PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 March 14; 13(10): 1528-1533
World Journal of Gastroenterology ISSN 1007-9327

COLORECTAL CANCER

Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran

Mohammad Reza Abbaszadegan, Alireza Tavasoli, Arash Velayati, Hamid Reza Sima, Hassan Vosooghinia, Mehdi Farzadnia, Hamid Asadzedeh, Mehran Gholamin, Ezzat Dadkhah, Azadeh Aarabi

Mohammad Reza Abbaszadegan, Arash Velayati, Hamid Asadzedeh, Mehran Gholamin, Ezzat Dadkhah, Azadeh Aarabi, Division of Human Genetics, Immunology Research Center, Bu-Ali Research Institute, MUMS, Mashhad, Iran
Alireza Tavasoli, Department of Surgery, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
Hamid Reza Sima, Department of Internal Medicine, Imam Reza Hospital, MUMS, Mashhad, Iran
Hassan Vosooghinia, Department of Internal Medicine, Ghaem Hospital, MUMS, Mashhad, Iran
Mehdi Farzadnia, Department of Pathology, Imam Reza Hospital, MUMS, Mashhad, Iran
Supported by a grant from the vice chancellor for research at Mashhad University of Medical Sciences, NO. 84082
Correspondence to: MR Abbaszadegan, PhD, Director, Division of Human Genetics, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Bu-Ali square, postal code: 9196773117, Mashhad, Iran. abbaszadegan@ams.ac.ir
Telephone: +98-511-7112343 Fax: +98-511-7112343
Received: 2006-11-20 Accepted: 2007-01-30

Abstract

AIM: To detect tumor-associated DNA changes in stool samples among Iranian patients with colorectal cancer (CRC) compared to healthy individuals using BAT-26, *p*16 hypermethylation and long DNA markers.

METHODS: Stool DNA was isolated from 45 subjects including 25 CRC patients and 20 healthy individuals using a new, fast and easy extraction method. Long DNA associated with tumor was detected using polymerase chain reaction method. Microsatellite studies were performed utilizing denaturating polyacrylamide gel to determine the instability of BAT-26. Methylation status of *p*16 promoter was analyzed using methylation-specific PCR (MSP).

RESULTS: The results showed a significant difference in existence of long DNA (16 in patients *vs* 1 in controls, $P < 0.001$) and *p*16 (5 in patients *vs* none in controls, $P = 0.043$) in the stool samples of two groups. Long DNA was detected in 64% of CRC patients; whereas just one of the healthy individuals was positive for Long DNA. *p*16 methylation was found in 20% of patients and in none of healthy individuals. Instability of BAT-26 was not detected in any of stool samples.

CONCLUSION: We could detect colorectal cancer related genetic alterations by analyzing stool DNA with a sensitivity of 64% and 20% and a specificity of 95% and 100% for Long DNA and *p*16 respectively. A non-invasive molecular stool-based DNA testing can provide a screening strategy in high-risk individuals. However, additional testing on more samples is necessary from Iranian subjects to determine the exact specificity and sensitivity of these markers.

Key words: Stool DNA; Colorectal cancer; Cancer screening; Long DNA; BAT-26; *p*16

Abbaszadegan MR, Tavasoli A, Velayati A, Sima HR, Vosooghinia H, Farzadnia M, Asadzedeh H, Gholamin M, Dadkhah E, Arabi A. Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran. *World J Gastroenterol* 2007; 13(10): 1528-1533

http://www.wjgnet.com/1007-9327/13/1528.asp

INTRODUCTION

Colorectal cancer is one of the most common forms of cancer in the world and is curable if diagnosed at an early stage[1]. Extensive research over the past 15 years has shown that a specific series of genetic changes drives the neoplastic transformation of normal colonic epithelium to benign adenomas and subsequently to malignant adenocarcinomas[2]. Colon cancers arise from at least three different genetic pathways: chromosomal instability, microsatellite instability, and CpG island methylation. Chromosomal instability accounts for about 85% of sporadic colorectal cancers. Microsatellite instabilities that are replication errors (RERs) caused by germline or somatic mutations of mismatch gene, are involved in the development of some colorectal cancers[3]. Loss of function of any of mismatch repair genes may lead to a failure in repair mutations and development of cancers[4,5]. One microsatellite, BAT-26, a single locus of 26 consecutive adenine nucleotides is strongly associated with failure of a mismatch gene. Thus, testing for mutations in BAT-26 is almost as effective as screening most microsatellite loci[6,7]. Several studies showed the relationship of this marker and colorectal cancers[8,9]. It is implicated in about 20% of right-sided colorectal cancers

while in only 1% to 2% of left-sided colon cancers.

