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Original Article

The correlation between the benign and malignant lesions of colorectal tumors and the CYP24A1 gene polymorphism

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Abstract: Objective: To explore the correlation between the CYP24A1 gene polymorphism and colorectal tumor patients' benign and malignant lesions and analyze the relationship between the CYP24A1 gene and colorectal cancer susceptibility. Methods: A total of 257 colorectal tumor patients diagnosed for the first time and admitted to our hospital were recruited as the study cohort and divided into the malignant group (n=139) and the benign group (n=118) according to their pathological examination results. A total of 110 healthy patients who visited our hospital during the same period for physical exams were recruited as the control group. The individual clinical data and blood samples from the patients in each group were collected and recorded. The gene fragments at the four loci (rs114368325, rs6068812, rs2296239, and rs1570670) in the CYP24A1 gene were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and inter-group difference and logistic regression analyses were performed to explore the relationship between the gene polymorphism and the benign and malignant lesions in the colorectal tumors. Results: The distribution frequencies of the loci rs114368325 and rs2296239 in the individuals in the three groups conformed to the Hardy-Weinberg equilibrium law. Two genotypes, GG and AG, were found in rs114368325, three genotypes, CC, CT, and TT, were found in rs2296239, and the GG genotype was found in rs6068812 and rs1570670. The malignant group had a significantly higher proportion of G in the locus rs114368325 and a higher GG distribution frequency than the other two groups ($P<0.05$). There was no significant difference in different phenotypes in the other gene loci among the three groups ($P>0.05$). Conclusion: There is no significant correlation between the genetic polymorphisms of the four loci (rs114368325, rs6068812, rs2296239, and rs1570670) at the CYP24A1 gene and the benign and malignant lesions of colorectal tumors, and the nature of the benign and malignant lesions in colorectal tumors can be evaluated by monitoring the locus rs114368325.

Keywords: Colorectal tumors, benign and malignant lesions, CYP24A1 gene polymorphism, correlation

Introduction

Colorectal cancer (CRC) is one of the most common malignancies, and its incidence rate ranks third among tumors occurring in males and second among tumors occurring in females [1]. In recent years, with the progress of China's industrialization and the changes in the Chinese diet and lifestyle, the incidence rate of CRC has been increasing yearly. Published studies indicate that one patient dies of CRC approximately every 30 seconds worldwide [2]. The epidemiological statistics regarding CRC show that there are 222,000 new CRC cases in the United States each year, of which approximately 157,000 die from CRC [3]. The statistics released by the World Health Organization (WHO) show that the number of new CRC cases is as high as 1.2 million globally every year, of which approximately 60% are concentrated in developed countries, with a male-to-female prevalence ratio of approxiamately 1.3:1. As many as 608,000 patients die from CRC each year, accounting for about 8% of all malignant tumor deaths and ranking fourth among lethal cancer deaths [4-6]. The early clinical symptoms of CRC are not obvious, and most CRC patients only present with dyspepsia and fecal occult blood. With the development of symptoms, CRC patients gradually reveal changes in their bowel habits, hematochezia, abdominal masses, intestinal obstructions, etc., and some

patients may also exhibit fever, anemia, emaciation, tumor infiltration, or metastasis, etc., affecting the functions of their affected organs. Currently, the pathogenesis of CRC remains unclear, but most empirical studies indicate that environmental and genetic factors are related to the occurrence and development of CRC, and poor diet structure, a CRC family history, gene mutations, etc. elevate the prevalence rate of CRC [7, 8].

Early identification and diagnosis are good ways to improve the clinical symptoms and prognoses of CRC patients. The pathological examination is a conventional method that is extensively implemented to identify benign and malignant colorectal tumors. Although pathological examinations are highly accurate, they cannot be performed on some patients because they are invasive. Laboratory tests can vary due to individual factors, and endocrine disorders and drugs can directly affect the examination's accuracy. Therefore, the molecular methods for identifying benign and malignant colorectal tumors have been extensively explored in clinical research [9]. Cytochromes P450 (CYPs), which play a significant role in drug metabolism and detoxification, are a superfamily of gene-coding isozymes associated with structures and functions. One CYP, CYP24A1, can effectively catalyze vitamin D degradation. Current empirical studies indicate that the genetic polymorphism CYP24A1 is closely related to the risk for the occurrence of multiple tumors, and some studies have shown that the polymorphism CYP24A1 is related to rectal cancer and associated with a higher risk of occurrence. A survey of a total of 231 colon cancer patients found that the gene polymorphism RS4809957 in CYP24A1 is related to a genetic predisposition for colitis, colon polyps, and colon cancer [10, 11]. The purpose of this study is to explore the correlation between the CYP24A1 gene polymorphism and benign and malignant CRC lesions, so as to provide a theoretical basis for the clinical diagnosis and prognosis evaluation of CRC patients.

