The Effect of *Helicobacter pylori* Infection on hMSH2 and P53 Proteins in Gastric Carcinogenesis

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OBJECTIVE To investigate the effect of *Helicobacter pylori* (*H pylori*) infection on expression of hMSH2 and P53 and its possible carcinogenic mechanism.

METHODS *H pylori* infection was detected using a rapid urease test and haematoxylin and eosin (H&E) staining. The hMSH2 and P53 proteins in gastric cancer (GC) tissue, its adjacent mucosa, gastritic mucosa and normal gastric mucosa were detected by the immunohistochemical SP method.

RESULTS (1) The positive rate of hMSH2 expression in GC tissue (62.7%)was higher than those in non-cancer tissues ($P<0.001$); the positive rate of hMSH2 expression in poorly differentiated adenocarcinoma (76.4%) was higher than that in other carcinomas ($P<0.05$). The positive rate of P53 expression in GC tissue (51.9%) was higher than that in non-cancer tissues ($P<0.001$); the positive rate of P53 expression in well-differentiated adenocarcinoma (32.6 %) was lower than that in other carcinomas ($P<0.01$). (2) The positive rate of hMSH2 expression in GC tissue with *H pylori* infection was lower than that without the infection (52.8% *vs.* 74.5%; $P<0.05$). The positive rate of P53 expression in GC tissue with *H pylori* infection was higher than that without the infection (61.4% *vs.* 40.6%; $P<0.05$).(3) The expression of hMSH2 and P53 in GC tissue correlated positively ($r=0.457$, $P<0.01$).

CONCLUSION High expression of hMSH2 and P53 as well as their interaction may be involved in gastric carcinogenesis; *H pylori* infection affecting expression of hMSH2 and P53 may be one of its carcinogenic mechanisms.

KEYWORDS: ***helicobacter pylori*, gastric cancer, hMSH2, P53, immunohistochemistry.**

Gastric cancer (GC) is one of the most fatal malignancies in the world, causing about 628,000 deaths annually. An accumulation of multiple genetic alterations of oncogenes and tumor suppressor genes are involved throughout the course of the multistep conversion of normal epithelial cells to gastric cancer. It has been generally accepted that the tumor suppressor gene p53 plays an important role in GC carcinogenesis.[1,2] In addition, alterations in DNA mismatch repair (MMR) genes result in mutation accumulation at target genes and cancer development. hMSH2 (human MutS homolog 2 gene), one of the MMR genes, has been considered to be strongly related to GC.[3,4] In recent years, a close association between *Helicobacter pylori* (*H pylori*) and GC has been found,[5,6] but the mechanism by which *H pylori* is involved in gastric carcinogenesis remains unknown. Whether alterations of the hMSH2 and p53 genes are partially related to *H pylori* -associated gastric carcinogenesis has yet to be defined. The aim of this study was to determine the effect of *H pylori* infection on hMSH2

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and P53 proteins in gastric mucosa and further clarify the role of H pylori in gastric carcinogenesis.

MATERIALS AND METHODS

Tissue samples

Tissue samples were obtained from upper gastrointestinal endoscopy during the period of April to November 2002 in the First Hospital Affiliated with Dalian Medical University. The results were comprised of 129 of gastric cancers, 74 of gastritis and 10 of normal gastric mucosa. GC samples included 46 of well-moderately differentiated adenocarcinoma, 51 of poorly differentiated adenocarcinoma, and 32 of mucoid carcinoma. GC samples were from 71 male and 58 female patients aged 39~89 years (median age 65). Gastritis samples were all chronic superficial gastritis, including 49 male and 25 female patients aged 24~69 years (median age 43). Normal mucosa samples had normal morphological structure without H pylori infection, including 4 male and 6 female patients aged 23~71 years (median age 40). Samples were stained with haematoxylin and eosin (H&E) for histopathological diagnosis. The diagnosis of each sample was based on agreement between two experienced pathologists.

Detection of *H pylori* infection

H pylori infection was detected by a rapid urease test and H&E staining. Samples were regarded to have a positive *H pylori* infection if both test results were positive.

Detection of hMSH2 and P53 Protein

For each case, 6 μm thick sections of 10% neutral buffered formalin-fixed and paraffin-embeded tissues were mounted on acid-cleaned glass slides. Sections were deparaffinized and rehydrated, then endogenous peroxidase activity was inhibited by incubation with 3% H_2O_2 for 30 min at room tempreture. Sections were heated at 100℃ for 10 min in antigen-retrieval citrate buffer (pH 6.0) in a microwave oven. To reduce non-specific background staining, sections were incubated with 10% goat serum for 40 min at room tempreture. Finally, sections were incubated with monoclonal antibodies against hMSH2 (dilution 1:50, Calbiochem) and P53 (dilution 1:50, Dako) overhight at 4℃. Immunostaining was performed using the standard streptavidin-biotin-peroxidase complex method according to manufacture's instructions (Zymed). For negative controls, the primary antibodies were replaced with PBS. Normal tissues were taken as internal positive controls. The stains were graded positive when there was brown staining in the cell nuclei. The number of positive cells accounting for more than 20 % in 5 high-power fields was regarded as positive expression.