One other pathway known to be involved in the pathogenesis of colorectal cancer is the methylation of the CpG islands located within the promoter regions of genes regulating cell proliferation, apoptosis, and DNA repair. The detection of hypermethylated fecal DNA has been reported by others in a few studies[10,11]. Methylation often affects multiple genes. It also occurs as an age-related phenomenon in morphologically normal mucosa. *p*16, a tumor suppressor gene silenced by hypermethylation of its promoter in early stages of cancer, provide a valuable approach to screening for early lesions[12].

The discovery of these genetic and epigenetic alterations has raised the possibility of detecting colorectal cancer through examination of the stool DNA as a healthy adult excretes approximately 10^{10} epithelial cells every day[13]. A large number of tumor cells will renew and exfoliate into the intestinal cavity of colonic cancer patients daily. A certain amount of DNA can maintain its stability due to the resistance of intestinal tumor cells to various degradation enzymes or due to the impairment of apoptotic mechanism of tumor cells. Therefore, molecular examination of the genetic composition of the colonic mucosal cells, which are exfoliated into the stool, brought new options for colorectal cancer screening. Sidransky *et al*[14], for the first time, detected the K-ras gene mutations in the fecal samples from early intestinal cancer patients. Since then, several studies have shown that it is possible to detect mutations of these genes in stool samples from patients with colorectal cancer. Stool-based DNA testing has gradually become a screening method for colorectal cancer[15-17].

The amount of human DNA in feces may be increased in individuals with colorectal cancer. Kelaassen *et al*[18] demonstrated increased amounts of human DNA in the feces of patients with colorectal tumors compared with healthy persons. Boynton *et al*[19] showed that the majority of DNAs isolated from the stool of patients with colorectal tumors were of high molecular weight, in contrast to the fragmented apoptotic DNAs found in stools from colonoscopy-negative patients. They proposed that the increased concentrations of human DNA could be explained by decreased apoptosis of bowel cells and/or increased shedding of cancer cells into the colonic lumen. There is evidence that transformed colonic mucosa cells have dysfunctional apoptotic mechanisms[20] and thus may shed cells that have not undergone apoptosis. Because one of the characteristics of apoptosis can be the cleavage of DNA into 180 to 210 bp fragments[21], dysfunction of apoptotic mechanisms will lead to presence of high-molecular-weight fragments (more than 1 Kb) of DNAs, which are named as Long DNA. Therefore, long DNA becomes a valuable marker in stool based DNA testing.

Newer assays examining more than one mutation are significantly more sensitive. They include more than 20 mutations on APC, *p*53 and k-ras, microsatellite analysis for BAT-26 and Long DNA and methylation markers[22,23]. In this study, we have established a stool-based DNA assay to detect Long DNA and BAT-26 markers and *p*16 methylation in patients with colorectal cancer in Iran.

MATERIALS AND METHODS

Sample collection

Human stool samples were collected from 45 individuals including 20 healthy colonoscopic volunteers and 25 patients with colorectal cancer without any dietary restrictions or antibiotic treatment. About 5 g stool was collected from each individual. All the samples were collected in dry clean plastic containers. Informed consent was obtained from every subject prior to the study. Stools were collected prior to any preparation for colonoscopy or 4-5 d following this procedure. Tumor characteristics such as location, size, histological features, stage and, in addition, age, sex and fecal occult blood test (FOBT) results were considered. The stool specimens were stored at -20℃ immediately after collection, to avoid potential enzymatic degradation of nucleic acids, and then transferred to a -70℃ refrigerator within 24 h until use. The information of 21 patients is shown in Table 1.

