

Relationship between Aberrant Expression of hMSH2 and Prognosis in Patients with Sporadic Colorectal Cancer

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Abstract

The aim of the study was to investigate relationship of hMSH2 aberrant expression and clinicopathological features of patients with sporadic colorectal cancer, and to explore effect of aberrant expression on prognosis. Clinicopathologic materials and postoperative samples of 327 patients with sporadic colorectal cancer in our hospital were collected. Immunohistochemistry PV-9000 two step method was performed to measure the situation of hMSH2 expression in 327 postoperative pathologic specimens. Relationship of hMSH2 aberrant expression and clinicopathological features of patients with sporadic colorectal cancer was analyzed. Prognosis difference between the groups of normal and abnormal protein expression of hMSH2 was compared. 10.7% of the patients showed abnormal nuclear staining for hMSH2 protein expression. Aberrant hMSH2 expression of sporadic colorectal cancer mostly indicated in right hemicolon, and its histological type mainly showed mucinous adenocarcinoma and low differentiated degree. The patients with loss of protein expression of hMSH2 displayed better prognosis than normal expression group. Consequently, aberrant expression of hMSH2 plays a significant part in occurrence and development for patients with sporadic colorectal cancer and its deletion may be an independent prognostic factor.

serious harm to people's health. Its occurrence and development is a very complex process and the exact

1. Introduction

Currently, colorectal cancer is a common digestive malignancy, and it has done more and more

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mechanism hasn't entirely clear. However, occurrence and development of tumor is a complex process of multi-factor, multi-step and multi-gene change, involving participation of multiple tumor-related factors. It includes oncogenes activation, inactivation of tumor suppressor genes, mismatch repair gene (MMR) mutations, gene promoter methylation, and so on[1-4]. With the discovery of MMR, its aberrant expression significance studies in hereditary non-polyposis colorectal cancer (HNPCC) and sporadic colorectal cancer, have been extensively carried out. Some studies have reported that aberrant MMR genes play an important role in occurrence and development of colorectal carcinoma[5-7].

Recent studies have found that hMSH2 protein expression can predict function aberrant expression of MMR gene and MSI presence[8]. One of important feature of MMR dysfunction is positive MSI. Compared with normal cells, gene mutation rate of microsatellite sequences in tumor cells with dysfunction of MMR gene is from 100 to 1000 times higher than normal cells. MSI can be found in a variety of tumors, of which colorectal cancer has been researched more. MSI performs positive expression in about 95% of HNPCC and 15% of sporadic colorectal. However, their mechanisms are different. MSI occurrence in sporadic colorectal cancer is mainly related with high hypermethylation of MMR gene promoter[9]. Some other studies showed there was significant different clinicopathological fetures between MSI-H (MSI-high) patients with colorectal cancer and normal expression of MMR. MSI-H tumors appeared more in female patients, in proximal colon, poorly differentiated and mucinous adenocarcinoma[10,11]. However, the majority of findings revealed that the higher MSI patients had a better prognosis[12,13].

Presently, there have been relatively less researches on hMSH2 aberrant expression, pathogenesis of sporadic colorectal cancer and its correlation with prognosis. In the study, we were to analyze relationship of hMSH2 aberrant expression and clinicopathological parameters of patients with sporadic colorectal cancer, to investigate its function in pathogenesis and to explore its effect on prognosis by collecting clinicopathologic data and partial follow-up data of 327 patients with sporadic colorectal cancer in our hospital and measuring hMSH2 expression level by immunohistochemistry.

2. Materials and Methods

2.1 Patients and Samples

Clinicopathological parameters and postoperative samples of 327 patients with colorectal cancer from January 1st 2005 to January 1st 2008 in Tumor Hospital of Xinjiang Medical University were collected. Data included age at diagnosis, gender, nationality, tumor size,

histological type, TNM stage, tumor location, lymphangion invasion and peripheral nerve infiltration (table 1 in detail). Diagnosis criterion as followings, for HNPCC, according to Amsterdam II; for TNM stage, according to American Joint Committee on Cancer (AJCC) / International Union Against Cancer (UICC) TNM staging system of colorectal cancer (2010, Seventh Edition). Patients with HNPCC, receiving preoperative chemoradiotherapy or lack of data, were all excluded.

2.2 Follow-up

All enrolled patients had complete personal follow-up files and explicit pathological diagnosis. After surgery, patients were followed up once a month for months, once every three months for within two years and then once every 6 months. Two follow-up ways were used, outpatient or inpatient review and telephone follow-up, including start time of postoperative chemotherapy, chemotherapy regimens, chemotherapy course count, side effects of chemotherapy, recurrence, survival time, and so on.

