**Name of journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 11851**

**Columns:** **RESEARCH REPORT**

**Comparative study of mutations in the SNP loci of *K-RAS*, *hMLH1* and *hMSH2* genes from** **neoplastic intestinal polyps and colorectal cancers**

Yan ZH *et al.* Mutations from neoplastic intestinal polyps and colorectal cancers

Zhi-Hui Yan, Li-Hong Cui, Xiao-Hui Wang, Chao Li, Xing He

**Zhi-Hui Yan, Li-Hong Cui, Xiao-Hui Wang, Chao Li, Xing He,** Department of Gastroenterology, Navy General Hospital of PLA, Beijing 100048, China

**Author contributions:** Yan ZH and Cui LH contributed equally to this work; all authors contributed to the manuscript.

**Correspondence to: Li-Hong Cui, MD,** Department of Gastroenterology, Navy General Hospital of PLA, 8 Fuxing Road, Beijing 100048, China. luckycui861@sina.com

**Telephone:** +86-10-66957541 **Fax:** +86-10-68780570

**Received:** June 8, 2014 **Revised:** July 12, 2014

**Accepted:** August 13, 2014

**Published online:**

**Abstract**

**AIM:** To clarify the molecular mechanism involved in pathogenesis as well as clinical significance of genetic analysis of histological samples.

**METHODS**: A total of 480 blood and tissue specimens were collected in our hospital from January 2011 to October 2012. In the observation group, there were 120 blood specimens and 120 intestinal tract tissue specimens collected from patients with neoplastic intestinal polyps. In the control group I there were 80 blood specimens and 80 intestinal tract tissue specimens collected from patients with colorectal cancer. In the control group II there were 40 blood specimens and 40 intestinal tract tissue specimens collected from healthy individuals. The gene segments were amplified using PCR and DNA gel electrophoresis along with DNA sequence analysis were employed for the detection of the following single-nucleotide mutations (SNPs): K-RAS codons 12 and 13; *hMLH1* gene missense mutation at Va1384Asp; *hMSH2* gene missense mutation at 2783C/A.

**RESULTS**: The mutation rate of the SNPs at Va1384Asp locus of the *hMLH1* gene from blood and tissue specimens in the observation group showed no statistical difference from the control group I. The mutation rates of SNPs in codons 12 and 13 of *K-RAS* and at 2783C/A locus of the *hMSH2* gene were lower in the observation group than that of the target gene in the control group I and the differences were statistically significant (*χ*2 = 15.476, 29.670, 10.811, 16.618, 33.538, 7.898, *P* < 0.05). The mutation rate of SNPs at Va1384Asp locus of the *hMLH1* gene was significantly higher in the observation group when compared to the control group II (*χ*2 = 10.486, 4.876, *P* < 0.05). The mutation rates of SNPs in codons 12 and 13 of K-RAS and at 2783C/A locus of the *hMSH2* gene did not show any statistical difference from the control group II.

**CONCLUSION**: There may be an important clinical significance and relevance between neoplastic intestinal polyps and colorectal cancer in terms of the mechanisms involved in the pathogenesis.

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**Key words:** Neoplastic intestinal polyp; Colorectal cancer; Single-nucleotide mutations; *K-RAS*; *hMLH1*; *hMSH2*

**Core tip:** This study selects the blood and tissue samples of neoplastic polyps, colorectal cancer and the normal for related gene sequencing. By comparing the differences in SNP, analyzes the early genetics pathogenesis of colorectal cancer.

Yan ZH, Cui LH, Wang XH, Li C, He X. Comparative study of mutations in the SNP loci of K-RAS, hMLH1 and hMSH2 genes from neoplastic intestinal polyps and colorectal cancers. *World J Gastroenterol* 2014; In press

**INTRODUCTION**

As a primary malignant neoplastic disease of the digestive system in China, colorectal cancer has a high incidence rate of 13.29 per 100000[1], second only to primary liver cancer. And the colorectal cancer remains one of the leading cause of cancer death[2,3]. The hereditary nonpolyposis colorectal cancer (HNPCC) accounts only for 5%-15% of all colorectal cancers[4], while the rest (more than 80%) of the cases of colorectal cancers evolve from neoplastic polyps. Since neoplastic polyps are precancerous lesions that can develop into colorectal cancer, Previous studies have confirmed that epigenetic phenomena caused by DNA hypermethylation may be early indicators of tumorigenesis[5-8]. Increasing attention has been paid recently to the fundamental and clinical studies on molecular genetics and tissue metabolism which are related to SNP loci[9]. Here we report the comparison of mutations in the *K-RAS*, *hMLH1* and *hMSH2* genes in the blood and intestinal tissue specimens collected from patients with neoplastic intestinal polyps and those with colorectal cancer.

