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Evaluation of multiparameter histologic diagnosis of gastric cancer

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Abstract

AIM: To evaluate multiparameter histologic diagnosis of gastric cancer (GC).

METHODS: Expression of the MGa23c7 antigen and the cerbBa22 receptor was detected in specimens of GC by ABC immunohistochemistry. The DNA content of specimens was determined by flow cytometry (FCM). The data of the multiparameter methodology were analyzed statistically.

RESULTS: The expression rates of MGa23c7 antigen and cerbBa22 receptor were observed in gastric tubular adenocarcinoma (GTA; 100% and 56% respectively), adenocarcinoma (AC; 58% and 50%) and normal gastric mucosa (NGM; 20% and 33%). The DNA heteroploid rate was 63% (12/19) in GTA, 75% (3/4) in AC and 0% (0/8) in NGM. In detecting GTA with FCM and the ABC method, the histological parameter analysis showed that sensitivity was 82% ± 3%, specificity was 88%, predictive value was 95% ± 4% and accuracy was 84%. Both methods were complementary.

CONCLUSION: Multiparameter diagnosis and studies of histomorphology and molecular biology may be a useful approach towards histologic diagnosis of GC.

Key words: Stomach neoplasms/diagnosis; Adenocarcinoma/diagnosis; Immunohistochemistry; Flow cytometry

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INTRODUCTION

Early diagnosis of gastric cancer (GC) is an important focus of the research field examining digestive tract tumors. The single parameter diagnosis is rather limited, and involves either histology or serology. Differences in laboratory test sensitivity and antibody specificity are common limitations to accurate tumor diagnosis. In this study, the self-made antihuman gastric tubular adenocarcinoma (GTA) monoclonal antibody (mAb) MG3c7 and the antihuman oncogene antibody cerbB-2 were used to investigate a series of paraffin-embedded GC tissues by ABC immunohistochemistry; the DNA content was analyzed by flow cytometry FCM as well. The aim of the study was to evaluate histological diagnosis of GC in clinical application by way of multiparameter analysis.

MATERIALS AND METHODS

Histological specimens

Specimens of GC (n = 51), gastritis (GS; n = 17) and normal gastric mucosa (NGM; n = 10, obtained through biopsy) were obtained from the surgically-resected samples and confirmed by pathological diagnosis. The paraffin-embedded tissue sections were evaluated by immunohistochemistry and tissue cell suspensions were detected by FCM to determine DNA content.

Reagents and instruments

The antihuman gastric tubular adenocarcinoma mAb MG-3c7 was made by our laboratory. The working dilution of antibody was 1:200 after purification. The ABC reagent, antihuman oncogene cerbB-2 IgG (1:200) and anti-rabbit IgG-HRP (1:40) were obtained from the Institute of Zhong Da Medical Applied Research (Shanghai, China). The fluorescein active cell selector (FACS, 420 type) was provided by the Institute of Cancer Research (Hebei Province, China).

Methods

Immunohistochemistry. Lactonase was dispelled from the sections after deparaffinization and re-hydration. Afterwards, the sections were blocked by 5% BSA and divided into 2 groups for respective incubation with McAb MG-3c7 or the oncogene cerbB-2 for 3 h at room temperature or overnight at 4 ℃. This was followed by reaction for 1 hour with ABC reagent and anti-rabbit IgG-HRP. The color reaction was visualized by DAB under microscopy, after which hematoxylin staining was completed and the sections were dehydrated, hyalinized and mounted.

FCM. The paraffin-embedded tissues were deparaffinized, re-hydrated, washed and shred to pieces. The cell suspension was then passed through a 300-millipore nylon sieve, centrifuged and fixed with 70% ethanol. Fluorescence staining of DNA interpolated with ethidium bromide and chicken red cells (as the internal refer-

Table 1 Distributions of MG-3c7 and CerbB-2 in gastric cancer tissues

Type	*n*	MG-3c7				*n*	cerbB-2			
		+ + +	+ +	+	-		+ + +	+ +	+	-
TACN	30	12	13	5		23	5	7	1	10
AC	12	3	2	2	5	6		1	2	3
SRCC	2				2	3				3
MAC	7				7	2			1	1
GS	4				3	4			2	2
NGM	10				8	6			2	4

TACN: Tubular adenocarcinoma of nipple; AC: Adenocarcinoma; SRCC: Signet-ring cell carcinoma; MAC: Mucous adenocarcinoma; GS: Gastritis; NGM: Normal gastric mucosa.