2.3 Immunohistochemical method

Neutral formalin-fixed(with concentration of 40g/L) and paraffin-embedded specimens were serially sectioned by thickness of 5 μ m, and PV-9000 two-step method was performed, using mouse anti-human monoclonal antibody of hMSH2 (from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) as primary antibodies with working concentration of 1:150. Universal two-step method (HRP) detection kit (from Fujian Maixin Biological Products Co., Ltd.) was utilized. PBS was instead of primary antibody as negative control, while normal colorectal mucosa and/or infiltrating lymphocytes were used as positive control. Positive expression of hMSH2 was in nucleus. Result judgement standard was according to Xu LZ's immunohistochemical method criterion[14]. Microscopic tumor cells showing positive nuclear staining was combined with staining intensity and percentage of positive cells, to determine positive expression levels. 5 high-power field with more cancer cells was selected from each slice by light microscope and each field counted 100 cells per field. According to grading of staining intensity, no coloring is 0 points, light yellow is 1 point, yellow is 2 points and brown is 3 points; according to grading of positive cell percentage, no positive cell is 0 point. Less than or equal 10% is 1 point, from 11% to 50% is 2 points, from 51% to 75% is 3 points and more than 75% is 4 points. If the result of two scores above multiplying is more than or equal 2 points, it will be judged as a positive expression case, meanwhile if less than 2 points, it will be judged as a negative expression case. Positive control was positive nuclei of

normal colorectal mucosa and/or infiltrating lymphocytes. Meanwhile, negative is judged in case of nucleus positive expression of positive control and tumor cell nuclei missing staining.

2.3 Statistical analysis

Univariate analysis between hMSH2 protein expression and clinicopathologic features was performed with chi-square test and Fisher's exact test, and multivariate correlation analysis between the two above was made with Logistic regression test. Univariate survival analysis was carried out by Kaplan-Meier survival curves, and Log-rank test was used for comparison between the groups. Multivariate survival analysis was performed by COX regression model. All above were carried out via SPSS for Windows Version 18 (SPSS Inc., Chicago, IL, USA). P values of less than or equal 0.05 were considered to be statistically significant.

3. Results

3.1 Immunohistochemical results of hMSH2

In the total of 327 cases with sporadic colorectal cancer, 35 patients (10.70%) showed aberrant nuclear staining of hMSH2 (Figure 1).

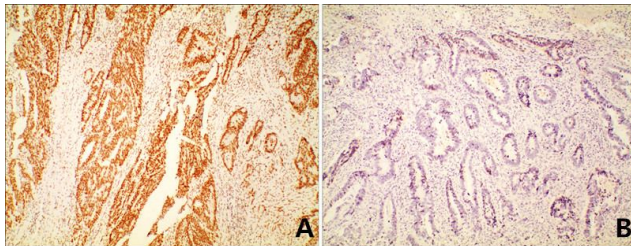


Figure 1 Showing of normal and aberrant hMSH2 expression by immunohistochemical staining (magnification, both 100×) A represents normal immunohistochemical staining, whose normal nuclear staining is not only in stromal cells but also notably in epithelial tumor cells, showing brownish accumulation of dye in the nucleus. B represents aberrant staining, whose abnormal nuclear staining is only in stromal cells, not in epithelial tumor cells.

3.2 Univariate analysis between aberrant hMSH2 expression and clinicopathologic parameters

By univariate analysis, hMSH2 aberrant expression of sporadic colorectal cancer was closely related with tumor location, histological type, differentiated degree, respectively with statistical significant difference ($P < 0.05$). There were no significant difference between hMSH2 aberrant expression and age, gender, nationality, tumor size, local infiltration depth, TNM staging, distant metastasis, regional lymphatic metastasis, peripheral nerve infiltration and lymphangion invasion,

respectively ($P > 0.05$) (Table 1).