**MATERIALS AND METHODS**

***General data***

All specimens were collected in our hospital from January 2011 to October 2012. A total of 120 blood specimens and 120 colon biopsy specimens were collected from patients with neoplastic intestinal polyps (the observation group). 80 blood specimens and 80 colon biopsy specimens were collected from patients with colorectal cancer (Control group I) and 40 blood specimens and 40 colon biopsy specimens were collected from healthy individuals (Control group II). In the observation group (120 patients), there were 71 males and 49 females with an average age of 38.3 ± 10.4 years ranging between 24‒68 years. The histological classification of the group is defined as follows: 85 cases of tubular adenoma, 17 cases of villous adenoma and 18 cases of mixed adenoma. Among the 80 patients with colorectal cancer (Control group I), there were 49 males and 31 females with an average age of 42.6±9.1 years, ranging between 33‒72 years with the following histological classification: 42 cases of tubular adenoma, 27 cases of mucinous adenocarcinoma, 8 cases of papillary adenocarcinoma and 3 cases of other types. Among the 40 cases of healthy individuals (Control group II) there were 26 males and 14 females with an average age of 34.6 ± 6.3 years ranging between 22-55 years. The detailed results are listed in Table 1.Peripheral blood was collected from each patient and focal tissue biopsy was conducted. All specimens were stored at -20°C for further analysis. Patients with a family history of familial adenomas, polyps or HNPCC were excluded from this study. Our study was performed in accordance with the Ethics committee of Navy General Hospital of PLA. Informed consent was obtained from all patients. The research protocol was approved by the institutional review board of the Ethics committee of Navy General Hospital of PLA.

***Materials***

Genomic DNA extraction kit (SBS Genetech, China), PCR amplifier PTC220 (US BIO-RAD Company, United States), DYY-10C electrophoresis apparatus (Beijing Liuyi Instrument Factory, China), Bio-Rad Gel Doc XR System Gel Imaging System, ABI 3730XL Sequencer, Eppendorf 5417R/5810R centrifuge, CodonCode Aligner sequence alignment analysis software.

***Methods***

**Collection of blood specimens:** All the patients were instructed to avoid spicy or excitant food one week prior to blood collection. They were also prohibited from drinking and asked to keep an empty stomach (fasting for 6 to 8 h) on the morning of blood collection. 5 ml venous blood was drawn from median cubital vein and stored in an anticoagulant tube (109 mmol/L sodium citrate, 0.4 mL). The collected blood specimens were either immediately sent to the laboratory for analysis or saved at -20°C. Before analysis, each blood specimen was thawed to room temperature and mixed well. DNA from the blood specimens was extracted using complete blood cells.

**Collection of intestinal tract tissue specimens**: All the patients were regularly examined and complete inspections including electrocardiogram, blood coagulation and immunity function after admission. Patients were asked to keep a soft liquid diet one day prior to the medical examination. They were orally fed the cathartic agent of the liquor mixed with 20% mannitol (500 mL) and 5% glucose normal saline (1000 mL) after supper in order to clean the intestines. They were also asked to stop eating on the morning of the examination and keep an empty stomach (for 6-8 h). The method of electrocoagulation assisted excision with nylon loop ligature was applied to conduct tissue biopsy on the tissue lesions of patients with neoplastic polyps and colorectal cancer. For healthy individuals, points 3, 6, 9 and 12 on intestinal mucosa were used for the collection of tissue specimen. All the specimens were stored in a 10% neutral formaldehyde solution until further analysis. The tissue specimens were grinded and DNA was extracted using the phenol-chloroform method.

