Advances in epigenetic biomarker research in colorectal cancer

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Abstract

Colorectal cancer (CRC) causes approximately 600000 deaths annually and is the third leading cause of cancer mortality worldwide. Despite significant advancements in treatment options, CRC patient survival is still poor owing to a lack of effective tools for early diagnosis and a limited capacity for optimal therapeutic decision making. Since there exists a need to find new biomarkers to improve diagnosis of CRC, the research on epigenetic biomarkers for molecular diagnostics encourages the translation of this field from the bench to clinical practice. Epigenetic alterations are thought to hold great promise as tumor biomarkers. In this review, we will primarily focus on recent advances in the study of epigenetic biomarkers for colorectal cancer and discuss epigenetic biomarkers, including DNA methylation, microRNA expression and histone modification, in cancer tissue, stool, plasma, serum, cell lines and xenografts. These studies have improved the chances that epigenetic biomarkers will find a place in the clinical practices of screening, early diagnosis, prognosis, therapy choice and recurrence surveillance for CRC patients. However, these studies have typically been small in size, and evaluation at a larger scale of well-controlled randomized clinical trials is the next step that is necessary to increase the quality of epigenetic biomarkers and ensure their widespread clinical use.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most widespread cancers in the world, accounting for over 1 million new diagnoses each year and over half of a million deaths[1,2]. Among all CRC cases, approximately 95% are adenocarcinoma. Less common types include lymphoma and...
squamous cell carcinoma. CRC patients are characterized by a lack of clinical manifestations until the late stages of cancer, leading to poor prognosis and a high mortality rate. Adenomas are the primary precursor lesion of colon cancer and often develop into colorectal carcinomas, but the process is slow, localized and asymptomatic, which is the primary factor contributing to late diagnosis. At the time of primary diagnosis, 80% of the patients are offered resection and potentially are cured by that. However, 40%-45% of these patients experience a later recurrence and was therefore not cured by resection.[8] Therefore, the identification of useful screening tools for CRC is a high priority. Currently, the detection of trace blood in stool using the fecal occult blood test (FOBT) and subsequent internal imaging of the colon by flexible sigmoidoscopy or colonoscopy represent the gold standard for CRC detection. Although widespread, these techniques suffer from several shortcomings. For example, the FOBT lacks specificity, often needs to be repeated, and is easily interfered by the contents of the bowel. Colonoscopy, on the other hand, is invasive, expensive, and has a high risk of complications, which often leads to poor patient compliance. As a result, the identification of biomarkers that are simple, noninvasive, cost-efficient and reasonably sensitive/specific is urgently needed. Over the past decade, the rapidly expanding field of epigenetics has shown great promise for the detection of CRC at earlier stages and the identification of resectable CRC lesions prior to metastasis, thereby providing patients with the highest chance of survival.

Epigenetic alterations are widely known to play an important role in tumorigenesis and are prevalent in CRC. Epigenetic changes in colorectal tumor tissues and CRC cell lines have been widely reported, and a substantial amount of information has been accumulated.[4,5]. These alterations include aberrant DNA methylation of promoter CpG islands, changes in microRNA (miRNA) expression profiles and various histone modifications. The exploration of epigenetic biomarkers in cancer for clinical use is a relatively new but rapidly developing field. Applications include screening, diagnosis, classification, surveillance and targeted therapies. If epigenetic factors are to be effective biomarkers in clinical practice, they must be detectable by noninvasive means and outperform the current gold standard. It should be emphasized that sample collection methods are a crucial factor. For example, miRNAs extracted from tissues should be evaluated separately from miRNAs isolated from serum and stool because their clinical potential is quite different. Serum and stool biomarkers are ideal for patient screening, but biomarkers from postoperative tissue may be more effective for prognosis, including the prediction of mean survival, resectability of the primary tumor and the administration of targeted therapies.

DNA METHYLATION

**In vitro and preclinical studies**

Generally speaking, in vitro studies are the first step in the discovery of new epigenetic biomarkers. Researchers often compare profiles of CRC cell lines with normal colorectal cells and then compile a list of candidate biomarkers for further study. Similarly, identifying valuable prediction biomarkers in CRC patients often begins with preclinical studies using xenograft tumors, which allow one to observe tumor growth and how it responds to different therapies. Nevertheless, a significant shortcoming of this approach is that the tumor and vasculature are of mouse origin rather than human. Studying tumors in a different growth environment makes it difficult to explain the results accurately and translate them into clinical application. However, in vitro and preclinical studies are still the foundations on which most clinical studies are built.

