**The Role of Expression of Mismatch Repair Proteins hMSH2 and hMLH1 in Gastric Carcinogenesis and Its Clinical Significance**

**OBJECTIVE** To investigate the expression of the mismatch repair proteins hMSH2 and hMLH1, and to examine the clinical significance of the intracellular expression site (ICES) in gastric carcinogenesis.

**METHODS** Specimens from 172 cases of gastric cancer, 151 tissues from paraneoplastic gastric mucosa and 34 from noncancerous gastric mucosa were collected in Dalain, China. An immunohistochemical method was used to determine the expression of the hMSH2, hMLH1 proteins and their ICES in the gastric mucosas.

**RESULTS** The rate of hMSH2 expression in gastric cancers, paraneoplastic gastric mucosas and noncancerous gastric mucosas were respectively 69.8%, 49.7% and 32.4%. The rate was significantly higher in gastric cancer compared to the latter two groups \((P=0.000)\), but there was no obvious difference in the expression between the two latter groups \((P=0.067)\). The hMLH1 protein expression rates were respectively 73.3%, 57.6% and 41.2% in the above three groups. The expression was significantly higher in the gastric cancer group compared to the two latter groups \((P=0.000)\), while there was no significant difference between the latter groups \((P=0.082)\). There was no obvious correlation between the hMSH2 and hMLH1 protein expression rates and related factors, such as gender, age and differentiated level of gastric cancer etc. The cell-nuclear expression of the hMSH2 protein was respectively 70.0%, 58.7% and 36.4% in the gastric cancer, paraneoplastic gastric mucosa and noncancerous gastric mucosa groups. The cytoplasmic expression rates were 30.0%, 41.3% and 63.6% in the three groups. The cell-nuclear expression rate of the hMSH2 protein gradually decreased in the gastric mucosas in the following order: cancer, paraneoplastic and noncancerous but cytoplasmic expression only increased slightly in these groups \((r=0.161, P=0.020)\). There was no significant difference in the ICES of the hMLH1 protein among the three different gastric mucosas \((P=0.659)\).

**CONCLUSION** Simultaneous determination of the expression and ICES of the mismatch repair proteins hMSH2 and hMLH1 in the gastric mucosa may be helpful in detecting early gastric cancer.

**KEYWORDS:** hMSH2, hMLH1, gastric cancer, immunohistochemistry.

**INTRODUCTION**

Integrity of the mismatch repair function is of fundamental importance for accurate copying of the cellular DNA and stability of the genome. Furthermore there is a close correlation between the loss of mismatch repair activity and oncogenesis and progression of several malignant tumors[1]. The mismatch repair proteins hMSH2 and hMLH1 are the key proteins for conducting mismatch repair activity. Under normal circumstances, these proteins are synthe-
sized in the cytoplasm and then are conveyed to the cell nucleus where they are involved in the mismatch repair reaction. Therefore, expression of the proteins can be detected in the cytoplasm and the cell nucleus. Moreover, changes in their expression or the intracellular expression site (ICES) will affect the mismatch repair function.

Gastric cancer is one of the most common malignant tumors in China, and gastric carcinogenesis also is related to the loss of cellular mismatch repair function[2-4]. However, it is still unclear if gastric carcinogenesis is affected by the expression and ICES of the hMSH2 and hMLH1 proteins. In our study we performed in situ assays of ICES, hMSH2 and hMLH1 protein expression in gastric cancers, paraneoplastic gastric and noncancerous gastric mucosa, in order to analyze the relationship among the ICES, expressed proteins, the occurrence and differentiation of gastric cancers, as well as the clinical significance.

MATERIALS AND METHODS

Specimens
The tissue specimens were collected in our hospitals as follows: 172 cases of gastric cancer, 151 paraneoplastic gastric mucosas and 34 noncancerous gastric mucosas from patients who had undergone surgery and gastroscopic biopsy. In the gastric-cancer group, there were 127 male and 45 female patients with ages ranging from 33 to 89 years and a mean of 61.4±11.2. The tissues were treated with a 4% neutral formalin fixation, paraffin imbedding, sectioning at 3 to 4 μm followed by H&E staining. Pathological diagnoses were performed by two experienced pathologists. In the gastric-cancer group, there were 14 cases with well-differentiated carcinoma, 48 with moderately-differentiated cancer, 83 with poorly-differentiated cancer and 27 with colloid carcinoma. Pathological diagnoses of the paraneoplastic gastric mucosas and the noncancerous gastric mucosas were chronic superficial gastritis or chronic atrophic gastritis.

Histological in situ assay of the hMSH2 and hMLH1 protein expressions
The immunohistochemical SP staining method was employed, and conventional deparaffinizing and hydrating of the sections and microwave coctoantigen repair conducted. Incubation of the mouse-antihuman monoclonal antibodies for hMSH2 (1:250 dilution, Zymed, clonal line: FE11) and for hMLH1 (1:50 dilution, Zymed, clonal line: 14) was performed overnight at 4°C. Other successional procedures were carried based on specification in the SP kit (the Fuzhou Maixin Biological Technology Co., Ltd.), with PBS as the negative control to replace the first antibody, and known positive sections as the positive controls.

Assessment of the results
The cells with pale-brown granules in the cell nucleus or cytoplasm were identified as positive. After assessment of 5 high-power fields or 500 cells, the specimen was scored as positive expression if the ratio of the positive cells to parenchymal cells was equal to or over 10%. Otherwise, there was negative expression. Based on the location of positive granules in the cells, the cases with a positive expression in the cell nucleus or a concurrent positive expression of the cell nucleus and cytoplasm were defined as positive nuclear-expression cases. The cases with only positive expression in the cytoplasm were identified as cytoplasmic-expression cases. A double-blind method was used to determine the results by two experienced pathologists who had no information concerning the patient clinical data.

Statistical analysis
The χ² test was adopted and the Spearman rank correlation analysis used for correlation statistics, by SPSS13.0 statistical software. The value of P<0.05 indicated a statistical significance in the tests.

RESULTS

The expression of the hMSH2 and hMLH1 proteins in various gastric mucosas
The positive expression rate of the protein hMSH2 in gastric cancer was 69.8% (120/172), which was significantly higher than that in the paraneoplastic gastric mucosas with 49.7% (75/151) and noncancerous gastric mucosas with 32.4% (11/34) (P=0.000). However there was no significant difference between the expression of the two latter groups (P=0.067). The positive expression rate of the mismatch repair protein hMLH1 in the gastric cancers was 73.3% (126/172), which was significantly higher than that found in paraneoplastic gastric mucosas with 49.7% (75/151) and noncancerous gastric mucosas with 32.4% (11/34) (P=0.000). However there was no significant difference between the expression of the two latter groups (P=0.067). The positive expression rate of the mismatch repair protein hMLH1 in the gastric cancers was 73.3% (126/172), which was significantly higher than that found in paraneoplastic gastric mucosas with 57.6% (87/151), and noncancerous gastric mucosas with 41.2% (14/34) (P=0.000). However there was no significant difference between the two latter groups (P=0.082, Fig.1). Table 1 shows there was no clear-cut correlation between the hMSH2 and hMLH1 expression rate and the related factors of gastric cancer patients, such as gender, age and level of cancer differentiation.