**Imaging, Diagnosis, Prognosis**

**Aberrant Methylation of APC, MGMT, RASSF2A, and Wif-1 Genes in Plasma as a Biomarker for Early Detection of Colorectal Cancer**

Bo Bin Lee,¹ Eun Ju Lee,¹ Eun Hyun Jung,¹ Ho-Kyung Chun,² Dong Kyoung Chang,³ Sang Yong Song,⁴ Joobae Park,¹,⁵ and Duk-Hwan Kim ¹,⁵

**Abstract**

**Purpose:** To identify epigenetic molecular makers in plasma for the early detection of colorectal cancer.

**Experimental Design:** We retrospectively analyzed the methylation status of 10 genes in fresh-frozen tissues and corresponding plasma samples from 243 patients with stage I and II sporadic colorectal cancer, 276 healthy individuals, and plasma from 64 colorectal adenoma patients using methylation-specific PCR. The methylation score (M score) was used to find molecular markers with high sensitivity and specificity.

**Results:** Of the 243 colorectal cancer tissues, methylation was detected in 18% for p14, 34% for p16, 27% for APC, 34% for DAPK, 32% for HLTF, 21% for hMLH1, 39% for MGMT, 24% for RARβ2, 58% for RASSF2A, and 74% for Wif-1. Receiver operator characteristic curve analysis in plasma from 243 patients with cancer and 276 healthy individuals showed that the M score of any single gene had a sensitivity of <40% after controlling for age, sex, and tumor location. The specificity of the M score was not different between multigene and single gene analyses, but the sensitivity of the M score was significantly increased by multigene analysis. For all patients, the M score in a model including APC, MGMT, RASSF2A, and Wif-1 genes had a sensitivity of 86.5% and a specificity of 92.1% when 1.6 was used as a cutoff. In this model, the M score had a positive predictive value of 90.6% and a negative predictive value of 88.8%.

**Conclusion:** The present study suggests that tumor-specific methylation of APC, MGMT, RASSF2A, and Wif-1 genes might be a valuable biomarker in plasma for the early detection of colorectal cancer. (Clin Cancer Res 2009;15(19):6185–91)

Colorectal cancer is one of the most common malignancies in the world. The prognosis for colorectal cancer has significantly improved because of advances in the treatment and detection of the disease. Despite significant improvements in screening methods for colorectal cancer, however, only 30% to 40% of patients are diagnosed at an early stage (1). Accordingly, an effective screening for premalignant adenomas and early stage cancers would have substantial clinical benefits, and would reduce the mortality of patients with colorectal cancer. Recent advances in molecular genetics have shown that colorectal cancer arises as a multistep process involving the progressive accumulation of genetic and epigenetic alterations, which could be surrogate markers for the monitoring of colorectal cancer.

Aberrant methylation of CpG islands at the promoter region of a gene is an epigenetic change that induces the transcriptional silencing of tumor suppressor genes (2). Hypermethylation of CpG islands in tumor suppressor genes has been reported for several human cancers, including colorectal cancers (3). Changes in the methylation pattern of CpG islands at the promoter region have obvious applications in both clinical diagnostics and therapeutics, as predictors of the response to therapy. It is known that patients with cancer have higher levels of circulating DNA in their sera or plasma than either healthy people or those with nonmalignant diseases. Analysis of the circulating cell-free DNA in the plasma or serum might be a promising noninvasive diagnostic tool for the early detection of cancer, as well as for monitoring the prognosis during follow-up. Recent reports have found that aberrant methylation of tumor suppressor genes in the plasma or serum of patients with colorectal cancer could be used as a surrogate marker for monitoring colorectal cancer (4–7).