DNA extraction

For DNA extraction, 1 g of stool, frozen at -70℃, was diluted in 10 mL of lysis buffer (0.5 mol/L Tris-HCl, 20 mmol/L EDTA, 10 mmol/L NaCl, 0.1% SDS, pH = 9.0) (TEN-9) in a 50 mL tube. After vortexing for 5 min, samples were homogenized by shaking for 10 min. A second dilution (1/2) step was performed with 10 mL lysis buffer and homogenized for 5 min. Particulate materials were removed by centrifugation at 4500 r/min for 10 min. The supernatant was transferred to a new tube, approximately 15 mL DNA was precipitated by addition of 7.5 mL ammonium acetate 7.5 mol/L (half the sample volume) and 30 mL of ice-cold ethanol 95%-100% (twice the sample volume). Incubation at -20℃ for 30-45 min rendered a better precipitation. DNA was collected following centrifugation at 4500 r/min for 15 min at RT. In this step, precipitated DNA was not colorless and contained the bile salts. The DNA pellet was re-suspended in 750 μL of TE (pH = 8) and incubated at 65℃ for 15 min. Then DNA was purified using the conventional single step phenol/chloroform protocol. Phenol would eliminate the colored impurities. After isopropanol precipitation, colorless DNA pellet was collected and dissolved in 300 μL of TE buffer following an overnight incubation at 37℃.

Long DNA analysis

A 1476 bp fragment including exons 6 to 9 of p53 gene was used for long DNA analysis. Primers were previously described by Beroud *et al*[24] (Forward: 5' GCCTCTGATTCCTCACTGAT 3' and reverse: 5' AAGACTTAGTACCTGAAGGGT 3'). The PCR reaction mixture consisted of 1 × CinnaGen PCR buffer, 500 nmol/L of each PCR primer, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs and 1 U of Taq DNA polymerase (CinnaGen, Tehran, Iran). Five hundred ng DNA diluted in 200 μL TE (pH = 8.0) was used in a reaction volume of 20 μL. PCR conditions were as follows: 3 min at 95℃ followed by 35 cycles of 40 s at 95℃, 120 s at 58℃ and 120 s at 72℃, and 5 min at 72℃ as final extension, with maximum heating and cooling settings in the Techne

Table 1 Demographic and clinicopathological characteristics of patients

Patients	Sex	Age (yr)	Tumor type	Tumor size (cm)	Tumor location	Stage	FOBT	Long DNA	Instability BAT-26	*p16*
1	M	37	A.C	10	AC	B2	+4	+	-	M
2	M	52	A.C	5	C	B2	+4	+	-	U
3	F	64	A.C	15	C	C2	+4	+	-	U
4	M	62	A.C	2	R	B1	+1	+	-	U
5	F	34	A.C	-	Sp	B2	-	+	-	U
6	M	50	A.C	10	Hp	B2	+4	-	-	U
7	M	52	A.C	7	R	C2	-	+	-	U
8	M	70	A.C	3	AC	B2	+2	-	-	U
9	M	83	A.C	1	R	-	+2	-	-	U
10	F	50	A.C	1.5	Sp	B2	+4	+	-	U
11	M	64	A.C	3	R	C2	+3	+	-	M
12	M	83	A.C	4.5	R	-	+4	+	-	M
13	M	72	A.C	8	C	B2	+4	+	-	U
14	M	64	A.C	3	R	C2	+2	+	-	M
15	F	73	A.C	6	-	B1	+4	+	-	U
16	M	43	A.C	7	AC	B1	-	+	-	U
17	F	67	A.C	3.5	AC	B2	+4	-	-	U
18	F	53	A.C	8	Sp	C2	+4	+	-	M
19	M	49	A.C	3	C	B2	+4	-	-	U
20	F	70	A.C	5	R	B2	+3	-	-	U
21	M	51	A.C	6	R	B2	+4	-	-	U

R: Rectum; C: Cecum; AC: Ascending colon; Sp: Splenic flexure; Hp: Hepatic flexure; A.C: Adenocarcinoma; FOBT: Fecal occult blood test. M: Methylated; U: Unmethylated.

Thermal Cycler (Techgene, Techne, UK).

Five microliters of amplified products were electrophoresed on 1.7% agarose gel, and stained with ethidium bromide. The presence of the 1500 bp band was considered as Long DNA. DNA extracted from blood was used as the positive control. We also used amplification of a short fragment (138 bp), including exon 9 of *p*53 gene, to evaluate the extraction method.