Statistical analysis

The statistical analysis was performed with the chi-square and Spearman rank correlation tests using the SPSS software 10.0. A probability P value<0.05 was considered statistically significant.

RESULTS

H pylori infection

H pylori infection rates in GC tissue, adjacent mucosa, gastritic mucosa were 54.2% (70/129), 60.4% (78/129) and 67.5 % (50/74), respectively (P>0.05).

hMSH2 and P53 expression in different tissues

The positive rate of hMSH2 expression in GC tissue was higher than that in non-cancer tissues (P<0.001); the positive rate of hMSH2 expression in poorly-differentiated adenocarcinoma was higher than those in other carcinomas (P<0.05). Gastritic mucosa and normal gastric mucosa showed negative staining for P53; the positive rate of expression in GC tissue was higher than that in adjacent mucosa (P<0.001); the positive rate of P53 expression in well differentiated adenocarcinoma was lower than that in other carcinomas (P< 0.01) (Fig.1~4, Table1).

Correlation between hMSH2 and P53 expression

The expression of hMSH2 and P53 correlated positively in GC tissue (r=0.457, P<0.01)(Table 2).

hMSH2 and P53 expression in tissues with and without *H pylori* infection

Positive rates of hMSH2 expression in tissues with *H pylori* infection were all lower than those without the infection, while positive rates of P53 expression in GC and adjacent tissues with *H pylori* infection were higher than those without the infection. The differences between cancer tissues with and without *H pylori* infection for the two genes were significant(P<0.05) (Table 3).

DISCUSSION

hMSH2, P53 and GC

The P53 protein is a transcriptional factor that arrests

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Table 1. Expression of hMSH2 and P53 in different tissues.

Groups	n	hMSH2		P53	
		n	Positve rate (%)	n	Positve rate (%)
Cancer tissue	129	81	62.7[a]	67	51.9[b]
Well-moderate differentiation	46	25	54.3	15	32.6[d]
Poor differentiation	51	39	76.4[c]	30	58.8
Mucoid carcinoma	32	17	53.1	22	68.7
Adjacent mcosa	129	38	29.4	4	3.1
Gastritic mucosa	74	24	32.4	74	0.0
Normal gastric mucosa	10	7	30.0	10	0.0

vs. non-cancer groups, [ab]P <0.001; *vs.* other cancer groups, [c]P <0.05, [d] P <0.01.

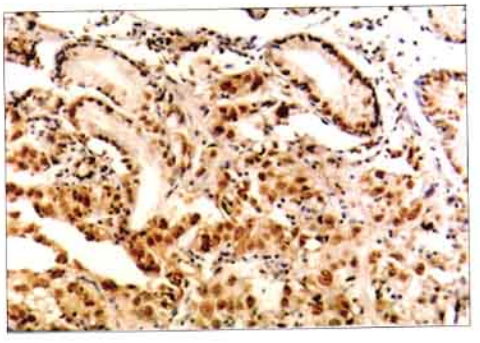

Fig.1. The expression of hMSH2 protein in GC and adjacent mucosa (SP × 200). The stronger positive staining of hMSH2 protein was detected in GC tissue compared with adjacent mucosa.

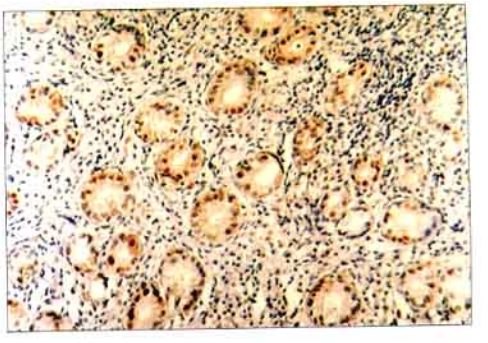

Fig.2. The expression of hMSH2 protein in gastritic mucosa (SP × 200). The weakly positive staining of hMSH2 protein was detected in gastritic mucosa.

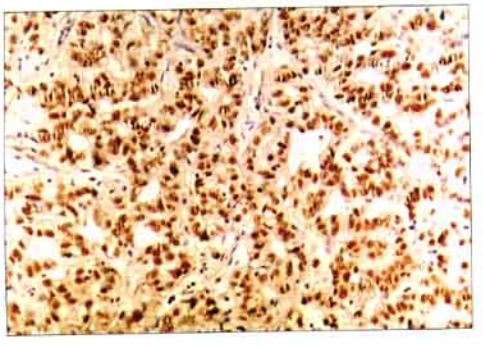

Fig.3. The expression of P53 protein in poorly differentiated GC (SP × 200). The strongly positive staining of P53 protein was detected in cell nuclei.