**DNA extraction**: The DNA was extracted from the centrifuged blood and grinded tissue specimens using the genomic DNA extraction kit. The purity of the extracted DNA was tested using the ultraviolet spectrophotometer.

**Primer design for target genes**: Primer3 online software was used to design primers for K-RAS codons 12 and 13, hMLH1 gene at Va1384Asp and hMSH2 gene at 2783C/A loci. The primer sequences sued in this study are as follows: K-RAS gene 5′-CGTCTGCAGTCAACTGGAAT--T-3′ (forward), 5′-CCTGACATACTCCCAAGGA-3′ (reverse); hMLH1 gene at Va1384Asp locus:5′-TGTGTGATATGTTTAGATGGAAATGA-3′(forward), 5′--TTGAAGTCACACTGCGAAGAA-3′ (reverse); hMSH2 gene at 2783C/A locus:5′-TCGGGCAGAATTGCTTCTAT-3′(forward),5′-ATTCCAGCACCATTCCAGAG-3′ (reverse).

**PCR amplified reaction**: PCR reaction mix consisted of 2.5 μL 10 × PCR Buffer, 16.775 μL double distilled water (dd H2O), 1.0 μL forward primer (10 μmol/L), 1.0 μL reverse primer (10 μmol/L), 2 μL template DNA, 1.6 μL 4 × dNT (2.5 mmol/L), 0.125 μL Taq enzyme (5 U/μL) and MgCl2 solution (1.5 mmol/L). PCR reaction conditions were as follows: initial denaturation at 95°C for 4 min, 30 amplification cycles, 95°C for 30 s, annealing (Ta) at 58°C for 30 s, amplification at 72°C for 30 s and a final extension at 72°C for 10 min. The PCR amplification products were separated on a 1% agarose gel using agarose gel electrophoresis (AGE) and imaged using the Gel Doc XR gel imaging system (Bio-Rad Company, United States).

**DNA sequence analysis**: 20 μL PCR product was sent to Beijing Yiming Fuxing Biotechnology Co., Ltd., China for sequencing. The sequencing was conducted in both directions (positive and negative) with respect to K-RAS codons 12 and 13, hMLH1 gene at Va1384Asp and hMSH2 gene at 2783C/A loci.

***Statistical analysis***

All data were analyzed using SPSS17.0 statistical software. The *χ*2 test for 2 × 2 fourfold table with the test level of 0.05 was used to conduct the mutation rate comparison of various gene loci; *P* < 0.05 was considered to be statistically significant. When the total specimen size N ≥ 40, but one of the theoretical frequencies was 1 ≤ T ≤ 5 or the *χ*2 value was slightly greater than 3.84 before verification, the correction formula was used to enumerate the *χ*2 value. If still *P* < 0.05, it suggested that the verification results were consistent and that the difference was statistically significant.

**RESULTS**

***Detection of target gene loci in blood specimens***

The mutations in the codons 12 and 13 of the *K-RAS* gene and the missense mutation of *hMSH2* gene at 2783C/A in the blood specimens of the observation group (neoplastic polyps) were significantly different (*χ2*= 15.476、29.670、10.811, respectively, and *P* < 0.05) compared to the mutations of relevant target gene loci of the control group I (colorectal cancer). No statistically significant difference was observed between the mutations in the codons 12 and 13 of the *K-RAS* and the missense mutation 2783C/A in the *hMSH2* gene from the blood specimens of the observation group (neoplastic polyp) and the target gene loci of the control group II (healthy population). The rates of missense mutation Va1384Asp in the *hMLH1* gene showed significant differences between the experimental group and the control group II (*χ*2 = 10.486, *P* < 0.05). No statistical difference was observed between the experiment group and the control group I. The detailed results are listed in Table 2.

***Detection of target gene loci in tissue specimens***

The results of the mutations are summarized in Table 3. Mutations in the codons 12 and 13 of the *K-RAS* gene and the missense mutation 2783C/A in the *hMSH2* gene from the tissue specimens of the observation group (neoplastic polyps) showed statistical differences (*χ*2 = 16.618, 33.538, 7.898, respectively, *P* < 0.05) when compared to the mutations of relevant target gene loci of the control group I (colorectal cancer). They showed no significant difference when compared with the mutations of relevant target gene loci of the control group II (healthy population). The rate of missense mutation of *hMLH1* gene at Va1384Asp, showed significant differences between the experimental group and the control group II (*χ*2 = 3.883, *P* < 0.05), while no statistical difference was observed between the experiment group and the control group I.

