RESEARCH ARTICLE

hMSH2 and nm23 Expression in Sporadic Colorectal Cancer and its Clinical Significance

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Abstract

Objective: To study the expression of the mismatch repair proteins hMSH2 and nm23 in sporadic colorectal cancer, determine any inter-relationship, and further investigate any clinical significance. Methods: Expression of hMSH2 and nm23 proteins was assessed in 87 colorectal cancer tissues by SP immunohistochemistry, with analysis of survival using follow-up data. Results: In the sporadic colorectal cancer tissues, nm23 protein expression appeared independent of the histological type ($P > 0.05$), but correlated with the invasion depth and lymphatic metastasis ($P < 0.05$). In contrast, hMSH2 protein expression was not significantly correlated with these clinicopathologic features ($P > 0.05$), although it positively correlated with that of nm23 protein in the sporadic colorectal cancers (rs=0.635, $P < 0.05$). Combined expression of the two was found to be related with invasion depth, lymphatic metastasis and prognosis of sporadic colorectal cancer ($P < 0.05$). Conclusion: nm23 protein level was related with the degree of malignancy, and could be used as an index to predict the invasion and metastasis potential. The expression of hMSH2 protein is positively correlated that of nm23 protein, and the combined expression of the two has certain guiding significance for the prognosis of sporadic colorectal cancer.

Keywords: Sporadic colorectal cancer - hMSH2 - nm23 - immunohistochemistry - prognosis

Asian Pacific J Cancer Prev, **14 (3)**, 1995-1998

Introduction

Colorectal cancer is a frequently seen malignant tumor of digestive tract. In recent years, its incidence is increased year by year, and it has become one of the main diseases resulting in deaths in various countries and regions. At present, the exact mechanism for the occurrence and development of colorectal cancer is still unknown, and it is considered as a complex process involving multiple factors and multiple steps, such as the activation of oncogenes, inactivation of antioncogenes and mutation of mismatch repair genes etc. Researches showed that, the occurrence, development, metastasis and prognosis of colorectal cancer is closely related to the difference in the expression of gene and protein, and therefore, discovering related sensitive indexes can better guide clinical screening of high-risk patients, thereby strengthening treatment and close follow-up, and improving the survival period of the patients. Recently, many experimental studies have showed that mismatch repair (MMR) of DNA and inactivation of tumor metastasis suppressor gene (nm23) plays an important role in the occurrence and development of colorectal cancer. This study detects the expression of mismatch repair gene (hMSH2) and tumor metastasis suppressor gene (nm23) in sporadic colorectal cancer tissues using immunohistochemical SP method (Overbeek et al., 2008), and investigates its relationship with the prognosis of colorectal cancer in the light of the survival data.

Materials and Methods

General data

87 colorectal cancer specimens collected from the patients treated through operation in Yingkou Central Hospital from 2004 to 2006. All these patients suffered from colorectal cancer I-III without family history of a hereditary disease, and did not undergo preoperative chemotherapy. 56 of them were male, and 31 of them were female. They were aged 35-82 years old with the average age of 59 years old, 45 of them with lymphatic metastasis, and 42 of them without lymphatic metastasis. All patients underwent postoperative observation or standardized treatment (according to the Guide of the NCCN), and were followed up for 5 years (before January 2012). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Yingkou Central Hospital. Written informed consent was obtained from all participants. Rabbit anti-human polyclonal antibody hMSH2, mouse anti-human monoclonal antibody nm23, and SP immunohistochemical ultrasensitive kit were all purchased from Fuzhou Maixin Biotechnology Development Co., Ltd.

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Table 1. Relationship Between the Expression of hMSH2 and nm23 Proteins and Clinicopathologic Factors of Sporadic Colorectal Cancer

Comparison items	hMSH2				nm23			
	+	-	χ^2	*P*	+	-	χ^2	*P*
Histological type								
WDAC	11	7			13	5		
Moderately differentiated adenocarcinoma	24	15	0.34	>0.05	25	14	3.35	>0.05
Poorly differentiated adenocarcinoma	14	8			12	10		
Mucinous adenocarcinoma	5	3			3	5		
Invasion depth								
Strata submucosa	2	2			4	0		
Muscle layer	19	12	0.3	>0.05	22	9	7.46	<0.05
Serosa and outside the serosa	33	19			27	25		
Lymphatic metastasia								
None	29	13	1.68	>0.05	31	11	5.67	<0.05
Available	25	20			22	23		