Microsatellite studies

For microsatellite analysis, BAT-26 was used as the microsatellite target. The purified stool DNA samples were subjected to PCR amplification of the BAT-26 sequence in 20 μL reaction mixture containing 1 μL (about 200 ng) of purified DNA (or diluted DNA), 1 × PCR buffer (Cinnagen), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 10 pmol BAT-26 sequence-specific primers, and 1 U of Taq DNA polymerase (Cinnagen). The primers have been described by Devouassoux-Shisheboran *et al*[25] (Forward: 5' TGACTACTTTTGACTTCAGCC 3'and reverse: 5' AACCATTCAACATTTTTTAACCC 3'). Amplifications were conducted in a Techgene thermocycler. After an initial denaturation at 94℃ for 5 min, PCR amplification was performed for 35 cycles, each consisting of 45 s at 94℃, 2 min at 54℃, and 2 min at 72℃, followed by a final extension of 30 min at 72℃. Appropriate amount of PCR-products was mixed with a formamide loading buffer and denaturated by boiling for 5 min at 95℃. The mixture was then loaded onto 6% polyacrylamide gel containing 7 mol/L urea.

Following the denaturating gel electrophoresis at 60 W (50 mA; 1200 V) for 60 min in 1 × TBE (89 mmol/L Tris; 2 mmol/L EDTA; 89 mmol/L Boric acid), the gels were stained with silver nitrate as described by Creste *et al*[26]. BAT26-associated instability was identified on the basis of comparison between electrophoretic patterns of tumor and their corresponding blood samples. Increased number of bands in tumor BAT-26 PCR products as compared to blood samples indicated microsatellite instability.

p16 methylation analysis

Stool DNA (2 μg) was treated with sodium bisulfite as previously described[12,27]. Modified DNA was purified using a Promega Wizard DNA Clean-Up System, according to the manufacturer's instructions and then was stored at -20℃ until it was used for PCR. Modified DNA was amplified using primers specific for methylated and unmethylated *p*16 sequences as previously described[12]. DyNAzyme Ⅱ Hot start Taq (Finnzymes, Finland) was used as DNA polymerase. PCR conditions were as follows: 10 min at 95℃ followed by 45 cycles of 45 s at 95℃, 45 s at 60℃ and 60 s at 72℃; and 5 min at 72℃ as final extension, with maximum heating and cooling settings in the Techne Thermal Cycler (Techgene, Techne, UK). Five microliters of amplified products were electrophoresed on 3% agarose gel, and stained with ethidium bromide. The presence of a 150 bp band was considered for methylated and a 151 bp band for unmethylated product. DNA extracted from blood and treated with CpG methylase (M.Sss1, Newengland BioLabs) was used as the positive methylated control.

Statistical analysis

The relationship between Long DNA, *p*16 methylation and BAT-26 instability and clinicopathological parameters, as listed in Table 1, was analyzed using SPSS version 11.5 and the *P* value was calculated using Chi-square and Fisher exact tests to find the significant relationships.

RESULTS

*p*53 exon 9, representing the short fragment (138 bp), was amplifiable in 24 out of 25 patient samples and 18 out of 20 control samples. It was revealed that the extraction

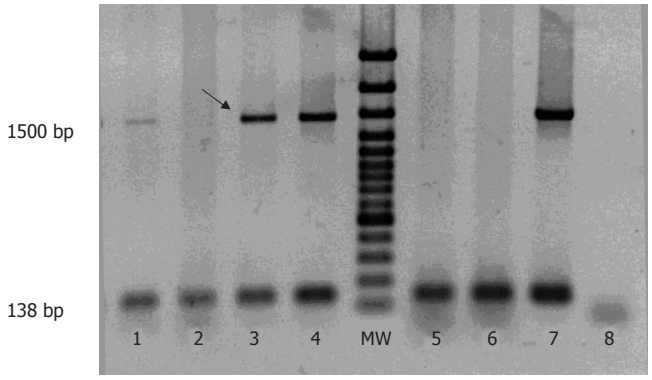

Figure 1 Long DNA analysis in patients with colon cancer in comparison with healthy individuals. 1, 2, 3, 4: Stool DNA of patients with colorectal cancer; MW: Ladder 100 bp; 5, 6: Stool DNA of healthy individuals; 7: Blood DNA as control; 8: Negative control. The arrow indicates Long DNA. Long DNA detected in 3 out of 4 patients in the picture. The 1.7% agarose gel plus ethidium bromide was used for electrophoresis at 120 volt.