Methylation-specific PCR (MSP) is most often used for detecting aberrant methylation of a CpG island in the plasma or serum from patients with colorectal cancer. Although the MSP is appropriate for detecting a few cell-free DNA molecules from...
Imaging, Diagnosis, Prognosis

Translational Relevance

Despite significant advances in screening methods for colorectal cancer, only 30% to 40% of patients are diagnosed at an early stage. Accordingly, more effective techniques for screening are needed to reduce the mortality for patients with colorectal cancer. The methylation statuses of 10 genes was retrospectively analyzed in fresh-frozen tissues and corresponding plasma samples from 243 patients with stage I and II sporadic colorectal cancer, 276 healthy individuals, and plasma from 64 patients with colorectal adenoma to identify tumor-specific methylation in plasma as a molecular marker for the early detection of colorectal cancer. The studies described in this article suggest that the analysis of methylation statuses of APC, MGMT, RASSF2A, and Wif-1 genes in plasma will allow us to detect colorectal cancer at the earliest stage.

Materials and Methods

Study population. A total of 243 patients who underwent curative surgical resection for colorectal cancer at the Department of Surgery in the Samsung Medical Center, Seoul, Korea between September 2004 and November 2007 were enrolled in this study. The surgically removed tumor tissues and corresponding plasma samples were collected after obtaining the appropriate Institutional Review Board permission and written informed consent from all of the patients. Plasma samples were also obtained from 276 healthy individuals and 64 patients that had visited the hospital for regular health checkups; these 64 patients had histologically confirmed colorectal adenoma including tubular, villous, or serrated adenoma. Normal colonic mucosas were obtained by colonoscopic biopsy from 64 of 276 healthy individuals. Pathologic stage was determined by tumor-node-metastasis staging.

DNA extraction from fresh tissue and plasma. The tumors were snap-frozen in liquid nitrogen and stored at -80°C until needed. Before DNA was extracted from the fresh-frozen tissues, the sections were placed on slides and stained with H&E to evaluate the admixture of tumorous and nontumorous tissues. Areas corresponding to the tumor were microdissected manually under a microscope. Tumors containing at least 75% neoplastic tissue were used in this study. The microdissected tissues were digested with proteinase K and resuspended in lysis buffer ATL (DNAeasy Tissue kit; Qiagen), and the genomic DNA was isolated according to the instructions of the manufacturer. Venous blood samples were collected in vacutainer plastic tubes coated with potassium EDTA (Becton Dickinson) for plasma preparation. The plasma was immediately separated from the cellular fluid by centrifugation at 2,500 rpm for 10 min and frozen at -80°C. The DNA was extracted from 400 μL of plasma using a QIAamp Blood Kit (Qiagen), dissolved in 50 μL of sterile distilled water and stored at -20°C.

MSP. The methylation status of the 10 genes was determined using MSP (Fig. 1), using two pairs of primers: one for the unmethylated promoter and the other for the methylated promoter, as described by Herman et al. (8). Briefly, 1 μg of genomic DNA from the fresh-frozen tissues or 40 μL of DNA extracted from the plasma was bisulfite modified, using a CpGenome DNA modification kit (Chemicon) according to the protocol described by the manufacturer, then dissolved in 20 μL of TE (10 mM Tris and 1 mM EDTA; pH 8.0). The PCR mixture consisted of 1× PCR buffer [50 mM/L KCl, 67 mM/L Tris (pH 8.8), 1.5 mM/L MgCl2], deoxynucleotide triphosphates (each 1.25 mM/L), primers (300 ng of each per reaction), 2.5 units of Taq polymerase, and bisulfite-modified DNA (50 ng of modified DNA from fresh-frozen tissue, or 1 μL of modified plasma DNA) as a template for MSP. Primer sequences and annealing temperatures for the MSP have been previously described by our group and others (9–12). DNA from the peripheral blood lymphocytes of healthy subjects was used as a negative control for the methylation-specific assays. Lymphocyte DNA from healthy volunteers was treated with SssI methyltransferase (New England BioLabs), and then with bisulfite. The resulting product was used as a positive control for methylated alleles. Bisulfite-modified DNA from normal lymphocytes was used as a positive control for unmethylated alleles, and the unconverted DNA from normal lymphocytes was used as a negative control for the methylated alleles. When a gene is amplified by a primer specific for methylated DNA in the presence of amplification of unmethylated DNA, the gene is considered methylated.