In the human genome, DNA methylation typically occurs on the cytosine of the sequence 5’-CpG-3’, which is found in promoter regions of approximately 70% of genes[6]. In this biochemical process, a methyl group (-CH$_3$) is added to cytosine nucleotides by a DNA methyltransferase (DNMT). A large body of evidence has demonstrated that promoter hypermethylation is associated with gene silencing, while hypomethylation results in gene-product upregulation. In this section, in vitro studies will be discussed first, followed by clinical studies that utilize blood or stool to identify DNA methylation in CRC patients.

Khamas et al.[7] conducted a genome-wide screen of 15 CRC cell lines and 23 paired tumor and normal samples from CRC patients to identify a set of methylation-silenced genes in CRC. Gene expression studies were then used to confirm whether the methylated genes were really regulated by their methylation status. The results of this study revealed that 139 genes showed greater than 1.5-fold up-regulation in at least one 5-aza-2-deoxycytidine-treated cell line and no less than a 1.2-fold change in other treated CRC cell lines. Among them, eight genes, DCAF4L1, DDX43, ICAM1, MIR615, PTPRO, ZFP42 and the cancer-germline antigen families, had previously been reported to be up-regulated by demethylation in CRC and were thus excluded from the analysis. Twenty genes with poor annotation, 20 genes located on the X chromosome, 16 genes with duplicated probes, two genes with no CpG islands, 8 genes with unknown function, 23 without a relevant function in tumorigenesis and 22 genes with potential oncogenic activity were also excluded, leaving 20 candidates (CAMK2B, CHAC1, THSD1, CSTA, COL1A1, GADD45B, DMR1B1, COL6A1, GAS5, GPRC5A, GPSM1, KLHL35, LTB2, NAAT1, RBP4, SEMA7A, SYCP3, TBRG1, TNSF39 and TXNIP) that had not been previously reported to be affected by epigenetic mechanisms in CRC. Therefore, from the 54613 genes analyzed, a much smaller set of genes was isolated as potential biomarkers for CRC.

In this study, two genes, THSD1 and GADD45B, were selected for further analysis. THSD1 methylation appeared to have the potential for diagnostic, prognostic or therapeutic use. Thrombospondin type-1 domain-containing protein 1 (THSD1) is located in a region that is strongly associated with the progression of colorectal
Table 1 Biomarkers of DNA methylation in blood of colorectal cancer patients, n (%)

| Markers       | Sensitivity | Specificity | Ref. |
|---------------|-------------|-------------|------|
| APC           | 3 (6)       | 0 (100)     | [20] |
| hMLH1         | 21 (43)     | 1 (98)      | [20] |
| HLTF          | 17 (34)     | 1 (98)      | [20] |
| HLTF          | 22 (21)     | 0 (100)     | [21] |
| ALX4          | 25 (83)     | 9 (70)      | [22] |
| TMEMF2        | 87 (65)     | 56 (69)     | [23] |
| NFGR          | 68 (21)     | 29 (84)     | [23] |
| 9-Sep         | 92 (69)     | 25 (86)     | [23] |
| 9-Sep         | 90 (72)     | 19 (90)     | [24] |
| 9-Sep         | 24 (72)     | 3 (90)      | [25] |
| 9-Sep         | 18 (60)     | 5 (89)      | [26] |
| 9-Sep         | 45 (90)     | 11 (89)     | [27] |
| NEUROG1       | 14 (52)     | 4 (91)      | [28] |
| 45 (64)       | 113 (67)    | 4 (94)      | [29] |
| SFRP2         | 12 (71)     | 0 (100)     | [30] |
| RUNX3         | 11 (65)     | 0 (100)     | [31] |
| TPEF/HP1      | 13 (13)     | 0 (100)     | [21] |

APC: Adenomatosis polyposis coli; hMLH1: Homo mutl, homolog 1; HLTF: Helical-like transcription factor; ALX4: ALX homeobox 4; TMEMF2: Transmembrane protein with EGF-like and two follistatin-like domains 2; NFGR: Nerve growth factor receptor; NEUROG1: Neurogenin 1; SFRP2: Secreted frizzled-related protein 2; CDKN2A: Cyclin-dependent kinase inhibitor 2A; RUNX3: Runt-related transcription factor 3; HP1: Hyperpigmementation, progressive; 1; TPEF: Transmembrane protein endothelial factor.