Table 1 Univariate analysis results between hMSH2 protein expression and clinicopathologic parameters

	Normal hMSH2 n=541	Aberrant hMSH2 n=101	Total n=642	χ^2	P value
Age(years)				0.103	0.749
<50	74	8	82		
≥50	218	27	245		
Gender				0.036	0.850
male	180	21	201		
female	112	14	126		
Nationality				0.103	0.991
Han	245	30	275		
Uyghur	27	3	30		
Hui	11	1	12		
Others	9	1	10		
Tumor location				12.53	0.002
right hemicolon	53	15	68	2	
left hemicolon	89	10	99		
rectal	150	10	160		
Tumor size(cm)				5.506	0.064
<4	108	11	119		
4-6	112	9	121		
≥6	72	15	87		
Tissue type				10.00	0.007
Glandular (well/moderate)	203	15	218	1	
Glandular (poorly)	36	8	44		
mucous gland/signet cell	53	12	65		
TNM staging				7.490	0.058
I	29	2	31		
II	90	18	108		
III	136	14	150		
IV	37	1	38		
T staging				0.864	0.834
T1	16	1	17		
T2	35	3	38		
T3	70	9	79		
T4	171	22	193		
N staging				2.743	0.254
N0	128	20	148		
N1	94	7	101		
N2	70	8	78		
Distant metastasis				2.931	0.087
M0	255	34	289		
M1	37	1	38		
Lymphangion invasion				0.238	0.626
yes	33	3	36		
no	259	32	291		

Peripheral nerve infiltration			0.057	0.812
yes	14	2	16	
no	278	33	311	

3.3 Multivariate analysis of aberrant hMLH1 expression and clinicopathologic parameters

By Logistic regression, independent risk factors of hMSH2 aberrant expression were tumor location and histological type, with statistical significant difference(P<0.05) (table 2)

Table 2 Multivariate analysis results between hMSH2 protein expression and clinicopathologic parameters

Variable	B value	OR	95% confidence interval		P value
			Lower bound	Upper bound	
Histological type poor/mucinous vs good/moderate adenocarcinoma	-1.128	0.324	0.156	0.671	0.002
Tumor location right vs left / rectal	-1.236	0.291	0.137	0.615	0.001

3.4 Survival analysis of normal and aberrant expression groups of hMSH2

All patients were followed up with 20 cases in lost midway. By survival analysis, 5-year survival rate in the group of hMSH2 aberrant expression was higher than normal expression group, with difference of statistical significance (P<0.05). By stratified statistic analysis for the patients with staging II and III, it revealed that 5-year survival rate of aberrant hMSH2 groups in both staging II and III were higher than normal expression groups, but there were no statistical significant difference (P_{II}=0.472, P_{III}=0.051)(Figure 2)

By COX regression analysis, age, histological type, TNM staging, lymphangion invasion and hMSH2 expression were all independent risk factors for prognosis(each, P<0.05). Level of hMSH2 aberrant expression was closely related with prognosis(RR=0.361, 95%CI, 0.156-0.834) (Table 3)

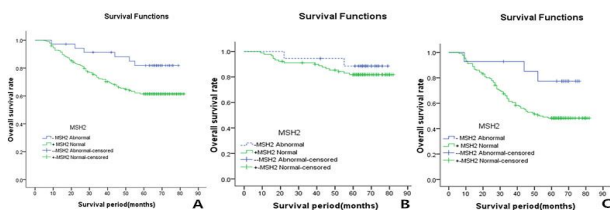
Figure 2 Comparison of 5-year survival rate between the groups of normal and aberrant hMSH2 expression A represents total patients: 5-year overall survival rate in group of aberrant hMSH2 expression was higher than normal group, with significant statistical difference (P<0.05); B stands for staging II patients: 5-year overall survival rate in group of aberrant hMSH2 expression was higher than normal group, with no statistical difference (P_{II}=0.472); C represents staging III: 5-year overall survival rate in group of aberrant hMSH2 expression was higher than normal group, with no statistical difference (P_{III}=0.051)

Table 3 COX regression survival analysis on prognosis

Variable	B value	RR	95% confidence interval		P value
			Lower bound	Upper bound	
Age	0.023	1.024	1.006	1.042	0.009
Tumor size	0.033	1.033	0.942	1.134	0.486
Glandular (well/moderately)	—	—	—	—	0.000
Glandular (poorly)	0.263	1.300	0.696	2.430	0.411
Mucinous adenocarcinoma	0.922	2.514	1.650	3.831	0.000
Left hemicolon	—	—	—	—	0.995
Right hemicolon	0.022	1.023	0.584	1.790	0.937
Rectum	0.022	1.022	0.652	1.601	0.925
T staging	0.022	1.022	0.797	1.311	0.862
N staging	0.248	1.282	0.912	1.802	0.153
TNM staging	0.755	2.127	1.199	3.772	0.010
Distant metastasis	0.317	1.374	0.606	3.112	0.447
Lymphangion invasion	0.728	2.071	1.258	3.411	0.004
Peripheral nerve infiltration	0.220	1.246	0.608	2.555	0.548
hMSH2	-1.020	0.361	0.156	0.834	0.017