***Sequencing results of target genes***

As indicated in Figures 1 and 2, in a typical sequencing peak map of codons 12 and 13 of *K-RAS* gene, the bases at loci 182 and 197 are both G (black), suggesting a normal wild type gene. After mutation, the original G peaks at loci 182 and 197 are replaced by A peaks (green). No displacement or deletion occurred in adjacent base loci, and the detection and analysis showed that G content was greater than the A content, indicating that the gene is mutant gene type. In a typical sequencing peak map of *hMLH1* gene at Val384Asp297 locus, T peak (brown) indicates a normal wild type gene. After mutation, the original sequencing peak at Val384Asp297 locus disappears, and the A peak showing the missense mutation (green) can be seen. No displacement or deletion is observed in adjacent base loci, and the detection and analysis showed that T content greater than A content, indicating a mutant gene type. In a typical sequencing peak map of *hMSH2* gene at 2783C/A233 locus, the base is C (blue) indicates a normal wild type gene. After mutation, the original sequencing peak at 2783C/A233 locus disappears and the A peak (green), indicating a missense mutation is observed. No displacement or deletion in adjacent base loci occurred and the detection and analysis showed that C content greater than the A content, indicating a mutant gene type.

**DISCUSSION**

***Genetic significance of SNP mutations of target genes***

In recent years, the biological relationship between the mutations in the SNP loci and pathogenesis of diseases is being gradually recognized and clarified. As a member of the RAS protein superfamily, *K-RAS* jointly constitutes the molecular switch regulating the GTPases and is responsible for the transition of extracellular signals, so as to trigger a series of cellular processes like proliferation, survival and differentiation[10,11]. Once the *K-RAS* gene has been mutated to a constitutively active form, it mediates signal transduction through the K-RAS-BRAF-MEK pathway and plays a role in inhibiting the epidermal growth factor receptor. Therefore, k-ras has been extensively studied, as it is one of the first cell markers found in tumors[12,13]. Numerous fundamental and clinical studies have shown increased[14,15,16,17] mutation rates of the *K-RAS* gene in human tumor cells (as high as 30% in some tumor cells). The most common type of *K-RAS* gene mutation is a locus-specific mutation, and the mutation loci primarily exist on the 12th and 13th codons of exon 2 (accounting for 90%) and the 61st codon of exon 3. Specific mutation types observed are: GGT>GAT (G12D), GGT>GTT (G12V), and GGC>GAC (G13D)[18]. The RAS protein-mediated signal transduction of GTP enzyme has a clear feedback control as GTP hydrolysis results in the inactivation of RAS protein. In an event of mutations in the SNP loci, activation of oncogenes results in the stabilization of RAS protein in a GTP binding activated form. This in turn affects the downstream signal transduction pathways resulting in malignant cell proliferation or differentiation, forming the basis for the occurrence of malignant tumors[19,20,21].

The *hMLH1* (human mutL homolog1) and *hMSH2* (human mutS homolog 2) genes, as two most important genes in the mismatch repair gene (MMR), have significant hereditary susceptibility and are involved in the base mismatch repair, fidelity of DNA replication, maintenance of gene stability and the reduction of spontaneous mutations at independent loci in the DNA replication process, so as to achieve the purpose of ensuring the biological stability of DNA. The lack of DNA-MMR protein increases both instability of repetition during DNA replication, as well as errors by DNA polymerase[22]. Their inactivation increases the incidence of base mismatch during DNA replication and leads to events like the occurrence of incipient tumors[23,24]. It’s important to point out that, as an MMR-defective tumor marker, MSI shows the distinct characteristic of an increased number of 1-4 bp repeat sequences[25]. MSI can rapidly cause frameshift mutations in the repeated sequences in target cancer suppressor genes, accounting for about 15% of sporadic CRCs[26]. The studies performed by Kim *et al*[27] and Li *et al*[28], in the case of sporadic colorectal cancer, although the allele frequency of SNPs is relatively low, these polymorphisms are closely related to the occurrence of colorectal cancer. Further studies are still required to explore the correlation between colorectal cancer and the location of SNPs. At present, studies on the role of *hMSH2* in CRC are very limited. As reported in relevant articles[29,30,31,32], *hMSH2* mutation is closely related to *p53* mutation. The *hMSH2* mutation may directly cause *p53* mutation. Studies conducted by Zhang *et al*[33] have shown that the incidence of MSH2 promoter methylation in sporadic CRCs can be as high as 18.3% (212/1160 cases). The reduction of *MSH2* expression in CRC patients is likely mainly caused by gene mutations of *hMSH2*, including missense mutations and also possibly mutations at the SNP site[34,35]. While the hypermethylation of *hMLH1* promoter may result in the loss of expression of *hMLH1* gene[25].

