Original Article

ความสำคัญของ Mismatch Repair Gene กับการพยากรณ์โรคในผู้ป่วยมะเร็งลำไส้ใหญ่และลำไส้ตรงที่ได้รับยาเคมีบำบัดชนิด 5-fluorouracil

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Prognostic Significance of the Mismatch Repair Gene in Colorectal Cancer Patients Treated with 5-Fluorouracil Chemotherapy.

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บทคัดย่อ

หลักการและเหตุผล: Mismatch repair gene เป็นยีนที่มีหน้าที่ป้องกันความผิดพลาดระหว่างการสังเคราะห์สาย deoxyribonucleic acid (DNA) หากยีนนี้เกิดความผิดปกติ จะเพิ่มความเสี่ยงต่อการเกิดมะเร็งหลายชนิด รวมถึงมะเร็งลำไส้ใหญ่และลำไส้ตรง ผลกระทบจากความผิดปกติของยีนนี้กับการพยากรณ์โรคของมะเร็งลำไส้ใหญ่และลำไส้ตรงยังคงไม่มีความชัดเจน

วัตถุประสงค์: เพื่อหาความสัมพันธ์ระหว่างความผิดปกติของ mismatch repair gene กับการพยากรณ์โรคในผู้ป่วยมะเร็งลำไส้ใหญ่และลำไส้ตรงซึ่งได้รับยาเคมีบำบัดชนิด 5-fluorouracil

วัสดุและวิธีการ: การศึกษานี้ทำในผู้ป่วยมะเร็งลำไส้ใหญ่และลำไส้ตรงระยะที่ 2 ถึงระยะที่ 4 ของโรงพยาบาลสงขลานครินทร์ ระหว่างปี พ.ศ. 2537-2546 จำนวนผู้ป่วยทั้งสิ้น 140 ราย ผู้ป่วยทุกรายได้รับการผ่าตัด และได้รับ

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ยาเคมีบำบัดชนิด 5-fluorouracil จากนั้นทำการศึกษาโดยนำชิ้นเนื้อของผู้ป่วยมาย้อม immunohistochemistry ตรวจการแสดงออกของโปรตีน mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*) (MLH1) และ mutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*) (MSH2) ในเซลล์มะเร็ง หากไม่มีการแสดงออกของโปรตีนชนิดใดชนิดหนึ่ง แสดงว่าผู้ป่วยมีความผิดปกติของ mismatch repair genes จากนั้นนำข้อมูลที่ได้มาคำนวณ Survival analysis โดย Kaplan-Meier method และ Cox Proportional Hazards regression

ผลการศึกษา: จากการศึกษาพบความผิดปกติของ Mismatch repair gene ในผู้ป่วย 36 ราย (ร้อยละ 25.7) แบ่งเป็นความผิดปกติของ MLH1 เพียงอย่างเดียวจำนวน 15 ราย (ร้อยละ 10.7) ความผิดปกติของ MSH2 เพียงอย่างเดียวจำนวน 10 ราย (ร้อยละ 7.1) และความผิดปกติของ MLH1 ร่วมกับ MSH 2 จำนวน 11 ราย (ร้อยละ 7.8) การวิเคราะห์ survival analysis พบว่าความผิดปกติของ mismatch repair gene ไม่มีความสัมพันธ์ที่มีนัยสำคัญทางสถิติกับการพยากรณ์โรคของผู้ป่วย (ช่วงความเชื่อมั่นที่ร้อยละ 95=0.35-0.67, p=0.54)

สรุป: ความผิดปกติของ mismatch repair gene ไม่สามารถบ่งบอกถึงการพยากรณ์โรคในผู้ป่วยมะเร็งลำไส้ใหญ่และลำไส้ตรงซึ่งได้รับยาเคมีบำบัดชนิด 5-fluorouracil

คำสำคัญ: 5-fluorouracil, colorectal cancer, Mismatch repair gene, MLH1 and MSH2

Abstract:

Background: Mismatch repair genes fix problems that arise during deoxyribonucleic acid (DNA) replication when bases are incorrectly paired, as well as preventing microsatellite instability. Mutations are associated with an increased malignancy risk, including colorectal carcinoma. There are conflicting reports concerning colorectal carcinoma with and without mismatch repair gene mutations in association with disease outcome.