4. Discussion

At present, a number of reports have confirmed immunohistochemical method is reliable for MMR gene measurement. The method has been put to use in vast majority of hospitals and research institutions. With low cost and stability, immunohistochemical method in detecting MMR gene expression and MSI of tissue specimens, has shown high sensitivity (77-100%) and specificity (98-100%) [11,15], so that recently immunohistochemical method has been suggested as the preferred method of MMR gene mutation analysis [16]. Immunohistochemistry PV-9000 two step method belongs to enzymatic biotin method. Monovalent Fab fragments of second antibody molecules polymerize with enzyme instead of traditional method of secondary and third antibody. Consequently, the antigen-antibody



binding signal is directly amplified. Compared with traditional SP third step method, it shows simple, fast and sensitive. Meanwhile, it also avoids background staining due to no biotin. Thus, Immunohistochemistry PV-9000 two step method is popularly used in clinical work. Thus, in the study immunohistochemistry PV-9000 two step method was performed to measure the situation of hMLH1 expression in 327 postoperative pathologic specimens.

Recently, MMR system includes genes MLH1, MSH2, MSH6, PSM1, PSM2, MSH3 and MSH5 [17]. Their expression product is a nucleic acid enzymes to correct mismatched basic group in DNA replication process for keeping more fidelity in copy process. Genes MLH1 and MSH2 have been researched more in the world, while the studies of genes MSH6 and PSM2 study are relatively less. Some studies reported there was low rate of aberrant hMSH2 in patients with sporadic colorectal cancer. In Kim's[18] study, its aberrant rate in sporadic colorectal cancer was about 9.375%. Lindor [11] reported that aberrant rate of hMSH2 was 8.8% by detecting 1114 patients with sporadic colorectal cancer. Nevertheless, Langner [19] pointed that hMSH2 mutation rate was 38.6% in the patients with sporadic colorectal cancer. In our study, aberrant expression of hMSH2 showed 10.7% in the patients with sporadic colorectal cancer, similar to most literature reports above.

Aberrant MMR expression were usually believed to be caused by point mutation. Long-term accumulation of mutations would ultimately lead to form replication error phenotype, which consequently caused secondary mutations and malignant transformation [20]. In addition, DNA methylation was an important way of causing MMR gene aberrant of both HNPCC and sporadic colorectal cancer, accompanied by MSI. What's more, DNA methylation was closely associated with specific expression of related gene[21]. Certainly, there weren't very few researches on hMSH2 methylation in sporadic colorectal cancer. Herman [22] didn't find hMSH2 island methylation in 34 patients with sporadic colorectal cancer. Zhang H[23] reported that hMSH2 methylation were found in 11 of 60 patients with sporadic colorectal cancer. However, Some researchers believed hMSH2 aberrant expression was due to missense mutations[24]. Obviously, mutation type of hMSH2 in sporadic colorectal cancer hasn't been reached final conclusion.

Some studies pointed out [10-11], missing expression of MMR protein was associated with some clinicopathological features. In our study, aberrant hMSH2 patients with sporadic colorectal cancer were mostly located in right colon. Their histological classification were mainly mucinous adenocarcinoma and poorly differentiated adenocarcinoma, which was

consistent with Perrin' study[25]. Right hemicolon carcinoma, poorly differentiated or mucinous adenocarcinoma were all relevant to poor prognosis, usually accompanied with histopathological feature of MSI-H. MSI incidence are mostly caused by aberrant MMR expression in sporadic colorectal cancer, which suggests that there is a certain relevance between its clinicopathologic characteristics and hMSH2 mutations. Some other related researches indicated molecular biologic characteristics were different in proximal colon and distal colon[26]. MMR aberrant expression was more common in right colon with no statistical difference between left colon and rectum. Pathopoiesia effect of MMR happened in incidence stage of tumor, with close relevance of molecular biology, and there might exist a higher rate of gene promoter hypermethylation in right hemicolon tissue. Patients suffering from colorectal cancer with clinicopathologic features of breakthrough membrane, regional lymphonodus metastasis, distant metastasis, peripheral nerve vascular invasion, staging III, staging IV, account for late stage and poor prognosis. Park' study[27] showed that MMR aberrant rate of colorectal cancer patients with regional lymphonodus metastasis, distant metastasis, positive lymphatic invasion, staging III or IV was very low. Yao's research[28] only confirmed hMSH2 aberrant expression were relevant to Dukes' staging, while Kruschewski[29] didn't confirm it. In our study, aberrant hMSH2 expression wasn't related with the clinicopathologic features representing late stage tumor. The patients with early-stage related clinicopathologic characteristics of negative lymphatic metastasis, staging I, staging II and no distant metastasis had lower hMSH2 aberrant rate. Obviously, it suggested that hMSH2 aberrant expression might occur in the early stage of colorectal cancer.

