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Mismatch repair gene expression in gastroesophageal cancers

ESOPHAGUS/STOMACH

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ABSTRACT

Background/Aims: Mismatch repair (MMR) genes play a critical role in maintaining genomic stability, and the impairment of MMR machinery is associated with different human cancers, mainly colorectal cancer. The purpose of our study was to analyze gene expression patterns of three MMR genes (MSH2, MHS6, and EXO1) in gastroesophageal cancers, a pathology in which the contribution of DNA repair genes remains essentially unclear.

Materials and Methods: A total of 45 Romanian patients diagnosed with sporadic gastroesophageal cancers were included in this study. For each patient, MMR mRNA levels were measured in biopsied tumoral (T) and peritumoral (PT) tissues obtained by upper endoscopy. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) with specific TaqMan probes was used to measure gene expression levels for MSH2, MSH6, and EXO1 genes.

Results: A significant association was observed for the investigated MMR genes, all of which were detected to be upregulated in gastroesophageal tumor samples when compared with paired normal samples. In the stratified analysis, the association was limited to gastric adenocarcinoma samples. We found no statistically significant associations between MMR gene expression and tumor site or histological grade.

Conclusion: In our study, MSH2, MSH6, and EXO1 genes were overexpressed in gastroesophageal cancers. Further investigations based on more samples are necessary to validate our findings.

Keywords: Gastric cancer, DNA, mismatch repair, gene expression, mutation

INTRODUCTION

Gastric cancer remains a major health problem worldwide. Despite advances in diagnosis and treatment, prognosis is often poor. During 2012, in Europe, the estimated number of new cases of gastric cancer were approximately 140,000, and approximately 107,000 died from gastric cancer in the same year, being the sixth most common cancer site and the fourth leading cause of cancer death in men and women (1).

Gastric carcinogenesis is a complex multistep process that involves various genetic and epigenetic alterations, including mutations in oncogenes, tumor suppressor genes, microRNA genes, and DNA repair genes (2,3). Multiple biological pathways are responsible for DNA repair and have important roles in the maintenance of genome integrity. Genomic instability has been associated with a variety of human cancers, including gastric cancer (4,5).

The mismatch repair (MMR) system recognizes and corrects mismatched bases and small insertion/deletion loops generated during DNA replication. Multiple genes that encode different proteins and enzymes, including MLH1, MSH2, MSH3, MSH6, PCNA, and EXO1, belong to the MMR system. MSH2 initiates DNA repair of mismatches by the following two different heterodimers: MutS alpha (MSH2-MSH6) is involved in the early repair stages of base–base mismatches and dinucleotide insertion/deletion loops, while MutS beta (MSH2-

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MSH3) is involved in the repair of larger insertion–deletion loops (6,7). Another component of the MMR pathway, EXO1, is an exonuclease with 5′ activity that participates in DNA replication, recombination, and repair (8).

MMR deficiency is characterized by a loss of function in the repair of replication-associated errors. As a consequence, mismatch mutations accumulate throughout the genome but are particularly concentrated in regions with short repetitive nucleotide sequences known as microsatellites (9-11). The deficiency in the MMR system leads to microsatellite instability and confers a mutator phenotype. This phenomenon has not only been identified in gastric cancer but also in intestinal metaplasia, indicating that it might be present in incipient stages of carcinogenesis (4,11,12). MMR gene variants may influence gene expression, leading to an increased susceptibility to mutations of more genes involved in gastric carcinogenesis (13). MMR deficiency is usually accompanied by the loss of MMR gene expression; however, MMR gene overexpression has been reported in gastric and other cancers. An imbalance in the relative amounts of the MSH3 and MSH6 proteins can lead to MMR deficiency. MMR impairment can also arise by MSH3 gene overexpression, and not only through mutation or transcriptional silencing (14,15).

We aimed to evaluate the expression profiles of MMR genes in gastroesophageal cancers, a pathology for which the contribution of DNA repair genes remains essentially unclear. The expression profiles of MSH2, MSH6, and EXO1 were compared in normal and gastroesophageal tumor tissues obtained from patients diagnosed with sporadic gastroesophageal cancers.

MATERIALS AND METHODS

Subjects

A total of 45 Romanian patients with gastroesophageal cancers were included in this study. The diagnosis was histologically confirmed on biopsied specimens obtained by upper gastrointestinal endoscopy at the Research Centre in Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Romania. All patients were infected with *Helicobacter pylori,* and none of them had received either chemotherapy or radiation therapy. The Ethics Committee of the University of Medicine and Pharmacy of Craiova approved the present study, and written informed consent was obtained from all included patients.