Adenoma to carcinoma and encodes a transmembrane molecule containing a thrombospondin type 1 repeat that might be involved in cell adhesion and angiogenesis. High THSD1 expression positively correlated with better distant metastasis survival in breast cancer. Therefore, its loss may be associated with metastatic tumor spread. Additionally, as one of the consensus radiation-response genes in primary human fibroblasts, THSD1 may play a role in radiation response in cancer stem cell. Moreover, a recent study has shown that THSD1 was expressed in CRC classified as D in Duke’s classification scheme for CRC and thus may be relevant to tumor progression. GADD45B functions as a tumor suppressor in many cancers, can inhibit cell proliferation at different stages and induce cell apoptosis, but its function in CRC is unknown.

In addition, Schuebel et al[1] described another genomewide, expression array-based approach for the identification of genes silenced by promoter hypermethylation in human CRC, and approximately 500 hypermethylated genes were identified. They analyzed the top-tier hypermethylome of each cell line (HCT116 and SW480) and then made a comparison of hypermethylation frequencies in cell lines, normal human tissues and human tumor samples. They found that BOLL, DKK3, C4BYR, EFFEMP1, GNB4, GSTM3, FOXL2, HOXD1, ITPH3, NEF3, NEURL, PPP1R1A, RAB32, TLR2, SALL4, TP53AP1 and ZFP42 were hypermethylated and underexpressed in both CRC cell lines and in colon cancers, but not in normal tissues. These genes possess great promise as useful biomarkers for molecular diagnostics, early detection and CRC therapy. Recently, Yi et al[18] also reported that hypermethylation of promoter DNA in the FBN2 and TCEG1L genes might provide excellent biomarkers for early detection of CRC. Both genes showed a high frequency of colon cancer cell lines, adenomas and carcinomas.

In addition, methylation of the hMLH1, p16INK4A, APC, MGMT, sFRP1, GATA-5, sFRP4, sFRP5, GATA-4, B4GALT1, TFF1, SOX17 and TMEM25 genes has been described in several studies[11-17]. These genes are hypermethylated and downregulated in CRC and thus may serve as excellent candidate biomarkers. In addition, insulin-like growth factor-binding protein 3 and Enah/Vasp-like have been validated as prognostic biomarkers for CRC and found to be useful in stratifying high-risk CRC patients who would benefit from adjuvant chemotherapy[19]. PPP2R2B was also found to be hypermethylated in CRC and was connected to therapeutic resistance[19]. These genes could serve as candidate biomarkers for prognosis. However, clinical studies are required to confirm these results, and it remains to be seen if these alterations can be detected in blood or stool.

Biomarkers of DNA methylation in blood

Biomarkers detected in patient blood samples would provide the most practical screening tool for CRC because of the ease with which these samples can be acquired. It has been well documented that genetic material can shed from tumor cells, and aberrant DNA methylation can be specifically quantified in blood despite the large amounts of normal DNA in circulation. Bisulfite treatment and methylation specific polymerase chain reaction (PCR) are the two most commonly used techniques. A blood biomarker with a high sensitivity and specificity for CRC can not only be used to segregate high-risk patients for further clinical tests but also be an excellent tool for monitoring CRC recurrence in patients who have undergone tumor resection (Table 1)[20-30].

The SEPT9 gene, encoding a guanosine triphosphate enzyme involved in cytokinesis and cell cycle control, has been reported to be associated with several cancers. The v2 region of the Septin 9 (SEPT9) promoter has been shown to be methylated in CRC tissue compared with normal colonic mucosa. Using highly sensitive real-time PCR assays, methylated SEPT9 was first detected in the plasma of CRC patients with an overall sensitivity of 72% and a specificity of 90%[20]. Significant validation has been performed for this methylation biomarker, and Warren et al[23] have confirmed a sensitivity of up to 90% and a specificity of up to 88% for SEPT9. Based on these results, SEPT9 methylation appears to have the highest probability of correctly distinguishing between the blood of cancerous and non-cancerous persons for CRC detection. Currently, two CRC detection kits using plasma SEPT9 methylation analysis are marketed for clinical application. Combining SEPT9 with other methylation biomarkers would improve the detection rate[33]. Further studies are needed to compare these panels and kits and discover their advantages and limitations.
most effective ones should be chosen for clinical use.