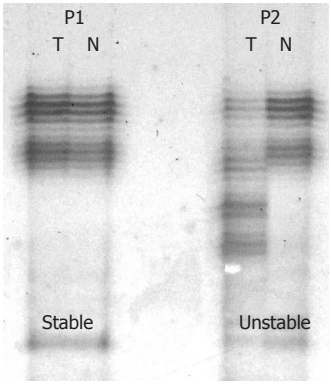

Figure 3 Microsatellite analysis of BAT-26 in two patients with colon cancer. BAT-26 instability detected in patient 2. P1: patient with stable BAT-26, P2: Patient with instable BAT-26, T: Tumor tissue, N: Normal margin. The gel was electrophoresed at 60 W (50 mA; 1200 V) for 60 min using 1 × TBE.

protocol produced enough amplifiable DNAs in most cases suitable for standard PCR amplifications. Long DNA (a nearly 1476 bp band) was detectable in 16 out of 25 colorectal cancer patients and only in 1 out of 20 healthy individuals. There was a significant difference of this marker in the stool samples of two groups ($P < 0.001$). The sensitivity for this marker was 64% and the specificity was 95%. Results of representative CRC patients and healthy individuals are shown in Figure 1. Methylation of *p*16 promoter was detected in 5 out of 25 patients compared to none in healthy controls ($P = 0.043$). The sensitivity for this marker was 20% and the specificity was 100%. Methylation analysis results are shown in Figure 2. Interestingly, all the hypermethylated *p*16 samples were also positive for Long DNA. Instability of BAT-26 was not found in any of colorectal cancer patients using stool DNA; whereas we could detect instability for BAT-26 using tumor tissue samples of the patients after surgery. The instability of BAT-26 was detected in two out of 25 patients (8%) using the tumor tissue. The results are shown in Figure 3.

Four samples were removed from statistical studies, one sample due to not producing the small fragment band (138 bp). It means the extraction protocol has not produced sufficient DNA for amplification. Three other samples were removed due to incomplete profile and lack of clinicopathological parameters. The data for the

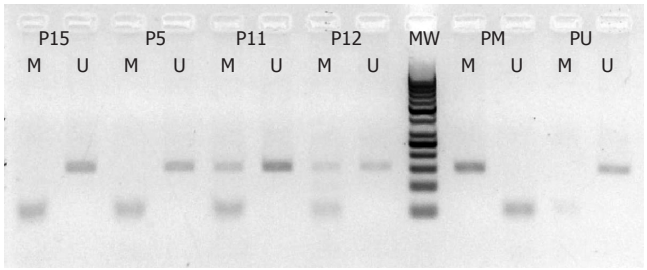

Figure 2 Methylation analysis of p16 among four patients. P15: patient 15; P5: patient 5; P11: patient 11; P12: patient 12; MW: Ladder 50 bp; PM: Methylated control; PU: Unmethylated control; M: Methylated PCR; U: unmethylated PCR. Methylated p16 detected in patients 11 and 12.

remaining 21 cases are presented in Table 1. The mean age of the patients was 59.2 years. Sixty six percent of patients were male and 34% were female. The most common tumor site was rectum (40%); other sites were cecum (20%), splenic flexture of colon (15%), ascending colon (20%) and hepatic flexture (5%), respectively. All the tumors were invasive adenocarcinoma. The tumor stages were reported as 15.8% B1, 57.8% B2, and 26.4% C2 in patients with available clinicopathological data. Tumor sizes were less than 3 cm in 35%, 3-6 cm in 30% and more than 6 cm in 35%. Although it seemed that there might be some relationship between the presence of Long DNA and *p*16 methylation in stool samples and clinicopathological parameters while reviewing Table 1, comprehensive statistical studies, using Chi-square and Fisher exact tests revealed that there was no statistically significant relationship.