Gene expression evaluation

Mismatch repair mRNA levels were evaluated in tumoral (T) and peritumoral (PT) tissue samples from biopsy specimens obtained by endoscopy. We collected the biopsy samples in RNAlater (Ambion, Austin, USA); then, the stabilized tissue samples were frozen until RNA isolation. Other specimens were collected in formalin for pathological examination. None of the biopsied PT samples showed macroscopic signs of malignancy; on histopathological examination, we found none of the PT tissues microscopically invaded by malignant cells.

Total RNA from samples was extracted using the SV Total RNA Isolation System (Promega, Madison, USA). A biophotometer (Eppendorf, Hamburg, Germany) and an Agilent 2010 bioanalyzer (Agilent Technologies, Santa Clara, USA) were used to evaluate RNA concentration and purity as well as RNA sample quality, respectively. Total RNA was reverse transcribed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA), according to the manufacturer's instructions. Quantitative reverse transcription polymerase chain reactions (qRT-PCR) were performed on a ViiA™ 7 Real-Time PCR System (Life Technologies, Carlsbad, USA) using the following specific TaqMan gene expression assays (Applied Biosystems, Foster City, USA): MSH2 (Hs00953523 _m1), MSH6 (Hs00264721 _m1), and EXO1 (Hs00243513 _m1); for the endogenous control, GAPDH (Hs99999905_m1) was used. To specifically detect and quantify only the targeted mature mRNA, we used TaqMan probes that span the splice sites.

Statistical analysis

MMR transcript expression was normalized to GAPDH (as housekeeping gene); then, gene expression difference was registered as fold changes using the $2^{-\Delta\Delta Ct}$ method. Chi-square test was performed to determine whether the difference between the number of patients in each group was significant. Variation of mRNA levels in paired samples higher than 1.8 fold were considered biologically relevant (mRNA levels of >1.8 indicates greater expression in tumor, <0.55 indicates greater expression in PT mucosa, and 0.55–1.8 indicates irrelevant difference between tumoral and adjacent mucosa).

To assess correlations between genes, Spearman's coefficients were calculated. GraphPad Prism 5 software (GraphPad, San Diego, USA) was used for statistical analyses.

RESULTS

A total of 45 patients diagnosed with gastroesophageal cancers were enrolled (30 men and 15 women; mean age, 62 years). The characteristics of the patients are summarized in Table 1. The sites of tumor origin in most cases were non-cardia (n=23), cardia (n=14), and esophagus (n=8). The histological type was adenocarcinoma for all gastric cancer specimens and squamous cell carcinoma for all esophageal samples.

Gene expression profiles of MSH2, MSH6, and EXO1 in T and PT specimens are shown in Table 2 and Figure 1. Chi-square test produced a two-tailed p value of <0.0001 for all the three genes analyzed. Spearman's coefficient revealed a strong positive association between tumor levels of MSH2 and MSH6 ($p<0.0001$) as well as between PT mRNA levels of MSH2 and both MSH6 and EXO1 ($p<0.0001$).

Table 1. Patient characteristics

	Cases
N	45
Male/Female	30/15
Age (years), mean	62
Location	
Esophagus	8
Cardia	14
Non-cardia	23
Histological type	
Squamous cell carcinoma	8
Adenocarcinoma	37
Histologic grade	
G1 - well	8
G2 - moderate	15
G3 - poor	20
Unknown/Not defined	2
Tumor stage	
T2	6
T3	23
T4	16

N: number

Table 2. The overall expression pattern for the investigated genes

Gene	Greater expression in tumor sample>1.8, n (%)	Greater expression in peritumor sample<0.55, n (%)	Irrelevant difference 0.55–1.8, n (%)
MSH2	14 (31.11)	3 (6.67)	28 (62.22)
MSH6	17 (37.78)	2 (4.44)	26 (57.78)
EXO1	21 (46.67)	0 (0.00)	24 (53.33)

When the gene expression was comparatively analyzed between paired T and PT tissues, there was a statistically significant difference in the expression of all the three genes between the paired samples (Figures 2-4). In a stratified analysis, a significant difference was observed only for gastric adenocarcinoma samples (Table 3 and Figure 5). We separately examined the association of MMR gene expression with tumor site (cardia and noncardia) or histological grade (well differentiated, moderately differentiated, and poorly differentiated), and no statistical differences were noted.

DISCUSSION

In this study, we evaluated the expression patterns of three MMR genes with important roles in DNA repair. We found a tendency toward increased expression of all the three MMR genes in gastroesophageal tumor samples compared with that in normal tissue.