Other genes, such as APC, hMLH1, ALX4, TMEFF2, NGFR, NEUROG1, SFRP2, CDKN2A/P16, TPEF/HPP1 and RUNX3, have also emerged as serum methylation markers for CRC, with sensitivities ranging from 6% to 83% and specificities ranging from 69% to 100% (Table 1). Among them, ALX4, TMEFF2 and NEUROG1 showed better performance relative to the others, and the use of these markers in combination can improve detection accuracy.[25,31]

In addition to the successful identification of DNA methylation-based blood biomarkers, it is important to find genes that have prognostic value in the blood of patients with CRC. Methylation of helicase-like transcription factor (HLTF) has shown a strong correlation with tumor size, metastatic disease and tumor stage and is also associated with an increased risk of disease recurrence in CRC patients. Therefore, the methylation of this gene can serve as an independent biomarker for the identification of CRC with an increased risk of death. These results indicate that detection of HLTF methylation in the blood of CRC patients has the potential as a pretherapeutic predictor of patient outcome.[32] Deafness, autosomal dominant 5 (DFNA5) is another candidate biomarker for the noninvasive screening and monitoring of CRC. DFNA5 methylation has been observed in DNA from the peripheral blood (PB) of CRC patients at a high frequency (48% or 12/25) relative to healthy controls (only 12% or 3/25). Moreover, the methylation of DFNA5 in PB samples from CRC patients was significantly correlated with lymph node metastasis and distant metastasis (P = 0.027)[33], which suggests that DFNA5 could potentially be an independent prognostic serum biomarker for CRC patients. It is clear, however, that further validation in large-scale prospective trials is necessary before these biomarkers are ready for use in the clinic.[30]

DNA methylation biomarkers in stool

As a more attractive alternative to tissue sampling, biomarkers from feces could be of great clinical value because sampling is noninvasive and has much higher specificity. These properties offer a distinct advantage over endoscopy- and FOBT-based screening strategies for the detection of both CRC and critical precursor lesions. Over the past decade, numerous studies have engaged in the development of methylation-based detection assays for stool biomarkers of CRC (Table 2)[13-66], though the fecal biomarker detection can only be performed in only less than 50% of patients due to very limited compliance. The best-studied and top-performing methylation biomarkers are secreted frizzled-related protein 2 (SFRP2) and vimentin.

SFRP2 was the first reported DNA methylation marker in stool, has shown a sensitivity of 77%-90% and specificity of 77%-100% and has since been studied extensively. SFRP2 methylation has been shown to be the most sensitive biomarker for CRC, with detection rates ranging from 77% to 94% (Table 2). When SFRP2 methylation was used in a multigene, fecal methylation panel, detection of CRC and a small number of advanced adenomas reached a sensitivity and specificity of 96%[30]. A follow-up study found that SFRP2 methylation was detectable in the stool of almost half of all patients with hyperplastic polyps or colorectal adenomas[53], further supporting its use in the detection of premalignant lesions. Fecal SFRP2 methylation also drops dramatically after surgery [postoperative: 8.7% (6/69) vs preoperative: 87% (60/69)][52], suggesting its possible utility as a biomarker for recurrence.

The vimentin gene, which encodes an intermediate filament protein involved in cell attachment, migration, and signaling, was identified in the stool of 83% of CRC patients with a specificity of 90%[52]. Since then, many studies have been devoted to vimentin methylation. Follow-up studies have obtained similar results and thus have reinforced the utility of vimentin as a standalone biomarker.[27,29,55,59,61]. This has led to the commercialization of a single-gene stool kit for CRC detection based on vimentin methylation. More recently, vimentin methylation has been used in combination with other methylation markers to further increase detection rates, and vimentin has also been found in urine, suggesting an alternative method of detection.[34,48,67]. Vimentin has a low detection rate in serum, however, and is thus most likely not suitable for use as a serum biomarker for CRC. Recently, Ahlquist et al.[36] reported that a panel of methylation markers from stool that includes vimentin has shown a significantly higher sensitivity for CRC, primarily because of higher detection rates in stage 1-III CRC (91% vs 50%).

In addition to SFRP2 and vimentin, several other methylation biomarkers have been identified; these include GATA4, HIC1, ITG4, NDRG4, OSMK, TFF1, E3SRI, SLIT2, PHACTR3, VPG20, JOST2 and MGMT. These genes have sensitivities for CRC ranging from 38% to 89% and specificities ranging from 79% to 100%. The combination of different methylation biomarkers (combinations of 2 to 7 genes including APC, ATM, CDKN2A, GSTP1, HLF1, hMLH1, HPP1, MGMT, RASSF1, SFRP2, MAL, P16, or vimentin) increased sensitivity from 55% to 100% and increased specificity from 87% to 100% (Table 2). However, more clinical studies are required to confirm these results.

