Expression of Mismatch Repair Enzyme hMLH1, hMSH2 and Cyclooxygenase-2 in Colorectal Cancer

Caili Han, Yihua Zhang, Yi Chen, Yu Liu

Department of Pathology, the School of Basic Medical Sciences, Hebei Medical University, Shijiazhuang 050017, China

Abstract **Objective** To investigate the expression of cyclooxygenase-2(COX-2), mismatch repair enzyme human mut-l homologue 1 (hMLH1), human mut-s homologue 2 (hMSH2) in colorectal cancer. **Methods** The expression of COX-2, hMLH1 and hMSH2 protein was analyzed in 78 colorectal cancer by using polyclonal antibody of COX-2, hMLH1 and hMSH2 with immunohistochemistry method. **Results** In the 78 colorectal cancer samples, the negative rates of hMLH1 and hMSH2 were 29.49% and 28.21%, respectively. The positive rate of COX-2 was 74.36%. **Conclusion** In colorectal cancer there was negative expression of hMLH1 and hMSH2, and a part of colorectal cancer demonstrated positive expression of COX-2, which implied that there were possibly at least two pathways to colorectal cancer: mismatch repair enzyme deficiency and high expression of COX-2.

Key Words colorectal neoplasm; gene deletion; immunohistochemistry

Human mutl homologue 1 (hMLH1) and human muts homologue 2 (hMSH2) are two mismatch repair enzymes. hMSH2 gene which is located in chromosome 2p21-22 was firstly found in mismatch repair gene and hMLH1 gene is located in chromosome 3p21.3-23. Evidence showed[1-2] that absence of hMLH1 and hMSH2 happened commonly in colorectal, gastric, endometrical, ovarian and hepatic carcinomas, which give rise to microsatellite instability(MSI) and cause tumor finally. Cyclooxygenase (COX) is the rate-limiting enzyme in prostaglandin (PG) synthesis. COX has two isoenzymes: COX-1 and COX-2. COX-1 participates physiological PG synthesis and maintains physiological functions. COX-2 is invoved in a diverse range of pathophysiological processes, including development of inflamation and tumor. A large body of evidence shows that COX-2 protein was highly expressed in colorectal carcinomas. This study was designed to exmine the expression of hMLH1, hMSH2 and COX-2 protein in 78 colorectal carcinoma samples to investigate the relationship between the expression of hMLH1, hMSH2 and COX-2 protein and colorectal carcinoma occurrence.

MATERIALS AND METHODS

Clinical materials

78 cases surgically resected gastric carcinoma and normal gastric mucosa (>10cm from carcinoma, next called normal tissues), which were collected from January 2002 to December 2002 in department of Gastrointestinal Surgery of the second hospital of HeBei Medical University. Among them, 46 cases were male, 32 cases were female; 58 cases were adenocarcinoma, 13 cases were mucinous adenocarcinoma, and 7 cases were signet-ring cell carcinoma. Among 78 cases, 38 were found having lymphatic metastasis and 62 cases infiltrated serous membrane. All cases were verified by pathologic diagnosis and all the patients had not received radiotherapy or chemotherapy before surgery.

Immunohistochemistry staining

Reagent and dispensing: COX-2, hMLH1 and hMSH2 polyclonal antibody were bought from Bo-Shide Bioengineering company. SP9001 Immunohistochemistry Chemical Kit, DAB Chemical Kit were bought from Zhong-Shan Biotechndogy company. Uni-antibody of COX-2, hMLH1 and hMSH2 were diluted by 1:80, 1:80, 1:50, antigen plerosis by microwave.

Methods: Investigating the expression of COX-2, hMLH1 and hMSH2 by using streptavidinhorsera–dish-peroxidase (SP) Chemical Kit and making positive contrast and negative contrast.

Determination of results: If there were brown particles in cell plasm or cell nucleus, it were positive cells. In high-mirrors, 5 sights were investigated in every slice, judging the expression by the

mean of the percentage of positive cells. 11%-50% were low expression and 51%-100% were high expression, it were negative if ≤10%.

Statistical analysis: SAS 6.12 software and the Chi-Square Check were used to performe Statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

The expression of three proteins: in 78 cases, the absent rate of hMLH1 and hMSH2 protein expression was 29.49%(23/78) and 28.21%(22/78), the positive rate of COX-2 protein expression was 73.08%(57/78).