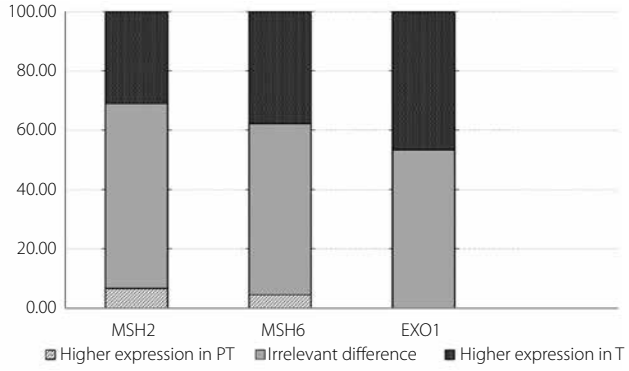

Figure 1. The overall expression pattern for MSH2, MSH6, and EXO1 genes.

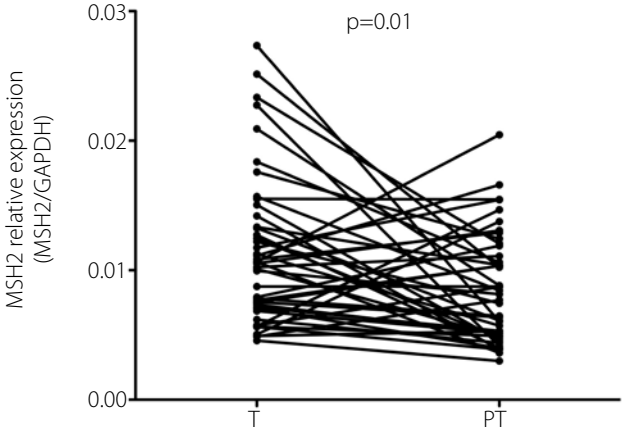

Figure 2. Comparative expression of MSH2 mRNA in paired tumoral and peritumoral mucosa. Data are presented as relative mRNA expression of the target gene to GAPDH estimated by the Wilcoxon matched-pairs signed rank test.

Several previous studies have implicated MMR genes in gastric cancer. Hypermethylation of the promoter region of MLH1 occurs frequently in gastric cancers, and the MLH1 methylation status is well correlated with MLH1 protein expression (16,17). In a study, the expression of MLH1 and MSH2 in gastric cancer was determined by immunohistochemical analysis. Loss of MLH1 expression was associated with old age, advanced nodal stage, and intestinal-type gastric cancer. It is notable that all cases in this study still expressed MSH2 (18). Also, in another study, MSH2 immunoreactivity was positive in all examined gastric cancer samples (19).

The role of MMR genes in cancer has also been studied in familial cases. Families with MMR mutation carriers are at a significantly increased risk of different types of cancer, including esophagus and gastric neoplasms, while noncarriers seem to be at a similar risk as that of the general population (20). Germline MSH2, MLH1, E-cadherin, and MYH mutations are more frequently found in patients with familial gastric cancer than in controls, and carriers of MYH gene mutations have a 4-fold increased risk of gastric cancer (21). Polymorphisms in

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Table 3. Expression pattern of investigated genes in gastric cancer samples

Gene	Greater expression in tumor sample>1.8, n (%)	Greater expression in peritumor sample<0.55, n (%)	Irrelevant difference 0.55–1.8, n (%)
MSH2	13 (35.13)	3 (8.11)	21 (56.76)
MSH6	14 (37.84)	2 (5.40)	21 (56.76)
EXO1	17 (45.95)	0 (0.00)	20 (54.05)

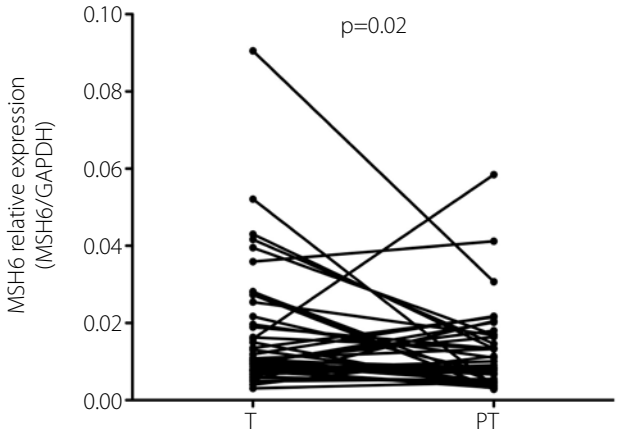

Figure 3. Comparative expression of MSH6 mRNA in paired tumoral and peritumoral mucosa. Data are presented as the relative mRNA expression of the target gene to GAPDH estimated by the Wilcoxon matched-pairs signed rank test.