Statistical analysis. The Wilcoxon rank sum test (or t test) and Fisher’s exact test (or the χ2 test) were used for univariate analysis of the continuous and categorical variables, respectively. Kappa agreement statistics was done to assess the concordance of methylation between tumor tissue and plasma. We calculated the methylation score (M score) to find molecular markers with high sensitivity and specificity for each sample as previously described (12). For overall patients, the M score was defined...
normal colonic mucosa was available from 148 individuals analyzed the methylation status in normal colorectal mucosa colorectal cancer, it is important to differentiate age-related individuals and in cancer tissues. For the early detection of I and II colorectal cancer, (87%) were moderately differentiated, and 5 (2%) were poorly differentiated. The 276 healthy individuals ranged in age from 28 to 76 years with a mean age of 58 years. There was no statistical differен toxicity was between the healthy individuals and patients with stage I cancer (P = 0.28; Wilcoxon rank sum test). The healthy individuals consisted of 167 men and 109 women, and no gender difference was found between the healthy individuals and the patients with cancer (P = 0.44). Prevalence of methylation in normal tissues from cancer-free individuals and in cancer tissues. For the early detection of colorectal cancer, it is important to differentiate age-related methylation and tumor-specific methylation. To do this, we analyzed the methylation status in normal colorectal mucosa from healthy individuals. Among 276 healthy individuals, normal colonic mucosa was available from 148 individuals that had normal colonoscopy and who did not have any known diagnosis of malignant disease. Aberrant methylation was not detected in DAPK, RARβ2, and RASSF2A genes in normal colonic mucosa from 148 healthy individuals, but methylation of p14, p16, APC, HLT, hMLH1, MGMT, and Wif-1 genes was found in 2%, 3%, 3%, 2%, 3%, 4%, and 2%, respectively. The methylation status of the 10 genes studied was not associated with patient age, whether age was treated as a continuous or categorical variable (data not shown). This indicated that the methylation status of the 10 genes was tumor-specific, so all genes were considered in further analyses. The prevalence of genetic methylation in 243 cancer tissues ranged from 18% to 74%. Wif-1 methylation was the highest (74%) and p14 methylation was lowest (18%; Fig. 2A). The mean number of methylated genes was 3.8 in cancer tissues, and 5 (2%) showed concurrent methylation in seven or more genes. Analytic sensitivity and specificity of tumor-specific methylation in plasma. Before building a model to find candidate plasma markers with high sensitivity and specificity, we examined analytic sensitivity and specificity in tumor tissues and matched plasma samples collected from 243 patients with colorectal cancer to differentiate genes showing false-positive methylation in plasma. Analytic sensitivity in plasma was defined as the ratio of the number of individuals with a true-positive result to those with a true-positive or false-negative result in plasma, whereas analytic specificity referred to the proportion of individuals without methylation in tumor tissue having a negative result in plasma (13). Analytic sensitivity ranged from 27% to 60% (Fig. 2B), and analytic specificity was 100% in all genes except HLT. The analytic sensitivity and specificity of HLT methylation in the plasma were 41% and 93%, respectively.

Table 1. Clinicopathologic characteristics (N = 243)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>139 (57)</td>
</tr>
<tr>
<td>Women</td>
<td>104 (43)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>44 (18)</td>
</tr>
<tr>
<td>II</td>
<td>199 (82)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>204 (84)</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>22 (9)</td>
</tr>
<tr>
<td>Others</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>56 (23)</td>
</tr>
<tr>
<td>Distal</td>
<td>187 (77)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Moderately</td>
<td>211 (87)</td>
</tr>
<tr>
<td>Poorly</td>
<td>5 (2)</td>
</tr>
</tbody>
</table>

*Mean ± SD.