Materials and methods

From January 2017 to January 2020, a total of 257 colorectal tumor patients diagnosed for the first time and admitted to our hospital were recruited as the study cohort and were divided into the malignant group (n=139) and the benign group (n=118) according to their pathological examination results. A total of 110 healthy patients who visited in our hospital for physical exams during the same period were selected as the control group.

Inclusion criteria: (1) The patients in the malignant and benign groups were examined using pathology and enteroscopy and had a definite pathological diagnosis. (2) Patients with complete medical records. (3) Patients with a clear consciousness and the ability to cooperate with the study procedures. The study was approved by the Hospital Ethics Committee. The enrolled patients all signed a written informed consent form.

Exclusion criteria: (1) Patients also suffering from mental disorders. (2) Patients also suffering from systemic multiple organ dysfunction syndrome (MODS). (3) Patients also suffering from severe cardiovascular diseases. (4) Patients with infectious diseases. (5) Patients also suffering from severe heart, liver, or kidney disease. (6) Patients with other malignant tumors. (7) Patients with autoimmune diseases. (8) Pregnant or lactating women. (9) Patients with poor compliance. (10) Patients also suffering from diabetes, hypertension, hyperlipidemia, or other chronic diseases.

Intervention method

A total of 2 ml of venous blood was collected from the individuals in each group, placed in EDTA-K2 anticoagulation tubes, and stored at -50°C after being mixed. DNA extraction was performed uniformly after the sample collection was completed. The whole blood DNA was extracted using the AxyPrep kit protocol. The kits were purchased from the Shanghai Kemin Biotechnology Co., Ltd. and the operations were carried out strictly in accordance with the kit instructions. After the DNA collection was completed, the CYP24A1 gene polymorphisms at different loci were detected using the SnaPshot method. After the detection was completed, a PCR amplification and purification of the PCR products was performed, and then a sequencing and result analysis of the PCR products was conducted using the SnaPshot method.

Attention was paid to avoid repeated freezing and thawing by aliquoting the blood samples and the DNA samples in the aforementioned operations. Meanwhile, the PCR reaction tubes,

Table 1. Comparisons of the general clinical data of the three groups ($\bar{x} \pm s$)/[n (%)]

General clinical data		Malignant group (n=139)	Benign group (n=118)	Control group (n=110)	*F/t*	*P*
Sex	M	70	59	55	1.291	0.581
	F	69	59	55		
Mean age (years)		56.22±4.33	56.31±4.21	56.33±4.51	1.322	0.545
Mean weight (kg)		63.38±3.22	64.01±2.31	63.87±3.31	1.434	0.521
Mean BMI (kg/m^2)		22.18±2.11	22.21±2.31	22.33±2.01	0.980	0.223
Degree of education	Illiteracy	19	10	10	0.879	0.431
	Primary school	40	28	20		
	Junior high school	50	60	60		
	High school and above	30	20	20		
Marital status	Married	128	105	100	0.445	0.878
	Not married	11	13	10		

Table 2. Hardy-Weinberg equilibrium analysis of the four gene loci of CYP24A1 in the three groups

Loci	Genotype	Malignant group (n=139)	Benign group (n=118)	Control group (n=110)	*F*	*P*
rs114368325	GG	135	110	107	0.982	0.541
	AG	4	8	3		
	AA	0	0	0		
rs2296239	TT	60	43	40	0.881	0.551
	CT	68	61	59		
	CC	11	14	11		
rs6068812	GG	139	118	110	0.789	0.581
	AG	0	0	0		
	AA	0	0	0		
rs1570670	GG	139	118	110	0.671	0.667
	AG	0	0	0		
	AA	0	0	0		

EP tubes, sample-loading guns, and the like used in the operations were sterilized under high pressure. All the operations were carried out in strict accordance with the PCR operation procedures to avoid microbial contamination. Finally, a negative control was set for each PCR amplification and enzyme digestion experiment.