Table 2 Results of positive coincidence rate determined by three markers

Type	cerbB-2	MG-3c7	DNA
	Positive rate (%)	Positive rate (%)	2c rate (%)
TACN	13/23 (56)	30/30 (100)	12/19 (63)
AC	3/6 (50)	7/12 (58)	3/4 (75)
MAC	1/2 (50)	0/2 (0)	2/2 (100)
SRCC	0/3 (0)	0/7 (0)	
GS	2/4 (50)	1/4 (25)	0/17 (0)
NGM	2/6 (33)	2/10 (20)	0/8 (0)

TACN: Tubular adenocarcinoma of nipple; AC: Adenocarcinoma; SRCC: Signet-ring cell carcinoma; MAC: Mucous adenocarcinoma; GS: Gastritis; NGM: Normal gastric mucosa.

ence standard) was carried out by staining for half an hour; the cells were then evaluated by FACS. The cell fluorescence photon appeared on the multichannel pulse analyzer in a combined point and digital pattern. The CV value remained steady between 3% and 5%. Ten-thousand cells from each specimen were determined and all of the data were analyzed statistically.

Experimental judgment standard

The stained cells were scored as negative or in accordance with the section background (-), with 1%-9% of cells showing light yellow coloration or weak positive reaction judged as (+), 10%-49% of cells showing yellow-brown coloration or positive reaction judged as (++), and 50%-100% of cells showing dark brown coloration or strong positive reaction judged as (+++). The DNA index (DI) G0/1 peak/channel average value of tumor tissues G0/1 peak/channel average value of normal tissues was determined according to the following:

Referring to the method of Fallenicus, *etc.* DI = 1 ± 0.1 was diploid (2c) and the excess was heteroploid.

Standard of multiparameter diagnosis: Sensitivity = TP/(TP + FN) × 100%.

Specificity = TN/(TN + FP) × 100%.

Predictive value = TP/(TP + FP) × 100%.

Accuracy = (TP + TN)/(TP + FN + TN + FP) × 100%.

TP: True Positive cases. FN: False Negative cases.

TN: True Negative cases. FP: False Positive cases.

RESULTS

The expression and distribution profiles of MG-3c7 antigen and cerbB-2 receptor in the different types of GC are shown in Table 1. The results of the positive coincidence rate of GC tissues determined by ABC and FCM for the three different markers are shown in Table 2.

The comparison of the positive judgment parameters determined by MG3c7 by immunohistochemistry and DNA content by FCM are shown in Table 3.

Table 3 Comparison of the positive judgment parameters determined by ABC and flow cytometry

Parameter	GC			GTB		
	A	B	A+B	A	B	A+B
Sensitivity	72.5	72.5	72.5	100.0	72.5	82.5
Specificity	80.0	100.0	88.8	80.0	100.0	88.8
Predictive value	94.8	100.0	96.9	94.8	100.0	95.4
Accuracy	73.7	73.7	75.2	95.0	68.9	84.0

A: Positive rate in immunoenzymology and histochemistry; B: Heteroploid rate in flow cytometry; A+B: Positive rate in immunohistochemistry and heteroploid rate in flow cytometry.

DISCUSSION

It is not unusual to make a clinical diagnosis of GTA with many markers and methods in histology or serology. We performed immunohistochemical staining with the self-made antihuman GTA mAb MG-3c7 and antihuman oncogene antibody cerbB-2.

The changes of DNA diploid status were determined by FCM in GC tissues. It was possible to evaluate the same specimen *via* different methods at the same time. We hope to make an early diagnosis by using analysis and comparison of the multiparameters as well as with complementation of laboratory tests. As a whole, we can combine histomorphology with molecular biology for the diagnosis of GC.

The mAb MG-3c7 reagent was generated from BALB/c mice that had been immunized, and fused with the NGCC-8310 cell line of human GTA. It was found to be specific to the GTA histo-antigen.

The function of cerbB-2 is very similar to that of the EGF receptor. Its over-expression is related to the occurrence of human tumor. Cell division and proliferation is based on DNA content. As normal cells transform into malignant ones, the DNA content and the expression rate of heteroploid status are elevated accordingly. Therefore, abnormal DNA content is a biological marker for diagnosis of malignant tumor.

It was shown in the current study that the mAb MG-3c7 was strongly sensitive to GTA and that its positive coincidence rate might reach 100%. The positive rate of cerbB-2 and heteroploid rate of DNA content were 56% and 63% respectively. The markers of MG-3c7, cerbB-2 and DNA showed rates of 80%, 50% and 68% for diagnosis of GC. Immunohistochemical staining showed that cerbB-2 was distributed in the cytomembrane and MG-3c7 was located in the cytoplasm. According to the different specificity profiles in histological analysis, we were able to observe the expressions of distinctly related antigens in the same tissues. As tumor markers, cerbB-2 and MG3c7 were found to be in direct proportion to the tumor differentiation degree. However, it was shown in the analysis on DNA diploid status that the poorer the tumor was differentiated, the higher the DNA heteroploid positive rate. The results showed that tumor malignant behavior, as well as its growth and decline, could be studied from different perspectives and have impact on cell immunology and molecular biology. No specific tumor marker has yet been obtained for the clinical diagnosis of GC. Therefore, we believe that this study provided very important knowledge towards making multiparameter analysis for the diagnosis of tumors.

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