Some studies showed that about 90% of patients with HNPCC could be detected out MSI [30], MSI incidence was accompanied with aberrant hMLH1 or hMSH2, and there was a close relationship between the two. Meanwhile, MSI occurrence was similarly caused by MMR missing in patients with colorectal cancer [9]. Staebler[31] reported that, hMSH2 aberrant rate was 25% in colorectal cancer patients with positive MSI. Although hMSH2 aberrant rate wasn't so high, it still suggested that it played an important role in positive expression of MSI, similarly to Parc's[32]. In addition, some studies showed the patients suffered from colorectal cancer with positive MSI had specific clinicopathological features of poor differentiation, mucinous adenocarcinoma, young individuals, positive regional lymph nodes, and so on, but they mostly had better prognosis[33]. Popat[13]found that the patients with MSI showed better prognosis than microsatellite stability (MMS) through researching the

prognosis of 7642 patients with colorectal cancer. Wang XF[34] reported survival analysis results of 146 patients with sporadic colorectal cancer 5-year survival rate of MSI patients was 92.3%, significantly higher than MMS patients of 63.5%, similarly to most other studies[35-41]. The reason might be that MSI-H limited tumor growth. However, some other studies remained negative views[42,43].

MSI in the patients with colorectal cancer predicted a better prognosis and MSI incidence was associated with aberrant hMSH2 expression. Therefore, aberrant hMSH2 expression might forebode a better prognosis. Park[27] reported that the group of the patients with aberrant MMR had better prognosis than normal MMR group and the patients in TNM staging III showed higher survival rate. Lanza[44] reported 6-year survival rate of negative group and positive group in staging II were 97% and 82% respectively by studying 718 patients with colorectal cancer in staging II and III. Similarly, those were 78% and 56% respectively in staging III. Consequently, difference was statistically significant. Some studies indicated patients with aberrant MMR expression of colorectal cancer had better prognosis[45,46]. Mostly for hMLH1, there was few researches on relationship of only aberrant hMSH2 expression and postoperative survival time. Jansson's study[47] showed that patients with aberrant hMSH2 expression of colorectal cancer had higher survival rate than normal ones, consistently with Kim's[18]. Nevertheless, by researching 68 patients with colorectal cancer, Langner's results[19] showed that 5-year survival rate of the patients with aberrant hMSH2 expression was higher than normal hMSH2 patients(74.8% vs 58.1%), with no statistical significance difference. Meanwhile, they also analyzed comparison among different Dukes staging, showing that aberrant group had higher survival rate than normal group in four stages, with no statistical difference. However, it was reported by some other studies[29,48]. In our study, hMSH2 expression of postoperative specimens of 327 patients with sporadic colorectal cancer were detected and all of them were postoperatively followed up. it showed aberrant hMSH2 patients had a higher 5-year survival rate than the normal (82.86% vs 62.33%). Simultaneously, patients in staging II and III were separately performed statistical analysis, and it showed that 5-year survival rate of aberrant hMSH2 group in the same staging II and III respectively were both higher than normal groups, nevertheless, with no difference of statistical significance(both, $P > 0.05$). Why do the patients with aberrant MMR have better prognosis? By large-scale and continuous research in the same institutions, Malesci [49] reported that aberrant MMR patients had better survival advantage partly

because of dependence on early cancer diagnosis, consistently with Park's[27]. Therefore, aberrant hMSH2 patients had higher survival rate maybe because of aberrant hMSH2 accompanied with MSI occurrence. And MSI-H in colorectal cancer also limited tumor growth. What's more, COX regression analysis indicated that aberrant hMSH2 was a good independent prognostic factor.

In short, our study indicated aberrant hMSH2 expression was closely correlated to tumor site and histological type. Aberrant hMSH2 expression suggested higher survival rate of patients with colorectal cancer and might be an important independent prognostic factor. Consequently, hMSH2 expression situation played an important role in occurrence and development of colorectal cancer. However, its detailed mechanism still requires deeper studies.

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Conflict of interest statement

We have no conflicts of interest to report.

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