***Analysis of SNP detection in target genes from neoplastic polyps of the intestinal tract***

In this study, the blood and tissue specimens of 120 cases of patients with tumorous polyp of the intestinal tract were collected to establish an observation group, while the blood and tissue specimens of 80 cases of patients with colorectal cancer and 40 cases of healthy individuals were collected to establish the control groups I and II, respectively. The PCR amplification and sequencing of the target genes (*K-RAS*, *hMLH1* and *hMSH2*) were conducted. As indicated by the results of blood specimens, except *hMLH1* gene at Va1384Asp which showed no statistical difference (*χ*2 = 0.568, *P* > 0.05) from that of patients with colorectal cancer in terms of the missense mutation rate, the mutation rate at SNP loci of *K-RAS* and *hMSH2* genes of the specimens from patients with neoplastic intestinal polyp were all significantly lower than those of the specimens from patients with colorectal cancer (*χ*2 = 15.476, 29.670, 10.811, *P* < 0.05). The results from the tissue specimens were consistent with the results of blood specimens, *i.e.,* as far as the missense mutation rate of *hMLH1* gene at Va1384Asp was concerned, no statistical difference (*χ*2 = 0.289, *P* > 0.05) was seen between the observation group (neoplastic intestinal polyp) and the control group I (colorectal cancer). Consistent with the incidence of *hMLH1* mutations in CRCs shown by Esteller *et al*[36] and Psofak *et al*[37] (1160 cases, 18.3%), the study has also pointed out that, in adenomas that do not necessarily progress to cancer, their hMLH1 mutation rate of is still higher than the *hMSH2* mutation rate in samples from the same period. However, Lee *et al*[38] have put forward a contradictory conclusion: besides mutations in the *hMLH1gene*, CpG island methylation at *hMSH2* and *MGMT* is more closely correlated to the traditional adenoma-tumorigenesis pathway. Therefore, whether the appearance of SNPs ultimately leads to the epigenetic phenomenon of *hMLH1* will be verified in further studies. On one hand, the mutation rates of the SNP loci of the K-RAS and *hMSH2* from the blood and tissue specimens of patients with neoplastic intestinal polyp showed no statistical difference when compared to the healthy individuals. On the other hand the mutation rate of *hMLH1* gene at Va1384Asp locus in the blood and tissue specimens of patients with neoplastic intestinal polyps was significantly higher than that of the healthy population (where *χ*2 = 10.486, 4.876, *P* < 0.05), indicating that the mutation rates of the SNP locus Va1384Asp of hMLH1 gene show similarities between the observation group and the control group I as well as the differences between the observation group and the control group II. What also needs to be pointed out is that, considering that there is still controversy with respect to the efficacy of immunocytochemical analysis of the MMR gene as an alternative molecular marker and also considering also the special hereditary definition of SNPs in target genes[39,40], the present study has adopted direct sequencing to achieve more direct results and avoid the influence of other genetic mechanisms. Considering the role of neoplastic polyps as an important precancerous lesion of colorectal cancer, it has been indicated that the genetic and histological changes must have been present during the course of development of the disease. At present, the molecular mechanisms of neoplastic polyps in pathogenicity are not clear. However, recent studies have shown that the surface migration and atypical hyperplasia of deep crypt cells play a primary role in pathogenesis [41]. Taking into account the biological and genetic functions of target genes, we suggest that the differences in SNP locus mutation of *KAS*, *hMLH1* and *hMSH2* genes between patients with neoplastic polyp and patients with colorectal cancer can be explained by the differences in the functional role of these genes. The *KAS* gene is primarily involved in cellular differentiation, proliferation and energy metabolism, while the *hMLH1* gene is an important gene in the mismatch repair system. The results of the study indicate that *hMLH1* gene might have been subjected to alteration during molecular events in the early stages of colorectal cancer or even in the precancerous lesions of neoplastic polyps. Although the SNP locus mutation of *K-RAS* gene has been shown to be an early molecular event in the onset of colorectal cancer, it was demonstrated in this study that the SNP locus mutation of *K-RAS* gene might not have play an important role in patients with intestinal neoplastic polyps. Similarly, *hMSH2* gene, which was also considered to be an important gene in the mismatch repair system, was not significantly changed in the neoplastic polyps. This might be explained by the differences between the “second hit” mechanisms of *hMLH1* and *hMSH2* genes; however, the specific mechanism needs to be further investigated. We also suggest that the hypermethylation of *hMLH1* gene is either easily facilitated or the modification occurs early during the course of disease progression. While in the domestic research, the Study conducted by Xiang *et al*[42] on the role of mismatch repair proteins (hMLH1 and hMSH2) in the intestinal polyps and sporadic colorectal cancer showed that the negative expression rates of hMLH1 and hMSH2 proteins was were 4.76% and 0 in the inflammatory intestinal polyp, 21.66% and 8.92% in the adenomatous polyp tissue of intestinal tract, and 27.27% and 13.64% in the sporadic colorectal cancer tissue, respectively. These results demonstrated the clinical significance of the negative expression of mismatch repair protein hMLH1 caused by the mutation of *hMLH1* gene in both neoplastic intestinal polyps and colorectal cancers. Additionally, in this study, the negative expression rate of hMLH1 protein was greater than the mutation rate of SNPs for *hMLH1* gene at Va1384Asp locus. As mentioned in the study by Xiang Fangfang and colleagues, the negative expression of mismatch repair proteins hMLH1 and hMSH2 cannot be attributed to a single factor of mutations in the SNP locus, but other factors such as protein translation also play an important role. It is also suggested that, the mutations in the SNP loci of the target genes were not a determining factor in the increased susceptibility of developing colorectal cancer and the exact role of mutations in the SNP loci and their involvement in the pathogenesis of colorectal cancer needs to be further evaluated.