Statistical analysis

The data collected were input into SPSS 20.0 for the statistical analysis. The measurement data were expressed as $\bar{x} \pm s$, and the differences between groups were compared using Student's t tests. The enumeration data were expressed as [n (%)], and the differences between groups were compared using chi-square or Fisher's exact tests. The differences among groups were compared using F tests, and the ages and sexes of the individuals in the different groups were corrected using logistic regression analyses. $P<0.05$ indicated a statistically significant difference [12].

Results

Comparison of the general clinical data in the three groups

There were no statistically significant differences in the general clinical data among the three groups, such as gender, age, mean weight, education level, or basic disease history ($P>0.05$), so the two groups were comparable (**Table 1**).

Hardy-Weinberg equilibrium analysis for the four gene loci of CYP24A1 in the three groups

The CYP24A1 gene polymorphisms of the individuals in the three groups conformed to the Hardy-Weinberg equilibrium law ($P>0.05$). The

Table 3. Comparisons of differences in the genotype and allele frequency distributions of the locus rs114368325 in the three groups (%)

Genotype	Benign group	Malignant group	Control group	*F*	*P*
G	98.64	86.61	68.56	20.192	<0.001
A	1.36	13.39	31.44	19.292	<0.001
GG	97.27	83.22	67.12	8.289	<0.001
AG	2.73	16.78	32.88	9.991	<0.001
AA	0.00	0.00	0.00	7.819	<0.001

Table 4. Comparisons of the differences in the genotype and allele frequency distributions of the locus rs2296239 in the three groups (%)

Genotype	Benign group	Malignant group	Control group	*F*	*P*
C	36.82	38.70	32.37	19.228	<0.001
T	63.18	61.30	67.63	18.229	<0.001
TT	36.36	36.44	43.17	10.292	<0.001
CT	53.64	51.69	48.92	18.229	<0.001
CC	10	11.87	7.91	14.198	<0.001

Hardy-Weinberg equilibrium law, also known as the genetic equilibrium law, is the most important principle in genetics and is mainly used to determine whether the distribution of different gene loci among the different groups conforms to the genetic law, that is, whether the gene loci of the analyzed subjects are in accordance with the population representativeness and comparability (**Table 2**).

Comparisons of the differences between the genotype and allele frequency distributions of the locus rs114368325 in the three groups

The locus rs114368325 at the CYP24A1 gene can be divided into the G and A alleles. The distribution frequencies of G and A were 98.64% and 1.36% in the control group, 86.61% and 13.39% in the benign group, and 68.56% and 31.44% in the malignant group, indicating no significant differences i among the three groups (*P*>0.05). The locus rs114368325 can be divided into GG and AG genotypes. The distribution frequencies of the GG and AG genotypes were 97.27% and 2.73% in the control group, 83.22% and 16.78% in the benign group, and 67.12% and 32.88%, in the malignant group, showing no significant differences among the three groups (*P*>0.05) (**Table 3**).

Comparisons of the genotype and allele frequency distributions of the locus rs2296239 in the three groups

The analysis showed that the locus rs2296239 in the CYP24A1 gene can be divided into C and T alleles, and the distribution frequencies of the C and T alleles were 36.82% and 63.18% in the control group, 38.70% and 61.30% in the benign group, and 32.37% and 67.63% in the malignant group, and there was little difference among the three groups (*P*>0.05). CYP24A1 can be further divided into the TT, CT and CC genotypes, and the distribution frequencies of the three genotypes were 36.36%, 53.64%, and 10.00% in the control group, 36.44%, 51.69%, and 11.87% in the benign group, and 43.17%, 48.92%, and 7.91% in the malignant group. There were slightly significant differences in the distribution frequencies of the TT, CT and CC genotypes among the three groups (*P*>0.05) (**Table 4**).