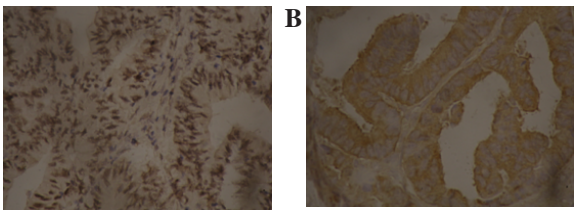

Figure 1. hMSH2 (A) and nm23 (B) Expression in the Colorectal Cancer Gland

Table 2. Relationship Between the Expression of hMSH2 Protein and That of nm23 Protein in Sporadic Colorectal Cancer Tissues

Expression of hMSH2 protein	Number of cases	Expression of nm23 protein		r_s	*P*
		+	-		
+	54	42	12	0.635	P<0.05
-	33	11	22		

Methods

All tissues were fixed with 10% formaldehyde, underwent conventional tissue processing, were embedded in paraffin, were made into serial section with the thickness of 4 um, and were subject to immunohistochemical staining as per the kit specification. Primary antibody was replaced with the PBS buffer as the negative control, and the known positive tissue section was used as the positive control.

Determination of positive immunohistochemical results

hMSH2 protein is located in the nucleus, that without staining or <1% nuclear staining is taken as (-), and that with >1% nuclear staining is taken as (+); nm23 protein is located in the cytoplasm, that without cell staining is taken as (-), and that with >1% cell staining is taken as (+).

Statistical analysis

SPSS13.0 statistical software is used for statistical data processing. Enumeration data are tested with χ^2; survival analysis are analyzed using Kaplan-Meier survival curves, the survival rates of each group are compared by log-rank test; relevant factors affecting the survival are analyzed using Cox multivariate regression analysis. $P < 0.05$ shows significant difference.

Results

Expression of hMSH2 and nm23 proteins

87 colorectal cancer specimens were collected, including 54 specimens with positive expression of hMSH2 protein, and 53 specimens with positive expression of nm23 protein. As can be seen under the microscope, hMSH2 is mainly expressed in the nucleus of colorectal cancer gland (Figure 1A); and nm23 is mainly expressed in the cytoplasm of colorectal cancer gland (Figure 1B).

Relationship between the expression of hMSH2 and nm23 proteins and clinicopathologic factors

Underexpression of hMSH2 and nm23 is independent of the histological type of sporadic colorectal cancer ($P > 0.05$), wherein, the underexpression of nm23 protein is related to the tumor invasion depth ($P < 0.05$) and lymphatic metastasis ($P < 0.05$). In the serosa with tumor invasion and cancer tissues with lymphatic metastasis, the underexpression rate of nm23 protein is respectively 48.1% (25/52) and 51.1% (23/45); and in the muscle layer with tumor invasion and cancer tissues without lymphatic metastasis, the underexpression rate of nm23 protein is respectively 29% (9/31) and 26.2% (11/ 42). In the cancer tissues with increased tumor invasion depth and lymphatic metastasis, nm23 protein is more significantly underexpressed (Table 1).

Relationship between the expression of hMSH2 protein and nm23 protein

In 54 sporadic colorectal cancer specimens with positive expression of hMSH2 protein, positive expression of nm23 protein was found in 42 specimens, and negative expression of nm23 protein was found in 12 specimens with the positive coincidence rate of 77.8% (42/54); in 33 sporadic colorectal cancer specimens with negative expression of hMSH2 protein, positive expression of nm23 protein was found in 11 specimens, and negative expression of nm23 protein was found in 22 specimens with the positive coincidence rate of 33.3% (11/33). In all 87 specimens, consistent expression of hMSH2 and nm23 proteins was found in 64 specimens, accounting for 73.6% (64/87). Statistical analysis showed that the expression of hMSH2 protein is positively correlated that of nm23

Table 3. Relationship Between Combined Expression of hMSH2 and nm23 Proteins and Sporadic Colorectal Cancer Stages

Combined expression of hMSH2 and nm23 proteins	Strata submucosa	Invasion depth Muscle layer	Serosa and outside the serosa	χ^2	*P*	Lymphatic metastasia -	Lymphatic metastasia +	χ^2	*P*
+	2	19	21	6.39	<0.05	20	22	5.34	<0.05
-	0	4	18			4	18		