In conclusion, there are similarities and differences between the mutation rates of *K-RAS,* *hMLH1* and *hMSH2* genes in the blood and tissue biopsy specimens of patients with neoplastic intestinal polyps and the patients with colorectal cancer. Our study indicates there may be a close relevance between neoplastic intestinal polyps and colorectal cancers in terms of the genetic alterations and pathogenic mechanisms, which may have important clinical significances.

**COMMENTS**

***Background***

The development process of colorectal cancer relates to a complex process with multi-gene, multi-stage, multi- step changes, mostly follows the mode of "normal intestinal epithelium→hyperplasia micro adenoma→early adenoma→mid adenoma→advanced adenoma→colorectal cancer→metastasis". At the same time, as the development of molecular genetics research, colorectal cancer prevention research is no longer limited to tissue pathology, and the significance of biological genetics between neoplastic polyps and colorectal cancer, as well as the disease prevention, epidemiology and etiology are paid more attention gradually. Related research at home and abroad has made a breakthrough in the pathogenesis and genetic susceptibility of colorectal cancer, which provides a new idea for the clinical prevention and treatment interventions.

***Research frontiers***

SNP is of high value in many research fields, such as genetic analysis, diagnosis and treatment of diseases, biomedical research, drug development and population ecology evaluation. At present, with the development of genetics, molecular biology and detection technology research, SNP has played a more important role in the correlation research of tumor pathogenesis, prognosis evaluation and judgment. Especially, the DNA mismatch repair system and the *K-RAS* gene SNP polymorphism have close relation with colorectal cancer susceptibility, so they are significant in early genetic events of colorectal cancer.