DISCUSSION

Many investigators studied different genetic alternations in stool-DNA of CRC patients and determined their sensitivities and specificities as a diagnostic tool. Ahlquist *et al*[15] analyzed stool samples in a blinded fashion from 22 patients with colorectal cancer, 11 patients with adenomas at least 1 cm in size, and 28 patients with endoscopically healthy colons. The assay targeted point mutations at any of 15 sites on K-ras, *p*53, APC, and the microsatellite instability marker BAT-26 and Long DNA. The sensitivity was 91% for cancer and 82% for adenomas 1 cm or larger; the specificity was 93%. They could detect Long DNA in 14 out of 20 cancers (70%) and 6 out of 11 adenomas (54.5%). However, Long DNA and BAT-26 were not detected in any of normal patients. Syngal *et al*[17] used a fecal-based assay to detect 23 DNA markers, including 21 point mutations in K-ras, APC, and p53; the microsatellite instability marker BAT-26; and Long DNA. The sensitivity was 68% for invasive colorectal carcinoma, 40% for adenomas with high-grade dysplasia, and 20% for adenomas with low-grade dysplasia. Overall, the sensitivity of multitarget DNA stool assays ranged from 68% to 91% for colorectal cancer and from 40% to 82% for advanced adenomas. The specificity of the assays was approximately 95%[28,29]. Methylation of *p*16 in stool samples of CRC patients has been reported[10]. Although it was detected in smaller percentages of patients compared to our study, it

still remains unconclusive for its sensitivity and specificity in a stool-based DNA testing.

We could detect 64% of colon cancer cases using three genetic markers, 64% were positive for Long DNA, 20% for methylation of *p*16 and none for instable BAT-26. However, additional testing of more samples is necessary from Iranian subjects to determine the exact specificity and sensitivity of these markers. We could detect the instability for BAT-26 in 2 out of 25 patients (8%) using tumor tissues after surgery, whereas we detected no instability when DNAs extracted from stool were applied for microsatellite studies. This may suggest that the proportion of unstable DNA to total extracted DNA was very low. It seems further procedures are required to increase the unstable DNA following our extraction protocol. Oligo capture was recommended by previous researchers to solve this problem[15].

There are still many obstacles for stool-based DNA testing to become a worldwide screening method. Stool-based DNA testing is noninvasive, and it is more sensitive than fecal occult blood testing. Only a single stool sample is needed, and the patient and physician do not need to handle it as much. The test does not require diet or medication restrictions, it evaluates the whole colon and rectum, and it is now generally available. However, it is expensive, it is less sensitive than colonoscopy, and if the stool-based test is positive, colonoscopy is still necessary. The positive predictive value is low, and there is uncertainty regarding how to manage patients with a positive test and a healthy colonoscopic test. It is unclear whether screening for extracolonic malignancies will prove to be an advantage of stool-based DNA testing.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Alireza Baradaran and Mr. Omeed Moaven for their kind cooperation in this study.