MIRNA BIOMARKERS

miRNA and cancer

In recent years, miRNA has been a relatively new but rapidly expanding field, as is evidenced by the increasing number of assays in development. miRNAs are small non-coding RNA molecules that function in transcriptional and post-transcriptional regulation of gene expression and control various cellular functions. Currently, more than 1000 miRNAs have been discovered in the human genome, and their activities and regulatory mechanisms are being intensively investigated. miRNAs typically function via base pairing with complementary sequences in mRNA molecules, resulting in gene silencing via translational repression or target degradation. It
Table 2  Biomarkers of DNA methylation in the stool of colorectal cancer patients

| Markers                              | Sample | Sensitivity | Specificity | Ref. |
|--------------------------------------|--------|-------------|-------------|------|
| AGTR1/WNT2/SLIT2/ITGA4/SFRP2/p16     | 214 CRC 25 IB 39 controls | 20%-78% | 80%-100% | [33] |
| Vimentin/EYA4/BMP3/NDRG4/ESR1        | 19 CRC 38 controls | 67%-100% | 89% | [34] |
| Vimentin/SLIT2/CRC                   | 60 CRC 32 IB associated CRC | 25% | 26 CRC | [35] |
| PHACTR3                              | 64 CRC 71 A 34 controls | 66% | 100% | [36] |
| SFRP2/HPP1/MGMT                      | 52 CRC 10 advanced A | 96% | 96% | [37] |
| CNRIP1/FBN/INA/MAL/SNCA/SFG20        | 78 CRC 61 A 48 controls | 65%-94% | 95%-100% | [38] |
| SPC20                                | 9 CRC | 67% | Unknown | [39] |
| MAL/CDKN2A/MGMT                      | 69 CRC 24 A 19 HP | 55.1%-78.3% | 96.2%-100% | [40] |
| 3OST2/ITGA4/SFRP2/p16                | 21 CRC 30 CRC 25 A 21 controls | 72.7% | 90% | [41] |
| RARB2/p16/INK4a/MGMT/ATM/CDKN2A/HIC1 | 26 CRC 20 A 16 IB | 62% | 100% | [42] |
| RASSF1/SFRP2                         | 84 CRC 27 advanced A 29 non-advanced A 12 HP 4 IC 2 UC | 75% | 44% | [43] |
| OSMR                                 | 69 CRC 81 controls | 38% | 95% | [44] |
| Vimentin                             | 22 CRC 20 advanced A 38 controls | 41% | 45% | [45] |
| MGMT/hMLH1/Vimentin                  | 60 CRC 22 advanced A 30 non-advanced A 37 controls | 75% | 46% | [46] |
| ITGA4                                | 13 A 75 CRC 75 controls | 69% | 53%-61% | [47] |
| NDRG4                                | 75 CRC 75 controls | 51%-71% | 84%-93% | [48] |
| GATA4                                | 75 CRC 75 controls | 51%-71% | 84%-93% | [49] |
| TFPI2                                | 26 CRC 45 controls | 76%-89% | 79%-93% | [50] |
| SFRP2                                | 69 CRC 34 A 26 HP 30 controls | 87% | 93% | [51] |