Comparisons of genotype and allele frequency distributions of the locus rs6068812 in the three groups

The analysis showed that the locus rs6068812 at the CYP24A1 gene could be divided into the G and A alleles. The distribution frequencies of G and A in the control, benign, and malignant groups were 100.00% and 0.00% respectively, and there were no differences in the distributions among the three groups (*P*>0.05). The locus rs6068812 can be divided into the three genotypes, namely, GG, AG, and AA. The distributions of GG, AG, and AA in the control, benign, and malignant groups were 100.00%, 0.00%, and 0.00%, respectively. There were no differences in the distribution frequencies among the three genotypes (*P*>0.05) (**Table 5**).

Comparisons of the genotype and allele frequency distributions of the locus rs1570670 in the three groups

The analysis demonstrated that the locus rs1570670 at the CYP24A1 gene can be divided into the G and A alleles. The distribution frequencies of the G and A alleles in the control, benign, and malignant groups were 100.00% and 0.00% respectively, and there were no dif-

Table 5. Comparisons of the differences in the genotype and allele frequency distributions of the locus rs6068812 in the three groups (%)

Genotype	Benign group	Malignant group	Control group	*F*	*P*
G	100	100	100	0.0	1.0
A	0	0	0	0.0	1.0
GG	100	100	100	0.0	1.0
AG	0	0	0	0.0	1.0
AA	0	0	0	0.0	1.0

Table 6. Comparisons of differences in the genotype and allele frequency distributions of the locus rs1570670 in the three groups (%)

Genotype	Benign group	Malignant group	Control group	*F*	*P*
G	100	100	100	0.0	1.0
A	0	0	0	0.0	1.0
GG	100	100	100	0.0	1.0
AG	0	0	0	0.0	1.0
AA	0	0	0	0.0	1.0

ferences in the distributions among the three groups ($P>0.05$). The locus rs1570670 can be divided into the three genotypes, namely, GG, AG, and AA. The distributions of GG, AG, and AA in the control, benign, and malignant groups were 100.00%, 0.00%, and 0.00%, respectively. There was no difference in the distribution frequencies among the three genotypes ($P>0.05$) (**Table 6**).

Analysis of the frequency distribution differences of the different loci in CYP24A1 in the three groups

The analysis showed that the distributions of the ACAG, ATAG, GCAG, and GTAG genotypes were 2.99%, 5.01%, 86.01%, and 141.99% in the benign group, 2.84%, 1.16%, 87.16%, and 186.84% in the malignant group, and 0.01%, 2.99%, 80.99%, and 136.01% in the control group. There were no statistically significant differences in the genotype distribution among the three groups ($P>0.05$) (**Table 7**).

Discussion

The early clinical symptoms of CRC are not obvious. Therefore, most CRC patients have reached the middle or advanced stages by the time they are diagnosed with CRC. Despite the advance sin the diagnosis and treatment technologies, the post-surgery, 5-year survival rates have not improved significantly. The lack of any early diagnosis methods for diagnosing CRC and the high specificity of precancerous lesions are the main reasons. If colorectal lesions can be identified and removed early, it will be conducive to reducing the disease's morbidity and mortality [13-16]. Currently, the clinical early diagnosis of CRC is performed primarily using colonoscopy, imaging examinations, pathological examinations, and serological marker carcinoembryonic antigen (CEA), etc. Due to its invasion or sensitivity, low specificity, and other shortcomings, it cannot be extensively implemented in clinical practice [17, 18]. In recent years, with the development of molecular biology, the important effects of genes in CRC have been extensively explored, and an early diagnosis and evaluation of CRC can be conducted through gene evaluation.

Currently, many studies suggest that vitamin D deficiency elevates the risk of the occurrence of multiple cancers, and the active ingredients in vitamin D may relieve intestinal inflammation and narrow the range of lesions. Meanwhile, an empirical experiment on rats with enteritis reveals that intervention with vitamin D in rats effectively shortens the duration of enteritis in rats and improves their vitality and motor functions [19]. Another CYP24A1 test conducted on 56 breast cancer specimens showed that the positive rate of CYP24A1 expression was 58.9% in breast cancer specimens. Further studies suggest that the positive rate of CYP24A1 expression is significantly correlated with the diameters, histological grades, and estrogen receptor expressions of breast cancer lesions, indicating that the gene is involved in the occurrence and development of breast cancer. This demonstrates that monitoring the CYP24A1 gene provides a certain theoretical basis for the evaluation of tumor status [20, 21].