Results

Clinicopathologic characteristics. The baseline characteristics of 243 patients with colorectal cancers are shown in Table 1. The patients ranged in age from 22 to 86 years, with a mean age of 61 years. The patients consisted of 139 (57%) men and 104 (43%) women. Forty-four (18%) of the patients had stage I disease and 199 (82%) had stage II disease. The histologic subtypes were adenocarcinoma (84%), mucinous adenocarcinoma (9%), and other (7%). Among the 243 colorectal cancers, 56 (23%) were located in the proximal colon and 187 (77%) in the distal colon, which included the rectum, sigmoid, and descending colon up to the splenic flexure. Twenty-seven (11%) of the colorectal cancers were well-differentiated, 211 (87%) were moderately differentiated, and 5 (2%) were poorly differentiated. The 276 healthy individuals ranged in age from 28 to 76 years with a mean age of 58 years. There was no statistical difference between the ages of healthy individuals and patients with stage I and II colorectal cancer (P = 0.28; Wilcoxon rank sum test). The healthy individuals consisted of 167 men and 109 women, and no gender difference was found between the healthy individuals and the patients with cancer (P = 0.44).
Out of 243 patients, 185 (76%) showed identical methylation changes in the HLTF gene in both the tumor and matched plasma DNA, but 12 out of 165 patients who did not show HLTF methylation in tumor tissue showed HLTF methylation in matched plasma (Table 2). The methylation status of the HLTF gene between tumor and plasma showed a statistically significant discordance (P<0.0001; Kappa statistics). Accordingly, the HLTF gene was excluded in further analyses due to the false-positive discordance between tumor and plasma DNA, but 12 out of 165 patients who did not show methylation of any of these genes in tumor tissue showed methylation at two or more loci. Methylation at two or more genes was found in 35% (85 of 243) of tumor tissues and in 20% (49 of 243) of plasma samples. Concurrent methylation in three or more genes was found in 14% (34) of tissues and in 7% (17) of plasmas. Methylation of four genes was found in two tumor tissues. Finally, we evaluated the M score as a predictive biomarker for colorectal cancer in plasma and analyzed the positive predictive value and negative predictive value of colorectal cancer detection using the model. At a cutoff value of 1.6, the positive predictive value was 90.6% and the negative predictive value was 88.8% using the model with four genes (Table 3A).

**Validation of four markers in the plasma of patients with colorectal adenoma.** Methylation of the four genes was reassessed in plasma from 64 patients with colorectal adenoma for validation of the predictive markers. Methylation of the APC, MGMT, RASSF2A, and Wif-1 genes was found in 18%, 14%, 37%, and 32% of plasma from 64 patients with colorectal adenoma, respectively. The overall M score in a model with APC, MGMT, RASSF2A, and Wif-1 genes was found to show a sensitivity of 86% of plasma showed methylation at one or more loci. Methylation at two or more genes was found in 35% (85 of 243) of tumor tissues and in 20% (49 of 243) of plasma samples. Concurrent methylation in three or more genes was found in 14% (34) of tissues and in 7% (17) of plasmas. Methylation of four genes was found in two tumor tissues. Finally, we evaluated the M score as a predictive biomarker for colorectal cancer in plasma and analyzed the positive predictive value and negative predictive value of colorectal cancer detection using the model. At a cutoff value of 1.6, the positive predictive value was 90.6% and the negative predictive value was 88.8% using the model with four genes (Table 3A).

**Target genes**

<table>
<thead>
<tr>
<th>Target genes</th>
<th>AUC</th>
<th>Cutoff (M score)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC + MGMT + RASSF2A + Wif-1</td>
<td>0.927</td>
<td>1.6</td>
<td>86.5 (81.7-90.8)</td>
<td>92.1 (88.2-95.0)</td>
<td>90.6 (86.0-94.1)</td>
<td>88.8 (84.4-92.2)</td>
</tr>
<tr>
<td>Wif-1</td>
<td>0.641</td>
<td>&gt;0</td>
<td>36.7 (30.6-43.7)</td>
<td>90.6 (86.4-93.2)</td>
<td>77.5 (68.6-84.7)</td>
<td>62.1 (56.9-66.1)</td>
</tr>
<tr>
<td>(B) Colorectal adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC + MGMT + RASSF2A + Wif-1</td>
<td>0.864</td>
<td>1.8</td>
<td>74.6 (62.6-85.0)</td>
<td>91.3 (87.5-94.6)</td>
<td>71.6 (57.3-80.1)</td>
<td>93.9 (90.2-96.5)</td>
</tr>
</tbody>
</table>