Objective: To correlate mismatch repair gene defects with the outcomes of colorectal carcinoma patients having undergone adjuvant chemotherapy containing 5-fluorouracil.

Materials and methods: The records of all stage II, III and IV colorectal carcinoma cases treated at Songklanagarind Hospital from 1994-2003 were examined for the study. There were 140 patients and all had received adjuvant 5-fluorouracil-based chemotherapy. The mismatch repair gene proteins mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*) (MLH1) and mutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*) (MSH2) in tumor cells were evaluated by immunohistochemistry. Negative staining confirmed abnormalities in or complete loss of mismatch repair genes. Survival analysis was carried out using the Kaplan-Meier method and the Cox Proportional Hazards regression.

Results: Mismatch repair gene defects were detected in 36 cases (25.7%): only MLH1 defects in 15 cases (10.7%), only MSH2 defects in 10 cases (7.1%), and both MLH1 and MSH2 defects in 11 cases (7.8%). Survival analysis showed no significant differences between mutated mismatch repair gene patients and normal mismatch repair gene patients (95% CI=0.35 to 0.67, p=0.54).

Conclusion: Mismatch repair gene defects do not predict the prognosis of colorectal cancer patients treated with 5-fluorouracil-based chemotherapy.

Key words: 5-fluorouracil, colorectal cancer, Mismatch repair gene, MLH1 and MSH2

Introduction

The incidence of colorectal cancer has been increasing every year in Thailand, and currently is the 3rd and 5th most common cancer in both Thai males and females respectively, according to the Cancer Report of Thailand of 1998-2000.[1] If this trend continues, it will soon become the major cause of death from cancer in Thailand. Colorectal carcinoma has high morbidity and mortality, so timely, effective diagnosis and treatment are necessary to improve the patient's survival chances.

Colorectal carcinoma is associated with two major genetic abnormalities: the adenomatous polyposis coli (APC)-β catenin gene mutation and microsatellite instability. The APC gene controls β-catenin to prevent the abnormal growth of colonic epithelium. If the APC-β catenin function is defective, the colonic epithelium will experience uncontrolled division leading to cancer development. The APC-β catenin gene mutation is found in 80% of sporadic colorectal carcinomas.[2]

A microsatellite is a repeat of 1-6 nucleotides on the deoxyribonucleic acid (DNA) sequence. The human genome has 50,000-100,000 microsatellites. If the mutation occurs in a sequence, which is called a microsatellite instability, it will lead to a DNA frame shift mutation. Such a frame shift mutation in the microsatellite area of the pro-apoptotic Bcl-2 associated X gene (BAX) or the transforming growth factor (TGF)-β gene causes colorectal cancer. Fortunately, the human genome has a prevention system to correct such microsatellite instabilities, the mismatch repair gene.[3]

The mismatch repair gene synthesizes a mismatch repair protein to repair incorrect DNA base pairs. If the mismatch repair gene mutates, however, the human genome will not have any tool to fix the microsatellite instability that leads to frame shift mutation in any microsatellite area. The mismatch repair gene has many locations such as MSH2, MLH1, mutS homolog 6 (*E. coli*) (MSH6), postmeiotic segregation increased 1 (S. cerevisiae) (PMS1) and postmeiotic segregation increased 2 (S. cerevisiae) (PMS2). Mutations of the MLH1 and MSH2 genes are found in 90% of all mismatch repair gene mutations.[3,4] MLH1 and MSH2 mutations are found in 15% of colorectal carcinomas, and are also related to hereditary nonpolyposis colorectal cancer (HNPCC), endometrial carcinoma, and urinary bladder carcinoma.[5,6]

In recent years, immunohistochemistry staining and molecular biological technique have been the main tests used to detect mismatch repair gene mutations.[7,8] The immunohistochemistry staining technique is convenient to perform in routine cases and has a low expense compared to the molecular technique. The immunohistochemistry staining test has high sensitivity and specificity to detect abnormal expression of a mismatch repair protein.[9] So the National Comprehensive Cancer Network (NCCN) Guideline of Colorectal Cancer Treatment 2010 recommends the immunohistochemistry staining method for detecting mismatch repair genes.[10]