In this study, three groups were established to analyze the correlation between the CYP24A1 gene polymorphism and the benign and malignant lesions of colorectal tumors. The results showed that there was no remarkable difference among the different alleles and genotypes of the three CYP24A1 gene loci of rs6068812, rs2296239, and rs1570670 in the benign, malignant, and control groups, but there was a

Table 7. Analysis of differences in the frequency distributions of the different loci at CYP24A1 in the three groups (%)

Genotype	Benign group	Malignant group	Control group	*P*	95% CI
ACAG	2.99	2.84	0.01	0.243	11.298-12.332
ATAG	5.01	1.16	2.99	0.443	0.371-6.554
GCAG	86.01	87.16	80.99	0.321	0.552-1.345
GTAG	141.99	186.84	136.01	0.541	0.918-1.891

significant difference in the distribution frequencies of the G and A alleles at the rs114368325 gene. The distribution frequencies of the G and A alleles were 98.64% and 1.36% in the control group, 86.61% and 13.39% in the benign group, and 68.56% and 31.44% in the malignant group. A further analysis of the differences in the genotypes among the groups showed that the distribution frequencies of the GG and AG genotypes were 97.27% and 2.73% in the control group, 83.22% and 16.78% in the benign group, and 67.12% and 32.88% in the malignant group. The distribution frequencies of the AG genotypes in the malignant group were markedly higher than the distribution frequencies in the benign and control groups, but the comparisons of the differences between the control and benign groups were not significant [22]. One study pointed out that the CYP24A1 gene is closely related to vitamin D metabolism. Normally, CYP24A1 is in a low expression state in many organs, but it has a very sensitive response to active vitamin D signals. The gene, which has been proved to be a modified enzyme of $1\alpha,25(OH)_2D_3$ metabolism, is a key factor for $1\alpha,25(OH)_2D_3$ physiological half-life [23]. However, $1\alpha,25(OH)_2D_3$ has been proved to have strong anti-tumor activity, and it not only inhibits the proliferation of tumor cells, but it can also induce cell differentiation and promote cell apoptosis. An analysis of the proliferation and cell cycle of gastric cancer cells shows that when the BGC-823 and SGC-7901 gastric cancer cell lines are respectively treated with $1\alpha,25(OH)_2D_3$ of different concentrations, then the cell inhibition rate can be quantified using an MTT assay, and the cell cycle can be quantified using flow cytometry. After $1\alpha,25(OH)_2D_3$ intervention, the BGC-823 and SGC-7901 gastric cancer cell lines exhibited significant increases in the G1 phase cell ratio and decreases in the S phase cell ratio, signaling that $1\alpha,25(OH)_2D_3$ markedly the affected cell cycle [24]. These studies all suggest that CYP24A1 is closely related to the metabolism of 1α, $25(OH)_2D_3$, and $1\alpha,25(OH)_2D_3$ can be combined with the vitamin D ligand. Previous empirical studies indicate that the vitamin D ligand can effectively reduce tumor volume and prevent lymph node involvement. If tumor patients are treated using a vitamin D ligand, the patients' survival times can be prolonged, so it can be inferred that CYP24A1 is closely related to tumor occurrence and development. However, through further investigation of the CYP24A1 gene polymorphism, we found that the G and A allele frequencies in the locus rs114368325 at CYP24A1 are significantly different in patients with different types of colorectal tumors, and the genotype distributions are also markedly different. Therefore, it can be inferred that the gene mutation in the locus rs114368325 at CYP24A1 may lead to the malignant transformation of colorectal tumors [25, 26].

In summary, the rs6068812, rs2296239, and rs1570670 polymorphisms at the CYP24A1 gene have no significant correlation with benign and malignant CRC lesions, and the nature of CRC patients' benign and malignant lesions can be evaluated by monitoring the locus rs114368325. The innovation of this study is our exploration of the relationship between genetic polymorphisms and the malignant transformation of CRC by associating the different gene loci of CYP24A1 with the pathological types of CRC, so as to provide a new strategy for the diagnosis and identification of CRC. However, the deficiencies lie in the lack of research on the expression of the gene-related proteins, which lacks comprehensiveness and rigor to a certain extent. Therefore, more in-depth studies will be conducted based on the deficiencies so as to provide more accurate theoretical data for the treatment and prognosis of CRC patients.

Disclosure of conflict of interest

None.

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