***Innovations and breakthroughs***

The target gene SNP sequencing is conducted by blood and tissue samples of neoplastic polyps, colorectal cancer and the normal. By directly comparing and analyzing the statistically difference of results, further clarify the significance of different genes and genetic event in the pathogenesis of colorectal cancer.

***Applications***

By comparing the differences of target gene SNP in different stages, further prove the potential clinical value of molecular markers and molecular diagnosis, thus, settle a theoretical basis and explore new clinical ideas for screening groups with high tumor risk as well as establishing an effective early warning mechanism.

***Peer review***

Through conducting the SNP sequencing by blood and tissue samples of neoplastic polyps, colorectal cancer and the normal, this study compares and analyzes the results theoretically, explores the role of different genes SNPs genetics in the pathogenesis, and provides new research directions for molecular genetics pathogenesis of colorectal cancer.

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**P-Reviewer:** Ponzetti A, Sinagra E **S-Editor:** Qi Y **L-Editor: E-Editor:**

**Table 1 Clinical data of three groups of patients**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Number****(*n*)** | **Sex (*n*)** | **Age (yr)** |
| **Male** | **Female** | **Range** | **X ± S** |
| Observation group (Neoplastic polyps) | Tubular adenoma | 85 | 54 | 31 | 27-68 | 39.2 ± 7.6 |
| Villous adenoma | 17 | 9 | 8 | 24-65 | 36.5 ± 11.2 |
| Mixed adenoma | 18 | 8 | 10 | 29-66 | 37.4 ± 10.9 |
| Control group I (colorectal cancer) | Tubular adenocarcinoma | 42 | 24 | 18 | 37-72 | 44.6 ± 11.3 |
| Mucinous adenocarcinoma | 27 | 17 | 10 | 34-70 | 40.2 ± 9.6 |
| Papillary adenocarcinoma | 8 | 5 | 3 | 33-58 | 41.1 ± 10.3 |
| Other types1 | 3 | 3 | 0 | 45-52 | 48.3 ± 7.1 |
| Control group II (normal) | 40 | 26 | 14 | 22-55 | 34.6 ± 6.3 |

1Other types include: Undifferentiated carcinoma.

**Table 2 Detection of target gene loci in the blood specimens of the observation group and the control groups I and II**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **K-RAS-12** | **K-RAS-13** | **hMLH1-Va1384Asp** | **hMSH2-2783C/A** |
| **+** | **-** | **+** | **-** | **+** | **-** | **+** | **-** |
| Observation group | 3 | 117 | 0 | 120 | 11 | 109 | 0 | 120 |
| Control group I | 15 | 65 | 18 | 62 | 10 | 70 | 7 | 73 |
| *χ*2 | 15.476 | 29.670 | 0.568 | 10.811 |
| P value | 0.000 | 0.000 | 0.451 | 0.001 |
| Control group II | 1 | 39 | 0 | 40 | 1 | 39 | 0 | 40 |
| *χ*2 | 0.000 | - | 10.486 | - |
| *P* value | 1.000 | - | 0.001 | - |

**Table 3 Mutation rates of target genes from tissue specimens of the observation group and the control groups I and II**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **K-RAS-12** | **K-RAS-13** | **hMLH1-Va1384Asp** | **hMSH2-2783C/A** |
| **+** | **-** | **+** | **-** | **+** | **-** | **+** | **-** |
| Observation group | 7 | 113 | 1 | 119 | 19 | 101 | 3 | 117 |
| Control group I | 21 | 59 | 22 | 58 | 15 | 65 | 10 | 70 |
| *χ*2 | 16.618 | 33.538 | 0.289 | 7.898 |
| *P* value | 0.000 | 0.000 | 0.591 | 0.005 |
| Control group II | 1 | 39 | 0 | 40 | 1 | 39 | 0 | 40 |
| *χ*2 | 0.702 | 0.335 | 3.883 | 1.019 |
| *P* value | 0.361 | 0.750 | 0.048 | 0.419 |

**Figure 1 Sequencing map of mutation locus of KRAS-12 (A) and KRAS-13 codon (B).**

**A**



**B**



**Figure 2 Sequencing map of mutation locus of hMLH1-Val384Asp (A) and hMSH2-2783C/A (B).**

**A**

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B