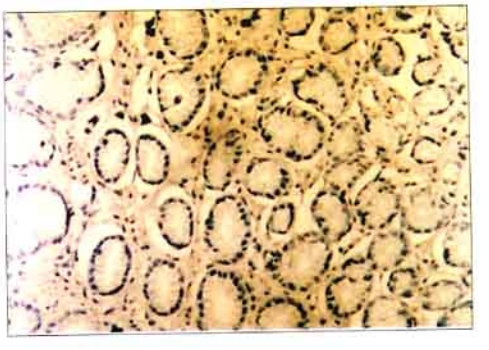

Fig.4. The expression of P53 protein in normal gastric mucosa (SP × 200). Negative staining of P53 protein was detected in cell nuclei.

Table 2. Correlation between hMSH2 and P53 expression in GC tissue.

hMSH2	P53	
	-	+
-	35	13
+	27	54

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Table 3. Relationship between hMSH2, P53 expression and H pylori infection.

Groups	n	hMSH2		P53	
		n	Positve rate (%)	n	Positve rate (%)
H pylori$^+$ Cancer tissue	70	37	52.8[a]	43	61.4[b]
H pylori$^-$ Cancer tissue	59	44	74.5	24	40.6
Hpylori$^+$ Adjacent mucosa	78	19	24.3	4	5.1
Hpylori$^-$ Adjacent mucosa	51	19	37.2	0	0.0
H pylori$^+$ Gastritic mucosa	50	14	28.0	0	0.0
H pylori$^-$ Gastritic mucosa	24	10	41.6	0	0.0

vs. H pylori-cancer tissue , [ab]P <0.05.

the cell cycle in the G1 phase when DNA is damaged. Thus, damaged DNA cannot replicate, allowing time for the repair system to act. The half-life of wild-type P53 is so short (20~30 min) that P53 protein expression is usually negative in normal tissues. Mutations render the P53 protein to be a more stable compound with a longer half-life (1.5~7 h). Thus, P53 protein overexpression is likely to represent mutant forms. Mutation of the p53 gene has been observed in more than 60% of GC. In the present study, P53 expression in GC tissue was increased significantly compared with those in non-cancer tissues, and its expression in poorly differentiated adenocarcinomas was stronger than that in well-differentiated ones, implying that P53 expression is related to the degree of malignancy of GC cells and clinical prognosis.

It is known that the MMR gene can correct DNA replication errors to avoid cumulative mutations of genes, keep genomic fidelity and stability, and reduce predisposition to cancer. So far, at least 6 human MMR genes have been identified (hMSH2, hMSH3, hMSH6, hMLH1, hPMS1,and hPMS2). hMSH2, ho mologous to MutS, is located on chromosome 2P21-22. hMSH2 can heterodimerize with either hMSH6 or hMSH3, the forming protein complexes designated a hMutS. This complex binds to the mis match site and triggers a series of enzymatic reactions resulting in the removal of the mismatched bases and insertion/deletion loops mistakenly incorporated dur ing DNA replication. hMSH2 protein is expressed mainly in normal epithelia of the digestive duct and genital gland. Some findings demonstrate the in creased expression of hMSH2 in certain types of cancer.[7,8] Here, we found hMSH2 expression in GC tissue was stronger than that in non-cancer tissues. Moreover, hMSH2 expression in poorly differentiated adenocarcinoma was stronger than that in well-differentiated ones. These results suggest abnormally active proliferation of tumor cells may contribute to up-regulation of hMSH2 expression, raising the possibility to use hMSH2 as a marker for malignant tumors. In this study, a significant positive correlation of hMSH2 and P53 expression was observed in GC tissue. It is hypothesized that mutated P53 can promote hMSH2 to be expressed compensatively to repair genetic mutations during DNA replication.

hMSH2, P53 and *H pylori* infection

H pylori, which was initially isolated from gastric tissue, has been well recognized as a causative factor in the pathogenesis of chronic gastritis and peptic ulcer. Furthermore, epidemiological analyses and experimental studies have revealed that H pylori is linked to gastric carcinogenesis. *H pylori* is regarded as a class I carcinogen in humans. It is suggested that chronic *H pylori* infection damages gastric barrier function and stimulates gastric cell proliferation. The chemical products from bacteria can induce cellular DNA damage.[9]

Thus far, correlation between P53 alteration and *H pylori* infection is a matter of debate. Some studies favored the view that mutated P53 had no association with *H pylori* infection;[10,11] others reported that *H pylori* infection can increase P53 expression.[12-14] In our study, the positive rate of P53 expression in GC with *H pylori* infection was significantly higher than that without the infection, and P53 expression in adjacent mucosa had a similar tendency, indicating that/*H pylori* infection, to some extent, perhaps impairs p53 gene and leads to increased mutated P53 products.

Park et al.[15] and Kim et al.[16] reported that the hMSH2 protein level was reduced in gastric epithelial cells after coculture with *H pylori* strains in vitro, and the level was increased after *H pylori* eradication. Our

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data showed that positive rates of hMSH2 expression in gastric tissues with *H pylori* infection were lower than those without the infection, suggesting that *H pylori* infection might lead to a deficiency of hMSH2 protein in gastric epithelial cells that may increase the risk of mutation accumulation (for example p53 gene) and the risk of GC during chronic *H pylori* infection. Further study is required to confirm the mechanism by which *H pylori* affects the expression of genes, which will be crucial for our understanding of GC pathogenesis.

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