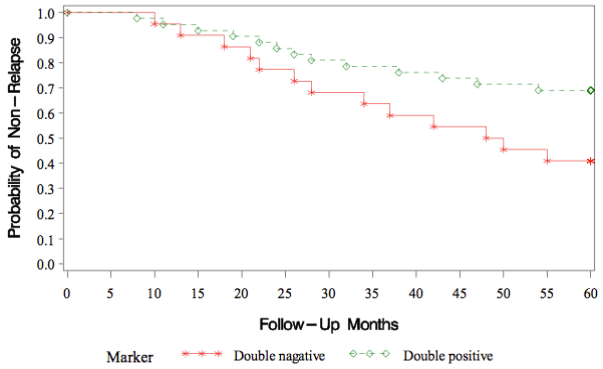

Figure 2. Kaplan-Meier Estimates for Probability of Non-Relapse During Follow-up

protein in sporadic colorectal cancer (rs=0.635, $P < 0.05$) (Table 2).

Relationship between combined expression of hMSH2 and nm23 proteins and sporadic colorectal cancer stages

Combined expression of hMSH2 and nm23 proteins is related to the tumor invasion depth of sporadic colorectal cancer ($P < 0.05$) and lymphatic metastasis ($P < 0.05$). In the serosa with tumor invasion and cancer tissues with lymphatic metastasis, the combined underexpression rate of hMSH2 and nm23 proteins was respectively 46.2% (18/39) and 45% (18/40); in the muscle layer with tumor invasion and cancer tissues without lymphatic metastasis, the combined underexpression rate of hMSH2 and nm23 proteins was respectively 17.4% (4/23) and 16.7% (4/24) (Table 3). In the cancer tissues with increased tumor invasion depth and lymphatic metastasis, the combined underexpression of hMSH2 and nm23 proteins was more significant.

Relationship between combined expression of hMSH2 and nm23 proteins and prognosis of sporadic colorectal cancer

Relationship between combined expression of hMSH2 and nm23 proteins and prognosis of sporadic colorectal cancer is compared by Kaplan-Meier survival analysis, followed up for 60 months, the numbers of patients with group of lost expression and combined expression of hMSH2 and nm23 proteins are 42 cases and 22 cases respectively, including 26 cases of patients with disease progression, the numbers of patients with group of lost expression and combined expression of hMSH2 and nm23 proteins are 13 cases and 13 cases respectively. The 5-year disease-free survival rate of combined expression group of hMSH2 and nm23 proteins is 69.0%, and the 5-year disease-free survival rate of lost expression group of hMSH2 and nm23 proteins is 40.9%, the two groups are significantly different ($P < 0.05$) (Figure 2). The sporadic colorectal cancer patients with combined expression of hMSH2 and nm23 proteins have a higher 5-year survival rate after surgery.

Table 4. Cox Multivariate Regression for 5-year Disease-free Survival of Colorectal Cancer Patients

Variables	RR	95% confidence interval	*P* value
Gender	0.951	0.561-1.498	>0.05
Age	0.936	0.548-1.465	>0.05
Tumour size	1.073	0.719-1.640	>0.05
T category	2.762	1.783-4.439	<0.05
N category	3.986	2.145-7.328	<0.05
Tumour grade	2.275	1.392-3.723	<0.05
hMSH2	0.917	0.503-1.436	>0.05
nm23	0.574	0.346-0.915	<0.05

Cox proportional hazards regression suggests, nm23, histological type, invasion depth and lymphatic metastasis are several important factors that impact prognosis of patients with colorectal cancer, $P < 0.05$ (Table 4). The relative risk (RR) of nm23 is 0.574 (95% CI 0.346-0.915), $P < 0.05$. The expression of nm23 and prognosis of patients have a positive correlation, the patients with higher expression level of nm23 have better prognosis.