**Table 3. Sensitivity and specificity of M score in colorectal adenomas (N = 64) and cancers (N = 243)**

| Abbreviations: PPV, positive predictive value; NPV, negative predictive value. |
sensitivity of 74.6% and a specificity of 91.3% (AUC, 0.864; Table 3B; Fig. 3D), when 1.8 was used as the cutoff value, after adjusting for age and sex. The positive predictive value was 71.6% (95% CI, 57.3-80.1) and the negative predictive value was 93.9% (95% CI, 90.2-96.5) using the model at a cutoff value of 1.8.

Discussion

The critical factor for the early detection of colorectal cancer is to differentiate age-or environment-related methylation in patients without cancer, from tumor-specific methylation in patients with cancer, because aberrant methylation of some tumor suppressor genes occurs after the onset of neoplastic evolution, and others become hypermethylated in normal epithelial cells from environmental factors such as exposure to folate and aging. Thus, such factors could be the source of false-positives in the study of tumor-specific methylation in colorectal cancer. In the present study, we did not observe statistically significant age-related methylation for the 10 genes studied. The lack of association of p14, p16, DAPK, and hMLH1 methylation with age in this study is contradictory to previous studies showing age-related methylation in normal colonic mucosa (14–17).

This may be due to the very small number (n = 148) of normal tissues, or differences in age distributions, tumor distributions, or CpG sites analyzed. Nakagawa et al. (15) reported that the prevalence of hMLH1 methylation was significantly higher in patients ≥80 years of age than in those <60 years of age (83% versus 44%, respectively), but in this study, the mean age was 61 years and only two patients were >80 years of age. They also found that full methylation of hMLH1 occurred at a high frequency in patients with MSI+ tumors, which are mainly found.
on the right side of the colon. In this study, 77% of colorectal cancers occurred in the left colon. Analysis of different CpG sites may also be the source of the conflicting results. Issa et al. (14) found substantial levels of p16 methylation in the exon 1 region, but not in the upstream region in nonneoplastic mucosa with ulcerative colitis and high-grade dysplasia. This suggests that age-related methylation might be limited to the edges of the CpG islands with protection against the spreading of methylation to CpG island from methylation center. Other possible explanations for the discrepant results between aging and methylation are (a) different rates and sites of aberrant methylation between populations because of genotypic variation (17), (b) application of different criteria (qualitative versus quantitative) in determining hypermethylation in normal colonic tissue, (c) differing exposure to environmental factors, such as diet or microorganisms, or (d) different methods of assessment. In this study, the methylation statuses of all genes were analyzed by qualitative assay. Age-related methylation needs to be reassessed in quantitative values of methylation.

The APC, MGMT, RASSF2A, and Wif-1 genes are known to be frequently methylated in colorectal adenoma, which is generally considered to be a precursor lesion of colorectal cancer. The defective APC protein is not able to bind and degrade β-catenin, and the resultant increased β-catenin levels lead to the activation of growth-promoting genes such as c-myc via the action of increased β-catenin/Tcf-4 transcription complex. Although mutation is the most common cause of APC inactivation, APC methylation was also detected in normal colon mucosa and colorectal adenoma (18–21). Aberrant methylation of the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) impairs the ability of the MGMT protein to remove alkyl groups from the O6-position of guanine and thereby increases the risk of cancer. MGMT methylation was reported at a high frequency in colorectal adenoma (20, 22). In addition, Shen et al. (23) found MGMT methylation at adjacent and distal mucosas of sporadic colorectal cancer, and reported that MGMT methylation could play a role as one of the mediators of field cancerization in colon mucosa.