The relationship between the expression of hMLH1, hMSH2 and COX-2 protein and clinic pathological features: The high expression rate of hMLH1 protein in adenocarcinoma was significantly higher than that in mucoid adenocarcinoma and signet-ring cell carcinoma ($P<0.05$). The expression of hMSH2 protein was not significant difference in sex, tissue types, with or not with serous membrane invasion and with or not with lymph node metastasis. The high expression of COX-2 protein in cases with lymph node metastasis was significantly higher than that in cases without lymph node metastasis ($P<0.05$). Further Spearman rank correlation test

Table 1 hMLH1 expression and clinicopathological features

Pathological feature	n	hMLH1 expression		
		Negative	Low	High
Sex				
Male	46	11	17	18
FemaleL	32	12	10	10
ymphatic metastasis				
Yes	38	13	11	14
No	40	10	16	14
Tissue Types				
Adenocarcinoma	58	12	21	25
mucoid adenocarcinoma	13	7	5	1*
signet-ring cell carcinoma	7	4	1	2*
Serious invasion				
Yes	62	18	21	23
No	16	5	6	5

* $P<0.05$, vs adenocarcinoma by χ^2 test

Table 2 hMSH2 expression and clinicopathological features

Pathological feature	n	hMLH1 expression		
		Negative	Low	High
Sex				
Male	46	10	16	20
FemaleL	32	12	14	6
ymphatic metastasis				
Yes	38	9	14	15
No	40	13	16	11
Tissue Types				
Adenocarcinoma	58	13	24	21
mucoid adenocarcinoma	13	6	4	3
signet-ring cell carcinoma	7	3	2	2
Serious invasion				
Yes	62	16	24	22
No	16	6	6	4

Table 3 COX-2 expression and clinicopathological features

Pathological feature	n	hMLH1 expression		
		Negative	Low	High
Sex				
Male	46	13	18	15
FemaleL	32	8	7	17
ymphatic metastasis				
Yes	38	8	6	24*
No	40	13	19	8
Tissue Types				
Adenocarcinoma	58	16	18	24
mucoid adenocarcinoma	13	4	6	3
signet-ring cell carcinoma	7	1	1	5
Serious invasion				
Yes	62	16	19	27
No	16	5	6	5

* $P< 0.05$, vs no lymphatic metastasis by χ^2 test

showed that the expression of COX-2 protein has positive correlation with lymph node metastasis (rs= 0.85493, $P<0.01$).

DISCUSSION

DNA repair includes excision repair and mismatch repair (MRR). The function of MRR is to correct mispaired nucleotides during DNA synthesis and the system exists not only in prokaryotic but also in eukaryotic cell. In mammals, mispaired nucleotides commonly are the dinucleotides and trinucleotides repetitive sequences which prone to polymerase slippage and errors. Human mut-l homologue 1 (hMLH1) protein and human mut-s homologue 2 (hMSH2) protein are the most important enzymes, which play a key role in mismatch repair.

The germline mutation of hMLH1 and hMSH2 accounted for 80% in hereditary nonpolyposis colorectal carcinoma (HNPCC). Researches showed that the germline mutation rate of hMLH1 and hMSH2, which were 22.5%(9/40) and 12.5%(5/40) in 40 Asia kindred, and were 37.4% (82/190) and 21% (38/180) in European, respectively. These studies suggested that germline mutation of hMLH1 and hMSH2 was common in HNPCC kindred. HMLH1 and hMSH2 with germline mutation expressed abnormal protein, giving rise to tumor occurrence.

Our study revealed that the deficient rate of hMLH1 and hMSH2 protein, which were 57.3% and 18.3% in other reports [4], were 29.49% and 28.21% in colorectal carcinoma, respectively. These suggested that absence of hMLH1 and hMSH2 protein was commonly found in colorectal carcinoma.

MMR family has nine mismatch repair genes, namely, hMLH1, hMLH3, hPMS1, hPMS2, hMSH2, hMSH3, hMSH4, hMSH5, hMSH6. Among them, hMLH1 and hMSH2 were the most important genes. The hMLH1 and hMSH2 proteins were always deficient in HNPCC patients. The absent or abnormal expression of mismatch repair enzymes due to two times heterozygositic loss of genes resulted in MSI and subsequently gave rise to HNPCC occurrence[5]. Since MRR genes played a role in the initiation and development of tumorigenesis, thereby, detecting MRR genes was important for screening of high risk population and early diagnosis of tumor[6]. Detecting hMSH2 protein, which was always high in sporadic colorectal carcinoma [7], could be a criterion to judging tumor types (hereditary or sporadic). Evidence [8] showed that positive expression of hMLH1 in colorectal carcinoma with lymph node metastasis is high, compared to the cases without lymph node metastasis. This study demonstrated that high expression of hMLH1 in adenocarcinoma was higher than that in mucoid adenocarcinoma and signet-ring cell carcinoma. So our study provide theoretic basis for screening of tumor types and judging prognosis of tumor.