The presence of mismatch repair genes mutation in colorectal carcinoma indicates a unique prognosis. Studies comparing mismatch repair gene mutations and tumor staging have shown a better prognosis in patients who have a mismatch repair gene mutation than patients who have normal mismatch repair genes.[11-15]

The treatment of colorectal cancer is composed of resection surgery with adjuvant chemotherapy. The major chemotherapeutic drug is a methylating drug such as 5-fluorouracil. The mismatch repair gene supports the effect of the methylating drug by separating the DNA base pair, then the methylating drug causes the double DNA strain to break, leading to apoptosis of the tumor cell.[16] According to this relation, if the mismatch repair gene mutates, the methylating drug will not have the effect of killing the cancer cell, leading to unresponsiveness to chemotherapy in the colorectal cancer patient. However, studies examining the relation between mismatch repair gene mutations and the prognosis of colorectal cancer patients treated with adjuvant chemotherapy remain controversial.[17-23]

The objective of this study was to determine the correlation between mismatch repair gene defects and the prognosis of colorectal cancer patients who were treated with adjuvant chemotherapy containing 5-fluorouracil.

Materials and methods

Study population

This retrospective study collected clinical and treatment data from stage II, III and IV colorectal carcinoma patients attending Songklanagarind Hospital during 1994-2003. Patients who had a follow up time less than 5 years and patients who did not receive a complete course of chemotherapy were excluded. From 185 patients who were diagnosed with colorectal cancer during the study period, there were 140 patients who met our criteria, and all of them had had adjuvant chemotherapy containing 5-fluorouracil. All patients had paraffin blocks from their colorectal surgery. The clinical and treatment data of these patients were reviewed.

Immunohistochemistry staining

MLH1 and MSH2 immunohistochemistry staining were performed on paraffin-embedded tissues from all cases. The staining procedure was done following a previously-described method.[9] The antibodies against MLH1 protein was mouse monoclonal antibodies (clone ES05 Leica Biosystems, Newcastle Ltd, England) and the antibodies against MSH2 protein was mouse monoclonal antibodies (clone 25D12 Leica Biosystems, Newcastle Ltd, England). An Envision Kit was employed as a secondary detection system, and the peroxidase reaction was developed using diaminobenzidine tetrachloride as a chromogen.

All cases were scored as negative (defined as less than10% tumor cell staining) or positive (defined as equal or more than 10% of tumor cell staining).[24] The example results of MLH1 and MSH2 immunohistochemistry staining were showed in Figure1.

Statistical methods

The relationships between tumor mismatch repair genes and clinical and pathological factors were assessed using the chi-squared test. The Kaplan-Meier method was used for survival comparisons among mismatch repair genes status groups. The Cox proportional hazard regression was used to calculate the relative risk ratios. Significance for all statistics was recorded if $p<0.05$. All statistical analyses were performed using Stata program version 6.

