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Analysis of promoter methylation in stool: A novel method for the detection of colorectal cancer

Konstanze Lenhard [1] • Guido T. Bommer [1] • Silke Asutay • ... Burkhard Göke • Rolf Lamerz • Frank T. Kolligs • Show all authors • Show footnotes

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Background & Aims: Detection of tumor-derived DNA alterations in stool is an intriguing new approach with high potential for the noninvasive detection of colorectal cancer (CRC). Because of heterogeneity of tumors, usually multiple markers distributed throughout the human genome need to be analyzed. This is labor intensive and does not allow for high through-put screening. Therefore, markers with high sensitivity and good specificity are needed. We explored the potential of a single epigenetic marker in comparison with fecal occult blood testing (FOBT) for the discrimination of s with CRCs and adenomas from those without. *Methods:* Methylation-specific era

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methylation status in a blinded fashion in stool samples from 26 patients with CRC, 13 with adenoma ≥1 cm, 9 with hyperplastic polyps, 9 with chronic inflammatory bowel disease, and 32 with endoscopically normal colon. *Results:* Ninety-seven percent of the stool samples contained amplifiable DNA. Forty-two percent of the samples from patients with CRC and 31% of the samples from patients with colorectal adenoma ≥1 cm were positive for HIC1 promoter methylation. No methylated HIC1 promoter DNA was detected in the fecal DNA from patients with endoscopically normal colon or hyperplastic polyps. *Conclusions:* The epigenetic marker HIC1 promoter methylation carries high potential for the remote detection of CRCs. We postulate that a panel of merely a few genetic and epigenetic markers will be required for the highly sensitive and specific detection of CRCs and adenomas in fecal samples from affected patients.

Abbreviations used in this paper:

CI (confidence interval), CRC (colorectal cancer), FOBT (fecal occult blood test), HIC1 (hypermethylated in cancer 1), MSP (methylation-specific PCR), PCR (polymerase chain reaction)

Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality.[1] About 40% of patients die within 5 years of being diagnosed. This mainly is attributable to late presentation with locally advanced or metastatic disease in one third of cases, precluding curative surgery.[2] The majority of CRCs follow the adenoma-carcinoma sequence, requiring a time period of usually more than 10 years.[3] Detection of early disease and precancerous adenomatous lesions leads to a decrease of CRC-related mortality.[4, 5, 6, 7] Therefore, there is a strong rationale for screening programs. Sigmoidoscopy, colonoscopy, and fecal occult blood test (FOBT) alone or in combination with endoscopy, are recommended for screening of the average-risk population.[8] However, FOBT, when applied regularly, can decrease CRC mortality by only 15%–33%.[5, 6, 7] Compliance with endoscopic screening is not satisfactory. Overall, in 1998, only 37% of eligible adults in the United States had been screened for CRC in the previous 3 years.[9]

Detection of tumor-derived genetic changes in stool is a promising new approach for CRC screening. Studies published so far focus on the detection of mutations in oncogenes,[10, 11, 12, 13, 14, 15, 16] and tumor-suppressor genes,[17, 18, 19] as well as changes in microsatellite markers.[20] Two recent studies required the analysis of up to 15 different genetic markers[21] or 144 aliquots from each stool[18] for the detection of 91% and 61% of cancers and 82% and 50% of adenomas ≥1 cm, respectively. This precludes their application for routine high throughput screening

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needed. So far, little attention has been paid to DNA hypermethylation as a potential stool marker for CRC.

Methylation of CpG islands of promoters leads to silencing of transcription of the affected gene.[22], [23] Methylation-specific polymerase chain reaction (PCR) (MSP)[24] has been used successfully to detect DNA methylation in primary tumors and in various body fluids.[25] The feasibility of amplification of methylated DNA from stool samples of patients with CRC has been reported.[26], [27] In one study evaluating several potentially methylated genes, SFRP2, SFRP5, and PGR were found to be methylated differentially in the stool of patients with CRC.[27] Of these markers, SFRP2 was found to be the most sensitive fecal methylation marker, detecting 77%–90% of CRCs. However, specificity of SFRP2 methylation was quite poor, at 77%. Therefore, identification of both sensitive and highly specific methylation markers is required before MSP can be evaluated as a stool-based screening procedure for CRC in prospective studies.

We studied the potential of hypermethylated in cancer 1 (HIC1) promoter methylation as a stool-based DNA marker. The promoter of HIC1, a candidate tumor-suppressor gene localized on 17p13.3 and the first gene cloned based on the finding of CpG island hypermethylation in cancer,[28], [29] frequently is methylated in CRC,[30] but not in normal or aging colonic tissue.[31] We show that HIC1 promoter methylation can be detected frequently and with high specificity in stool samples from patients with CRCs. The combination of HIC1 methylation analysis with FOBT allowed for the detection of two thirds of CRCs.

Materials and methods

Patients and stool samples

The investigation was approved by the ethical committee of the Medical Faculty of the University of Munich. Stool samples were collected preoperatively from patients with verified CRCs (Table 1), and before endoscopy from patients with adenomas ≥1 cm (Table 2), endoscopically normal colons, hyperplastic polyps, and chronic inflammatory bowel disease (Table 3). Most patients with CRCs had been referred with a known diagnosis, all persons with adenomas and normal colons were asymptomatic and underwent colonoscopies for surveillance reasons.

Table 1 Clinicopathologic Data and HIC1 Methylation Status of Patients

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No.	Sex	Age (*y*)	UICC stage	Localization	Symptoms [a]	HIC me
1	M	71	IV	Sigmoid colon/rectum	1	−
2	F	75	III	Sigmoid colon	1, 3	−
3	M	66	IV	Descending/sigmoid colon	0	−
4	M	69	II	Ascending colon	0	+
5	M	65	III	Transverse colon	0	+
6	M	56	IV	Rectum	1	+
7	M	63	IV	Ascending colon	2	−
8	F	72	III	Cecum	2	+
9	M	74	III	Sigmoid	0	−

UICC, International Union Against Cancer; M, male; F, female; NA, no amplification.

a 0, no symptoms; 1, change in bowel habits; 2, blood admixed with stool; 3, abdominal pain; 4, anemia.

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Table 2 Clinicopathologic Data and HIC1 Methylation Status of Patients With Adenomatous Polyps

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No.	Sex	Age (*y*)	Diagnosis	HIC1 methylation	FOBT
27	M	72	Tubular adenoma, ascending colon, 3 cm	+	−
28	F	63	Tubulovillous adenoma, ascending colon, hepatic flexure, sigmoid colon, 1–1.5 cm	−	−
29	M	63	Tubulovillous adenoma, ascending colon, 2 cm	−	−
30	M	76	Tubulovillous adenoma, ascending and transverse colon, 1, 3, 4 cm	+	+
31	M	83	Tubulovillous adenoma, transverse colon, 3 cm	−	−
32	F	65	Tubulovillous adenoma, sigmoid colon, 1.5 cm	−	−

M, male; F, female.

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Table 3 HIC1 Methylation Status of Healthy Controls and Patients With Nonneoplastic Disease

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