Discussion

Human DNA mismatch repair (MMR) mainly involves 6 repair proteins, namely hMSH2, hMSH3, hMSH6, hMLH1, hMLH3 and hPMS1 (Iyer et al., 2006). Their mechanism of action may be that the mismatch repair protein encoded by the MMR gene forms the heterodimers involved in mismatch repair (Marsischky et al., 1996). HMSH2 protein, hMSH6 and hMSH3 form two heterodimers: hMhutSα and hMhutSβ (Gradia et al., 1997), which respectively identify individual and more than 2-4 base deletion/insertion mismatch. hMSH2 protein is required to identify mismatch gene (Duncop et al., 2000; Lipkin et al., 2000). The hMutLα dimer formed through combination of hMLH1 and hPMS1 proteins forms a compound with hMhutS bound to the DNA chain, so as to start the mismatch repair, cut off the DNA fragments containing mismatched base, synthesize new fragments, and then complete the repair process (Sarasin and Stary, 1997). MMR gene defect will lead to the loss of mismatch repair function, increase cell mutation frequency, and continuously enlarge and accumulate the mutation events, so that the error information is throughout the genome, and finally results in the occurrence and development of tumor (Hsieh and Yamane, 2008). The research findings of this study showed that in 87 patients with sporadic colorectal cancer, underexpression of hMSH2 was found in 33 patients, accounting for 37.9%, which was close to the result of Gafa et al. (2000). In colorectal cancer tissues, the expression of hMSH2 protein was not significantly correlated with the histological differentiation degree of tumor, invasion depth and lymphatic metastasis, and the difference was not statistically significant.

nm23 gene is a tumor metastasis suppressor gene that is separated from melanoma cells of the rat K-1735 with differential hybridization technology (Steeg et al., 1988). It is known that human nm23 gene family includes 8 members, wherein, only nm23 H1 and nm23 H2 are proved to have the ability to inhibit tumor metastasis (Leone et al., 1991). The mutation, deletion and underexpression of nm23 allele is closely related to the metastasis of many malignant tumors (Dursun et al., 2002; Suzuki et al., 2004). It is reported that nm23 H2 point mutation arises from CTG→GTG of 48th codon, so that the leucine zipper structure is damaged, and this structure is considered to be likely to act as the transcription factor controlling cell proliferation (Leone et al., 1991; Okada et al., 1994). The research findings of this study showed that the expression of nm23 gene in colorectal cancer is negatively correlated with the tumor invasion depth and lymphatic metastasis, which is consistent with the literature report (Chen et al., 2007). With the underexpression of nm23 gene, the probability of deep invasion and lymphatic metastasis of colorectal cancer tissues is increased. As the tumor metastasis suppressor gene, nm23 gene can restrict invasion and metastasis of cancer cell. When nm23 gene is underexpressed, the organism has decreased constraint force on cancer cell, so as to greatly facilitate its invasion to surrounding tissues and distant metastasis.

Besides, the research findings of this study showed that: the expression of hMSH2 is positively related to that of nm23 in sporadic colorectal cancer, suggesting that the two may have positive feedback regulation mechanism in the regulation of cell proliferation. The mutation of hMSH2 mismatch repair gene can cause wide somatic mutation and DNA replication error, which results in the increase of mutation rate of antioncogene (Gryfe and Gallinger, 2001), so as to infer that related factors are likely to down-regulate nm23 gene expression. A number of studies showed that, MMR gene defect mainly promotes tumorigenesis through the following approaches (Kolodner et al., 1995): a. by increasing the mutation frequency of cancer gene or tumor suppressor gene (Loeb, 1994); b. allowing some important functional gene to have genetic instability (Rhyu, 1996); c. promoting tumorigenesis by cell injury caused by some chemicals. The survival analysis results of this study showed that, the probability of postoperative recurrence and metastasis in the patients with combined underexpression of hMSH2 and nm23 proteins is far higher than that in the patients with high expression of hMSH2 and nm23 proteins, and therefore, the combined expression of hMSH2 and nm23 proteins can be used as an independent marker to identify the patients with colorectal cancer with high invasion and metastasis potential, so as to facilitate objective and accurate evaluation and prognosis.

In conclusion, in sporadic colorectal cancer progression, nm23 protein level is associated with the malignancy degree, and could be used as an index to predict the tumor invasion and metastasis potential. hMSH2 protein expression is positively correlated with nm23 protein expression; combined detection of the two can be used as an important index to judge the malignancy degree and prognosis of tumor, and has important significance for selection of the treatment method and prognostic judgment of the disease. It is necessary to further study the specific mechanism of underexpression of HMSH2 protein and nm23 protein in colorectal cancer.

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