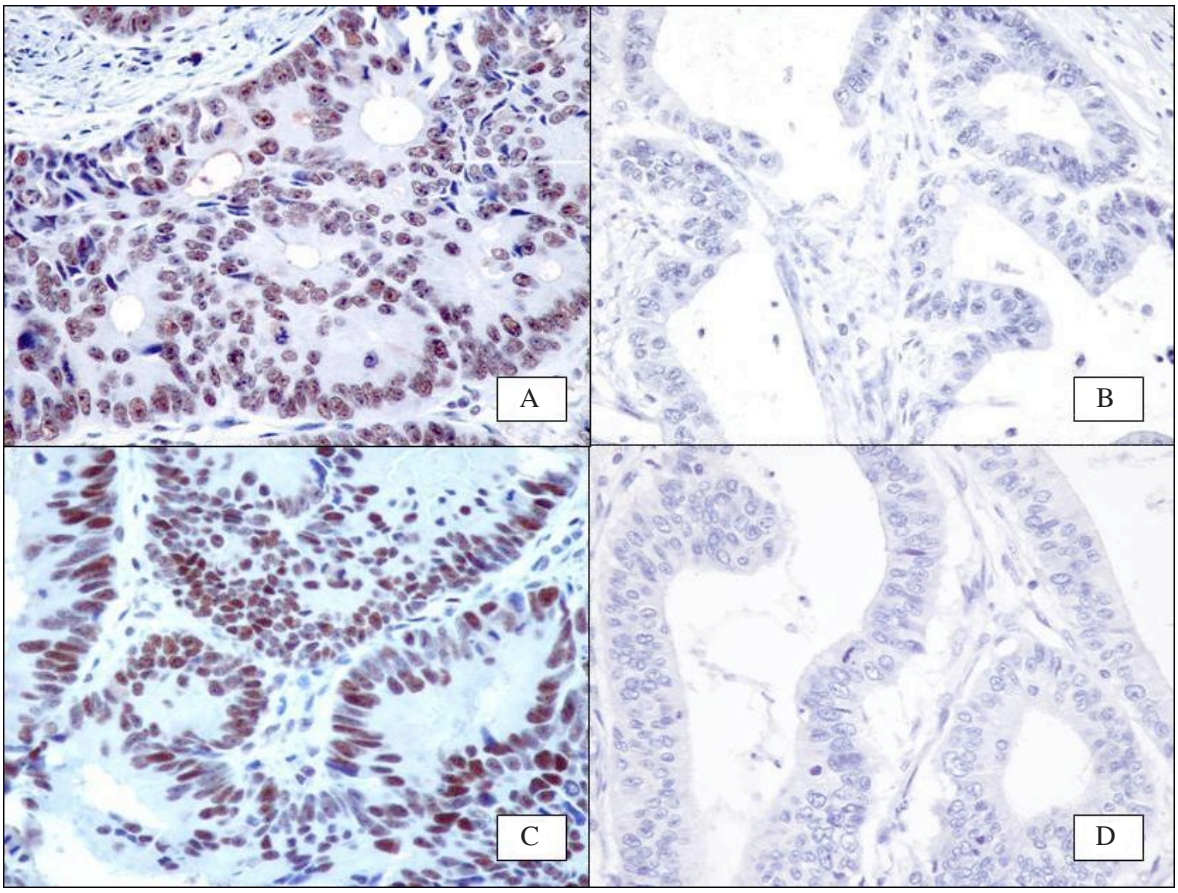

Figure 1 A, Adenocarcinoma with positive staining of MLH1 protein. B, Adenocarcinoma with loss of expression of MLH1 protein. C, Adenocarcinoma with positive staining of MSH2 rotein. D, Adenocarcinoma with loss of expression of MSH2 protein

Results

All slides from the 140 cases were stained with MLH1 and MSH2 proteins. Mismatch repair protein non-expression was detected in 36 cases (25.7%): the only MLH1 non-expression in 15 cases (10.7%), the only MSH2 non-expression in 10 cases (7.1%), and both MLH1 and MSH2 non-expression 11 cases (7.8%).

Tumors with mismatch repair gene protein defect (non-expression of MLH1 protein and/or MSH2 protein) were significantly more common in the cecum and colon than in the rectum (X^2 p=0.03). The median age of the patients was 59 years old, but no significant relationship was found between age group and mismatch repair gene status (p=0.16 from Mann-Whitney test). 19 female and 17 male patients were found with mismatch repair gene defect, indicating gender was not associated with mismatch repair gene status (X^2 p=0.43). The mismatch repair gene

defect was found significantly more in well-differentiated carcinomas than in moderately and poorly differentiated carcinomas (X^2 p<0.01).

Of the mismatch repair gene defect patients, there were 18 cases of colorectal cancer stage II, 14 cases of colorectal cancer stage III, and 4 cases of colorectal cancer stage IV. The mismatch repair gene defect significantly decreased from colorectal cancer stage II through stage IV (X^2 p<0.01) (Table 1).

Table 1 Characteristics of the patients

Characteristic	All patients (N=140)		Patients with mismatch repair gene normal (N=104)		Patients with mismatch repair gene defect (N=36)		P-value
	NO.	%	NO.	%	NO.	%	
Location							0.03
Cecum or colon	64	45.7	39	37.5	24	66.7	
Rectum	76	54.3	65	62.5	12	33.3	
Age (years)							0.33
Median	59		59		59		
Range	20-74		30-76		20-71		
Sex							0.43
Male	66	47.1	47	45.2	19	52.8	
Female	74	52.9	57	57.8	17	47.2	
Tumor grading							<0.01
Well differentiated	85	60.7	70	67.3	15	41.7	
Moderately differentiated	42	30.0	30	28.8	12	33.3	
Poorly differentiated	13	9.3	4	3.9	9	25.0	
Chemotherapy							0.50
Only 5-fluoruracil	69	49.3	53	50.9	16	44.4	
5-FU and Leucovorin	71	50.7	51	49.1	20	55.6	
Radiation							0.08
No radiation	84	60.0	58	55.7	26	72.3	
5-FU and radiation	56	40.0	46	44.3	10	27.7	
Tumor staging							0.04
Stage II	47	33.6	29	27.9	18	50	
Stage III	67	47.9	53	50.9	14	38.9	
Stage IV	26	18.6	22	21.2	4	11.1	