COX was the rate-limiting enzyme in the process of the synthesis of PG, there were two isozyme: COX-1, COX-2. In mostly normal tissues, there were no expression of COX-2, but it was in-

duced by some factors: cytokine, growth factor, oncogene, cocarcinogen, NO, bile acid, acceleration factor of tumor and so on. In a lot of pathological process, such as inflammation, the occurrenc and development of tumor, COX-2 posses the activity of peroxidase, it could make the oxidation of the oncogene and catalyse a series of xenia oxidation. If there were excessive expression of COX-2, the xenia oxidation would be reinforced, the oxidizing amine would act with DNA and alter its constitution, thus, the role of anti-occogene was alterd and resulted in the occurrenc of tumor. In addition, COX-2 could elongate G1 of cell, resulting in the lasting proliferation and mutation, thereby, the probability of two-hit increased. In a lot of reports, there were the high expression of COX-2 in large intestine tumor, which replied that the over-expression of COX-2 would result in the occurrenc of large intestine tumor. It was certificated that the occurrenc of large intestine tumor revolving in the inactivation of the tumor suppressor gene APC, the activation of protooncogene K-ras, the inactivation of DCC or p53 and so on. So, the over-expression of COX-2 mag play an important role in the occurrenc of large intestine tumor.

The result of experiment replied that there were no expression of COX-2 in normal tissues and pigmenting in the cell plasm and nucleus of carcinoma cells. The expression rate of COX-2 was 73.08%, and the high expression rate was 41.02%, which is identical with Murata and Sheehan [10,11]´s experimental results. Conjugating Akhtar[12]´s study, in tumor cells, the demethylation of COX-2 gene promoter could result in the high expression of COX-2.

The result of experiment replied that the level of COX-2 had positive correlation with the metastasis of lymph node (r_s>0). In the cases having the lymph node metastasis, the high expression rate of COX-2 was 63.16%, which replied that in large intestine tumor, the high expression rate of COX-2 was the molecular mark of the metastasis of lymph node. Murata [10]´s study manifested that in gastric carcinoma, if there were the high expression rate of COX-2, the infiltration and metastasis of lymph node were reinforced and the prognosis was bad; the use of Aspirin would ameliorate the prognosis of the gastric carcinoma. Thus, we could judge the prognosis of tumor and offer the theoretic base in the field of clinical medication.

Taken together, there were at least two pathway in the development of colorectal carcinoma, including overexpression of COX-2 and deficient of MMR enzymes. This study provide theoretic basis for diagnosis of colorectal carcinoma and judging prognosis of tumor.

REFERENCES

1. Chiaravalli AM, Furlan D, Facco C, et al. Immunohistochemical pattern of hMSH2/hMLH1 in familial and spo radic colorectal, gastric, endometrial and ovarian carcinomas with instability in microsatellite sequences. Virchows-Arch, 2001, 438(1): 39-48
2. Cong Wen-Ming, Wu Meng-Chao, Zhang Shu-Hui, et al. Expression of hMLH1 and hMSH2 protein in hepatic cancer and its significance. Chin J Dig, 2002, 22 (11): 697-698
3. Yuan-Ying, Huang Jian, Zheng Shu. Mutation of human mismatch repair genes in hereditary nonpolyposis colorectal cancer (HNPCC) families. Chin J Oncol, 1999, 21(2): 105-108.
4. Furukawa T, Konishi F, Shitoh K, et al. Evaluation of screening strategy for detecting hereditary nonpolyposis colorectal carcinoma. Cancer, 2002, 94(4): 911-920.
5. Peltomaki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet, 2001, 10(7): 735-740.
6. Liu Xiao-ru, Cui Xian-ya. Studies on DNA mismatch repair gene hMSH2 in gastric cancer. Med J Chin PLA, 2000, 25(2): 125-126.
7. Leach FS, Hsieh JT, Molberg K, et al. Expression of the human mismatch repair gene hMSH2: a potential marker for urothelial malignancy. Cancer, 2000, 88(10): 2333-2341.
8. Weng Yang, Wang Xiao-Ying, CAI Wang-Wei, et al. Expression of hMLH1 in colorectal carcinoma tissue and its clinical significance. Journal of HaiNan Medicine, 2003, 14(9): 3-5.
9. Coffey RJ, Hawkey CJ, Damstrup L, et al. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenesis in polarizing colon cancer cells. PNAS, 1997, 94(2): 657-662.
10. Murata H, Kawano S, Tauji S, et al. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric garcinoma. Am J Gastroenterol. 1999, 94(2): 451-455.
11. Sheehan KM, Sheahan K, O´ Donog hue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA, 1999, 282(13):1254-1257.
14. Akhtar M, Cheng Y, Magno RM, et al. Promoter methylation regulates Helicobacter pylori stimulated COX-2 expression in gastric epithelial cells. Cancer Res, 2001, 61(6): 2399-2403.