The RAS association domain family 2 (RASSF2) is a novel tumor suppressor that regulates the Ras signaling pathway and is involved in actin cytoskeleton organization and apoptosis (24). The RASSF2 gene contains 11 exons that span ~43 kb at chromosome 20p13, and three isoforms have been identified: RASSF2A, RASSF2B, and RASSF2C. RASSF2B and RASSF2C do not have CpG islands, but RASSF2A has a CpG island between -105 bp and +1075 bp relative to the transcription start site (NM_014737; ref. 11). RASSF2A methylation was found in 16 of 16 adenomas (25), and in 7 of 8 colon adenomas (11). Wnt-inhibitory factor 1 (WIF-1) is a secreted extracellular molecule that blocks the Wnt signaling pathway primarily by directly binding to Wnt proteins and preventing their access to cell surface receptors. Belshaw et al. (26) reported significantly higher median levels of WIF-1 methylation in mucosal samples from patients with adenomatous polyps than those from patients with cancer (19.18% versus 1.75%, respectively). Taniguchi et al. (27) also observed the down-regulation of WIF-1 expression in 32 of 44 colorectal adenoma tissues and found WIF-1 methylation in all tumor samples with WIF-1 down-regulation. Based on these observations, aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes is considered to be related to tumor initiation, but not to tumor progression.

Several studies suggest that the failure to detect methylation in plasma or serum might result from the low amount of DNA present in plasma or serum, with a consequent diminished sensitivity of the assay. In addition to low plasma DNA levels, the sensitivity of the methylation assay may be affected by the fragmentation of cell-free circulating DNA. Small pieces of fragmented DNA, ~100 bp in size, were found with the cell-free DNA from pancreas, colon, ovarian, and prostate cancer patients, but not in normal cell-free DNA (28, 29). The MSP in this study produced PCR products of 200 to 400 bp for the methylated and unmethylated segments, respectively. Accordingly, fragmentation of the cell-free DNA might have reduced the sensitivity in this study. DNA degradation by bisulfate modification may be another source of low sensitivity. DNA is usually incubated in a high concentration of bisulfite salt at high temperature and low pH for the complete conversion of unmethylated cytosines. These harsh conditions may lead to a high degree of DNA fragmentation during conversion, and DNA loss during purification, which subsequently affects the detection of methylation in plasma.

In this study, false-positive HLFTE methylation was found in 14 of 162 matched plasma samples. The mispriming of methylation-specific primers may be a technical source of false-positives, and might pose a greater problem when higher numbers of PCR cycles with a small amount of methylated DNA, or two-stage nested primers, are used. Recently, Shaw et al. (30) reported that MSP using a low concentration of methylated DNA and a high number of PCR cycles resulted in the mispriming of methylation-specific primers in ~10% of cases, with subsequent false-positives. Although the number of PCR cycles used for MSP in this study was not high, the possibility that the cycle number might play a role in producing false-positives in a minute amount of cell-free DNA cannot be ruled out. Accordingly, further evaluation for mispriming is needed using assays such as methylation enrichment pyrosequencing or bisulfite DNA sequencing (31).

This study was limited by the small number of normal tissues from healthy individuals and plasma from patients with colorectal adenoma. Although HLFTE methylation was not included in the final model because it gave false-positives in plasma, HLFTE methylation is known to be associated with early stages of colorectal cancer (10). Therefore, additional work is needed to evaluate the role of HLFTE methylation as a biomarker in plasma. Additionally, the present results need to be validated using quantitative real-time methods such as MethyLight for methylation analysis. In conclusion, the present study suggests that methylation of APC, MGMT, RASSF2A, and Wif-1 genes may be a valuable molecular marker in plasma for the early detection of colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Hee-Jin Jo and Hyang-Suk Jung for data collection and management, and Suh-Kyu Park for sample collection.
Aberrant Methylation in Plasma of Colorectal Cancer

References


www.aacrjournals.org 6191 Clin Cancer Res 2009;15(19) October 1, 2009

Downloaded from clincancerres.aacrjournals.org on April 20, 2017. © 2009 American Association for Cancer Research.
Colorectal Cancer
Genes in Plasma as a Biomarker for Early Detection of Colorectal Cancer

Bo Bin Lee, Eun Ju Lee, Eun Hyun Jung, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-0111

Cited articles
This article cites 26 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/19/6185.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/15/19/6185.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.