The Kaplan-Meier curve showed no significant difference in overall survival between normal mismatch repair gene patients and defective mismatch repair gene patients (p=0.54, 95% CI=0.35 to 0.67) (Figure 2). The median survival of the normal mismatch repair gene (MMR) patients was 62 months (95% CI=0.33 to 0.65) and median survival of the MMR defect patients was 40 months (95% CI=0.40 to 0.59).

Univariate Cox regression analysis showed no significant difference in 5-year survival between the mismatch repair gene normal group and the mismatch repair gene defective group (p=0.93) (Table 2). The only significant survival factor was tumor staging (p<0.01). Tumor stages II, III and IV had 57.5 %, 46.3% and 3.9% 5-year survival rates, respectively. The other observed factors, tumor location, sex, tumor grade, leucovorin chemotherapy and radiation, had no significance impact on survival.

Multivariate analysis showed no significant difference between mismatch repair gene status and hazard ratio of survival. Tumor staging revealed patients with colorectal cancer stages III and IV had 1.37 and 4.74-fold higher risk of death respectively than stage II patients (Table 3).

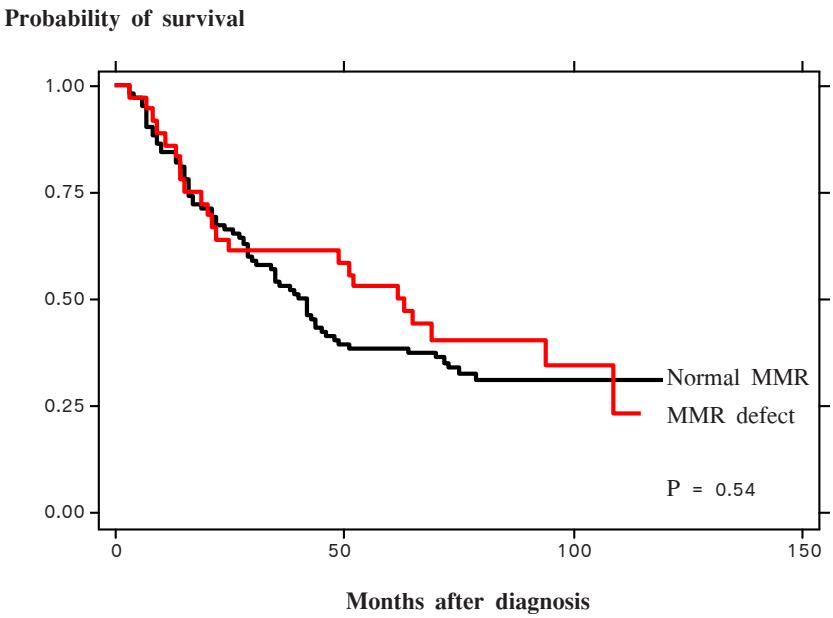

Figure 2 Kaplan-Meier curve of survival times between normal and defective mismatch repair genes

Table 2 Parameters of 5 year survival (Univariate analysis)

Characteristic	Probability of 5-year survival (%)	95% CI	P-value
MMR status			0.934
MMR normal	38.46	29.16-47.67	
MLH1 defect	53.33	26.32-74.38	
MSH2 defect	60.00	25.27-82.72	
Both MLH1 and MSH2 defects	45.45	16.66-70.69	
Location			0.443
Cecum or colon	48.44	32.80-59.96	
Rectum	36.84	26.16-47.54	
Sex			0.823
Male	42.42	30.42-53.91	
Female	41.89	30.59-52.77	
Tumor grading			0.424
Well differentiated	42.35	31.77-52.53	
Moderately differentiated	45.24	29.92-59.37	
Poorly differentiated	30.77	9.50-55.43	
Treatment			0.180
Only 5-fluorouracil	44.93	32.99-56.14	
5-fluorouracil and leucovorin	39.44	28.13-50.53	
Radiation			0.229
No radiation	39.29	28.89-49.51	
Radiation	46.43	33.05-58.76	
Tumor staging			<0.01
Stage II	57.45	42.15-70.07	
Stage III	46.27	34.06-57.61	
Stage IV	3.85	0.28-16.43	

