer and lymphatic metastasis. The results indicated that HLA class I antigen (B/C locus) was lowly expressed in gastric cancer

and in lymphatic metastasis compared with normal gastric mucosa, which was similar to the report from Klein[12]. HLA class I antigen is a cell surface glycoprotein composed of heavy chain, $\beta 2m$ and a peptide. Any defect in the antigen processing progress such as LMP2 can result in the downregulation or loss of HLA class I antigen. In gastric cancer, we found that the change of $\beta 2m$ and LMP2 were relatively slight and there was no statistical relationship between the downregulation of HLA class I antigen and that of $\beta 2m$ and LMP2. That is to say, other mechanisms may contribute to this downregulation. In our observation it was the change of HLA heavy chain at DNA and transcription level that lead to HLA class I antigen downregulation (to be published).

We also found that the downregulation of HLA class I antigen (B/C locus) was statistically correlated with pathologic stage in gastric adenocarcinoma. The data shown in Table 1 demonstrated that the expression of HLA class I antigen was higher in high-differentiated adenocarcinoma, while it decreases at advanced stage. The low-differentiated adenocarcinoma, which had lower expression of HLA class I antigen, may have more opportunity to escape from host immune surveillance. This may contribute to its rapid progression and poor prognosis.

In conclusion, the expression of HLA class I antigen (B/C locus) was obviously downregulated in gastric cancer and in lymphatic metastasis. This abnormal expression would provide the tumor cells with a way to avoid immunological recognition. Because recognition of tumor cells by cytolytic T lymphocytes depends on cell surface MHC class I expression, the downregulation might be an obstacle for T-cell-based immunotherapy or peptide vaccination that is of great interest at present[13,14]. For this reason, it is necessary to select the patients who positively express HLA antigen before clinical therapy[15] and it is important to find the mechanism underlying this abnormal expression and a way to promote it in gastric cancer.

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