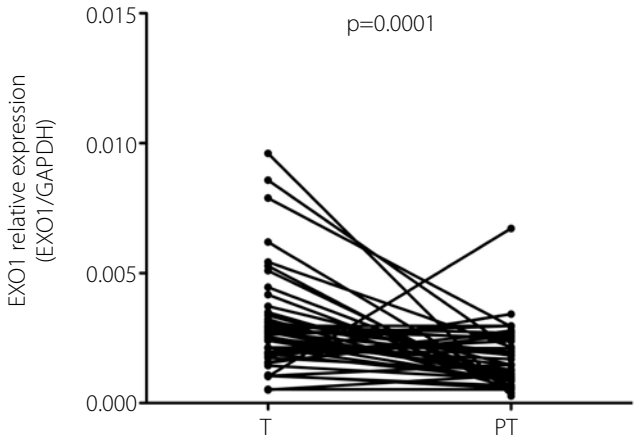

Figure 4. Comparative expression of EXO1 mRNA in paired tumoral and peritumoral mucosa. Data are presented as the relative mRNA expression of the target gene to GAPDH estimated by the Wilcoxon matched-pairs signed rank test.

MSH2 (IVS10+12G>A and IVS12-6T>C) and EXO1 (K589E) were shown to increase the risk of gastric cancer in Asian populations (22,23).

Our findings of MMR gene overexpression are in accordance with those obtained by Li et al. (24) regarding increased MMR gene expression in gastric cancer. Li's study showed an over-

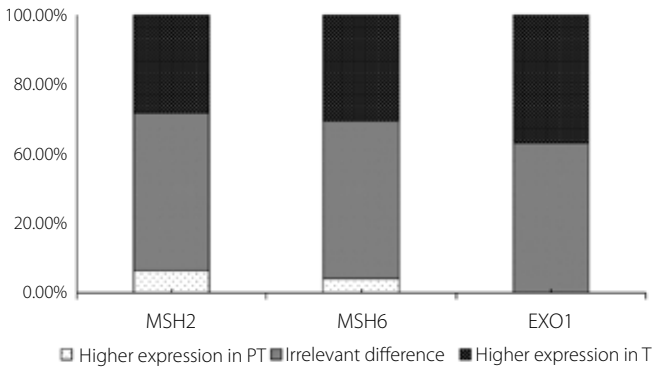

Figure 5. Expression pattern for MSH2, MSH6, and EXO1 genes in gastric adenocarcinoma samples.

expression of MSH2 in gastric tumor samples and in the surrounding mucosa compared with that in the gastric mucosa of non-cancer patients.

Increased MMR gene expression in tumors may result from several possible causes. Overexpression may be induced by genetic variants that alter gene expression, thereby leading to an increased level of MMR proteins with impaired functions (25). Because cancerous cells accumulate many genetic alterations, it is also possible that an increased level of MMR proteins could be a cellular adaptation aimed to repair DNA lesions (26). Another explanation may be that the overexpression of these genes in tumor cells could represent a possible response to the rapidly growing number of replication errors in a tissue with an increased rate of cell divisions. An upregulation of MSH2 protein has been detected during the replicative phase of the cell cycle when DNA synthesis occurs as well as in the post-replicative stages (14).

MSH2 was found to be expressed in highly proliferative cells of the human gastrointestinal tract, and mutations in this gene could, therefore, be expected to favor the progression of adenoma to carcinoma in this tissue (27,28).

In conclusion, our study offers new evidence that MMR genes have a tendency toward overexpression in gastroesophageal carcinomas. Future studies with larger sample sizes are needed to determine whether MMR gene expression can be considered a useful marker in the early prediction of gastroesophageal cancers and to validate the association between MSH2, MSH6, and EXO1 expression and pathological characteristics.

Ethics Committee Approval: This study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania.

Informed Consent: Written informed consent was obtained from patients participants in this study.

Peer-review: Externally peer-reviewed.

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A.D., M.D., M.I.; Data Collection &/or Processing - A.D., M.D., M.C.; Analysis &/or Interpretation - A.D., C.A., M.I.; Literature Search - A.D., M.D., M.C., F.B.; Writing - A.D., C.A., F.B.; Critical Reviews - M.I., F.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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