Table 3 Multivariate analysis according to microsatellite status and tumor staging

Characteristic	Hazard ratio	95% CI	P-value
Mismatch repair gene status			0.935
Normal	1 ref.	-	
MLH1 defect	1.07	0.55-2.11	
MSH2 defect	0.93	0.40-2.57	
Both MLH1 and MSH2 defects	1.29	0.58-2.86	
Tumor staging			<0.01
Stage II	1 ref.		
Stage III	1.37	0.83-2.26	
Stage IV	4.75	2.62-8.57	

Discussion

The relationship between a mismatch repair gene defect and colorectal cancer survival is controversial. Several studies have found a better prognosis in mismatch repair gene defect patients than mismatch repair gene normal patients,[11-15] but other studies have found no difference in survival between these groups.[25,26]

The main treatment for stages II, III and IV colorectal cancer is surgery with adjuvant 5-fluorouracil-based chemotherapy. Many studies have found a poor prognosis in mismatch repair gene defect patients who received adjuvant chemotherapy,[17-20] but many studies have also reported the opposite.[21-23]

Currently, the main hypothesis explaining the effect of a mismatch repair gene defect is reduction of apoptosis. Treatment of cells with methylating agents produces DNA O6-methylguanine (O6-meG). Similarly, during growth in 6-thioguanine (6-TG), the thiopurine is extensively incorporated into the DNA and undergoes a rare non-enzymatic methylation to produce a small number of S6-thiomethylguanines (6-meTG). The methylated bases code ambiguously during replication to generate structures that resemble mismatches and that are processed by MMR. This processing is incomplete, however, and leads to the generation of DNA double-strand breaks that have a high probability of inducing apoptosis. Inactivation of MMR prevents damage processing and reduces the connection between DNA damage and apoptosis induction. In the clinic, this would lead to treatment failure.[16]

The relationship between mismatch repair gene defect and characteristic of patient in this study showed no statistic significant except the location of tumor, differentiation of tumor and staging of tumor. The mismatch repair gene defect generally found in the proximal part of colon more than distal part of colon and found in early stage of cancer more than advance stage of cancer. In this study, the mismatch repair gene defect was found in the well differentiated tumor more than poorly differentiated tumor that was difference from previously study.[13] The mismatch repair gene defect probable to be found in the early development of tumor that usually has well differentiation and has early stage.

This study showed no difference in 5-year survival between the mismatch repair gene normal group and the mismatch repair gene defect group. These results correlated with other previous studies which explained this phenomenon by the sequence of carcinogenesis. The mismatch repair gene mutation is the initial factor that increases the risk of other gene mutations such as with the p53 and K-ras genes.[3,4] After the mismatch repair gene causes a frame shift mutation, it does not have any further role in the colorectal cancer carcinogenesis.

The only significant factor is tumor staging, which shows a decreased 5-year survival from stage II to stage IV. This indicates that colorectal cancer progression depends on the colorectal cancer staging more than the mismatch repair gene.

The study employed no molecular technique to confirm the mismatch repair gene mutations. However, in general the immunohistochemical study of expression of the mismatch repair proteins

we used has a greater ability than the molecular technique to detect the function of mismatch repair genes in cases of hypermethylation of mismatch repair genes that do not produce the mismatch repair protein in spite of no gene mutation.[27,28]

Conclusion

In conclusion, in this retrospective study of 140 colorectal cancer patients who received 5-fluorouracil-based chemotherapy, we detected the non-expression of mismatch repair protein in 36 cases (25.7%), but the relation between overall survival and mismatch repair protein expression was not statistically significant.