REFERENCES

1 **Markowitz AJ**, Winawer SJ. Screening and surveillance for colorectal cancer. *Semin Oncol* 1999; **26**: 485-498
2 **Vogelstein B**, Kinzler KW. The multistep nature of cancer. *Trends Genet* 1993; **9**: 138-141
3 **Parsons R**, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res* 1995; **55**: 5548-5550
4 **Leach FS**, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen P, Aaltonen LA, Nyström-Lahti M. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993; **75**: 1215-1225
5 **Fishel R**, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993; **75**: 1027-1038
6 **Traverso G**, Shuber A, Olsson L, Levin B, Johnson C, Hamilton SR, Boynton K, Kinzler KW, Vogelstein B. Detection of proximal colorectal cancers through analysis of faecal DNA. *Lancet* 2002; **359**: 403-404
7 **de la Chapelle A**. Testing tumors for microsatellite instability. *Eur J Hum Genet* 1999; **7**: 407-408
8 **Shitoh K**, Konishi F, Masubuchi S, Senba S, Tsukamoto T, Kanazawa K. Important microsatellite markers in the investigation of replication errors (RER) in colorectal carcinomas. *Jpn J Clin Oncol* 1998; **28**: 538-541
9 **Lynch HT**, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet* 1999; **36**: 801-818
10 **Belshaw NJ**, Elliott GO, Williams EA, Bradburn DM, Mills SJ, Mathers JC, Johnson IT. Use of DNA from human stools to detect aberrant CpG island methylation of genes implicated in colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1495-1501
11 **Ahlquist DA**, Klatt KK, Harrington JJ, Cunningham JM, Han J, Shuber AP. Novel use of hypermethylated DNA markers in stool for detection of colorectal cancer: a feasibility study. *Gastroenterology* 2002; **122**: A40
12 **Abbaszadegan MR**, Raziee HR, Ghafarzadegan K, Shakeri MT, Afsharnezhad S, Ghavamnasiry MR. Aberrant p16 methylation, a possible epigenetic risk factor in familial esophageal squamous cell carcinoma. *Int J Gastrointest Cancer* 2005; **36**: 47-54
13 **Wan J**, Zhang ZQ, You WD, Sun HK, Zhang JP, Wang YH, Fu YH. Detection of K-ras gene mutation in fecal samples from elderly large intestinal cancer patients and its diagnostic significance. *World J Gastroenterol* 2004; **10**: 743-746
14 **Sidransky D**, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992; **256**: 102-105
15 **Ahlquist DA**, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, Thibodeau SN, Shuber AP. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000; **119**: 1219-1227
16 **Dong SM**, Traverso G, Johnson C, Geng L, Favis R, Boynton K, Hibi K, Goodman SN, D'Allessio M, Paty P, Hamilton SR, Sidransky D, Barany F, Levin B, Shuber A, Kinzler KW, Vogelstein B, Jen J. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J Natl Cancer Inst* 2001; **93**: 858-865
17 **Syngal S**, Chung D, Willett C. Stool DNA analysis for the detection and follow-up of colorectal cancer (CRC) and advanced adenomas (AA): sensitivity in a prospective series. *Am J Gastroentero* 2002; **97** suppl: S109
18 **Klaassen CH**, Jeunink MA, Prinsen CF, Ruers TJ, Tan AC, Strobbe LJ, Thunnissen FB. Quantification of human DNA in feces as a diagnostic test for the presence of colorectal cancer. *Clin Chem* 2003; **49**: 1185-1187
19 **Boynton KA**, Summerhayes IC, Ahlquist DA, Shuber AP. DNA integrity as a potential marker for stool-based detection of colorectal cancer. *Clin Chem* 2003; **49**: 1058-1065
20 **Bedi A**, Pasricha PJ, Akhtar AJ, Barber JP, Bedi GC, Giardiello FM, Zehnbauer BA, Hamilton SR, Jones RJ. Inhibition of apoptosis during development of colorectal cancer. *Cancer Res* 1995; **55**: 1811-1816
21 **Kerr JF**, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; **26**: 239-257
22 **Tagore K**, Ross M, Shuber AP. Stool-based DNA multi-target assay for the detection of colorectal cancer (CRC) and advanced adenomas. *Gastroenterology* 2002; **122**: A481
23 **Brand RE**, Shuber AP, Laken SJ. Stool-based DNA mutation testing for colorectal cancer detection: sensitivity and reproducibility. 66th Annual Scientific Meeting of the American College of Gastroenterology; 2001 Oct 22-24, Las Vegas, Nevada
24 **Béroud C**, Soussi T. p53 gene mutation: software and database. *Nucleic Acids Res* 1998; **26**: 200-204
25 **Devouassoux-Shisheboran M**, Mauduit C, Bouvier R, Berger F, Bouras M, Droz JP, Benahmed M. Expression of hMLH1 and hMSH2 and assessment of microsatellite instability in testicular and mediastinal germ cell tumours. *Mol Hum Reprod* 2001; **7**: 1099-1105
26 **Creste S**, Tulmann Neto A, Figueira A. Detection of Single Sequence Repeat Polymorphisms in Denaturing

Polyacrylamide Sequencing Gels by Silver Staining. *Plant Molecular Biology Reporter* 2001; **19**: 299–306

27 **Herman JG**, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 1996; **93**: 9821-9826

28 **Deenadayalu VP**, Rex DK. Fecal-based DNA assays: a new, noninvasive approach to colorectal cancer screening. *Cleve Clin J Med* 2004; **71**: 497-503

29 **Ahlquist DA**. Stool-based DNA tests for colorectal cancer: clinical potential and early results. *Rev Gastroenterol Disord* 2002; **2** Suppl 1: S20-S26

S- Editor Liu Y **L- Editor** Zhu LH **E- Editor** Che YB