References

1. Srivatanakul P, Attasara P. Cancer incidence and leading sites. In: Khuhaprema T, Srivatanakul P, Sriplung H, et al, editors. Cancer in Thailand. Vol IV, 1998-2000. Bangkok: BM Publisher; 2007; 9 - 21.
2. Liu C, Crawford JM. The gastrointestinal tract. In: Robbins SL, Cotran RS, editors. Robbins and Cotran's Pathologic Basis of Disease. 7th ed. Philadelphia: Elsevier Sauders; 2004: 862 - 8.
3. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev 2007; 21: 2525 - 38.
4. Mitchell RJ, Farrington SM, Dunlop MG, et al. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: a HUGE review. Am J Epidemiol 2002; 156: 885 - 902.
5. Watson P, Lynch T. Cancer risk in mismatch repair gene mutation carriers. Fam Cancer 2001; 1: 57 - 60.
6. Umar A, Boyer JC, Thomas DC, et al. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem 1994; 269: 14367 - 70.
7. Blanes A, Diaz-Cano SJ. Complementary analysis of microsatellite tumor profiles and mismatch repair defects in colorectal carcinomas. World J Gastroenterol 2006; 12: 5932 - 40.
8. Dietmaier W, Wallinger S, Bocker T, et al. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997; 7: 4749 - 56.
9. Lanza G, Gafa R, Maestri I, et al. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol 2002; 15: 741 - 9.
10. National Comprehensive Cancer Network. Clinical practice guidelines in oncology. Colorectal cancer screening. Version 1.2010 [homepage on the Internet]. Fort Washington: National Comprehensive Cancer Network [cited 2010 April 12]. Available from: http://www.nccn.org/professionals/physician_gls/PDF/colorectal_screening.pdf
11. Parc Y, Gueroult S, Mourra N, et al. Prognostic significance of microsatellite instability determined by immunohistochemical staining of MSH2 and MLH1 in sporadic T3N0M0 colon cancer. Gut 2004; 53: 371 - 75.
12. Columbino M, Cossu A, Manca A, et al. Prevalence and prognosis role of microsatellite instability in patients with rectal carcinoma. Ann Oncol 2002; 13: 1447 - 53.
13. Smyth EF, Sharma A, Sivarajasingham N, et al. Prognostic implications of hMLH1 and p53 immunohistochemical status in right-sided colon cancer. Dis Colon Rectum 2004; 47: 2086 - 91.
14. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. J Clin Pathol 2008; 61: 561 - 9.
15. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000; 124: 979 - 94.
16. Bignami M, Casorelli I, Karran P. Mismatch repair and response to DNA-damaging antitumour therapies. Eur J Cancer 2003; 39: 2142 - 9.
17. Jover R, Castells A, Llor X, et al. Predictive value

of microsatellite instability for benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55: 1819 - 20.

18. Hemminki A, Mecklin JP, Jarvinen H, et al. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology 2000; 119: 921 - 8.
19. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003; 349: 247 - 57.
20. Clark AJ, Barnetson R, Farrington SM, et al. Prognosis in DNA mismatch repair deficient colorectal cancer: are all MSI tumours equivalent? Fam Cancer 2004; 3: 85 - 91.
21. Lamberti C, Lundin S, Bogdanow M, et al. Microsatellite instability did not predict individual survival of unselected patients with colorectal cancer. Int J Colorectal Dis 2007; 22: 145 - 52.
22. Benatti P, Gafa R, Barana D, et al. Microsatellite instability and colorectal cancer prognosis. Clin Cancer Res 2005; 11: 8332 - 440.
23. Iacopetta B, Watanabe T. Predictive value of microsatellite instability for benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55: 1671 - 2.
24. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: cancer and leukemia group B protocol 89803. J Clin Oncol 2009; 27: 1814 - 21.
25. Kruschewski M, Noske A, Haier J, et al. Is reduced expression of mismatch repair genes MLH1 and MSH2 in patients with sporadic colorectal cancer related to their prognosis? Clin Exp Metastasis 2002; 19: 71 - 7.
26. Gafa R, Maestri I, Matteuzzi M, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. Cancer 2000; 89: 2025 - 37.
27. Esteller M, Corn PG, Baylin SB, et al. A gene hypermethylation profile of human cancer. Cancer Res 2001; 61: 3225 - 9.
28. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the hMLHl promoter in colon cancer with microsatellite instability. Cancer Res 1998; 58: 3455 - 60.