A: Adenoma; HP: Hyperplastic polyp; HR: High risk; LR: Low risk; IBD: Inflammatory bowel disease; IC: Ischemic colitis; UC: Ulcerative colitis; CRC: Colorectal cancer; AGTR1: Angiotensin II receptor; type I; WNT2: Wingless-type MMTV integration site family member 2; SLIT2: Slit homolog 2; VIM: Vimentin; EYA4: Eyes absent homolog 4; BMP3: Bone morphogenetic protein 3; NDRG4: NDRG family member 4; ESR1: Estrogen receptor 1; PHACTR3: Phosphatase and actin regulator 3; TFPI2: Tissue factor pathway inhibitor 2; CNRIP1: Cannabinoid receptor interacting protein 1; FBN: Fibrillin; INA: Internexin neuronal intermediate filament protein, alpha; MAL: Mal, T-cell differentiation protein; SNCA: Synuclein, alpha; SPC20: Spastic paraplegia 20; MGMT: O-6-methylguanine-DNA methyltransferase; 3OST2: Heparan sulfate (glucosamine) 3-O-sulfotransferase 2; ITGA4: Integrin, alpha 4; RARB2: Retinoic acid receptor, beta 2; RASSF1: Ras association (RhoGDS/AF-6) domain family member 1; OSMR: Oncostatin M receptor; GATA4: GATA binding protein 4; TFPI2: Tissue factor pathway inhibitor 2; CDKN2A: Cyclin-dependent kinase inhibitor 2; ADIA: DNA integrity assay; MSI: Microsatellite instability; GSTP1: Glutathione S-transferase pi 1; HIC1: Hypermethylated in cancer 1; ATM: Ataxia telangiectasia mutated; APC: Adenomatosis polyposis coli; hMLH1: Homo mult. homolog 1; HIF: Helicase-like transcription factor; SFRP2: Secreted frizzled-related protein 2.
has been well documented that many miRNAs are regulated by the methylation of their promoter region, and some miRNAs target epigenetic activity. For example, miR-29b has been reported to induce DNA hypomethylation and the re-expression of tumor suppressor genes in acute myeloid leukemia by targeting DNMT[74]. These results suggest that there is a strong relationship between miRNA expression and epigenetic mechanisms. Notably, many miRNAs have been found in CRC, and researchers have quantified specific miRNAs for the purpose of CRC diagnosis and prognosis in patient blood, stool and tissue samples. In vitro studies have also been conducted to identify any correlation between epigenetic aberrations and therapy response.

**In vitro studies**

Currently, 54 miRNAs have been identified that are regulated either up or down in CRC cells relative to non-tumor cells (Table 3)[77-79]. Of these, miR-17, miR-20, miR-21, miR-31, miR-92a, miR-93, miR-183 and miR-203 were upregulated in CRC cells, while miR-30a, miR-30c, miR-133a, miR-143, miR-145 were downregulated. These observations have been validated in subsequent studies. The upregulated miRNAs were associated with chromosomal regions that are often amplified in CRC, and the downregulated miRNAs often associated with chromosomal regions that were typically deleted. These changes may be closely related to genetic alterations as well as epigenetic modification.

However, there are some discrepancies between studies. For example, miR-34a, miR-191 and miR-378 were reported to be upregulated in some studies[71-73] and yet were down regulated in others[74-76]. This may have been caused by heterogeneity between the different studies with regards to tumor stage, tumor location, genetic background and technical issues. We believe that the accumulation of further studies will allow us to determine which miRNAs will be the most effective biomarkers and also better understand their role in colorectal cancer.

**miRNA biomarkers in blood**

It is widely believed that miRNAs can shed from tumor cells via exosomes and survive in a stable form in the circulation. Many studies have been performed to quantify miRNAs in the blood for use as a biomarker (Table 4)[77-88]. miR-92a, located on chromosome 13q13, is a member of the miR-17-92 gene cluster. This cluster promotes cell proliferation, suppresses apoptosis, induces angiogenesis and accelerates tumor progression. miR-92a was first identified by Ng et al[80] as a potential noninvasive biomarker for CRC detection with a sensitivity of 89% and specificity of 70%. miR-17-3p, another member of the miR-17-92 gene cluster, was also evaluated in this study as a detection biomarker. This miRNA produced a sensitivity of 89% and specificity of 70%.

To follow this study, Huang et al[81] performed a receiver-operating characteristic (ROC) analysis on 120 CRC patients, 37 patients with advanced adenomas and 59...
would predict poor survival, and thus miR-133b, miR-143, miR-145, miR-17-92, miR-18a, miR-601, miR-760
Diagnosis
miR-133b, miR-143, miR-145, miR-17-92, miR-18a, miR-20a, miR-21, miR-31, miR-92, miR-96, miR-155, miR-183
Prognosis
miR-18a, miR-21, miR-20a, miR-31, miR-143, miR-145, miR-153, miR-181b, miR-200c, miR-203, miR-106a, miR-17-92, miR-135a, miR-335, miR-206, miR-10b, miR-146a/b, let7a/b
Treatment
miR-21, miR-17, miR-215, miR-125b, miR-137, miR-143, miR-145, miR-192, miR-622, miR-630

**Table 5 Relationship between microRNAs and screening, diagnosis and prognosis in colorectal cancer**

| Screening | miR-17-92, miR-20a, miR-21, miR-92, miR-96, miR-106a, miR-135, miR-144, miR-203, miR-326, miR-181b, miR-601, miR-760 |
|-----------|---------------------------------------------------------------|
| Diagnosis | miR-133b, miR-143, miR-145, miR-17-92, miR-18a, miR-20a, miR-21, miR-31, miR-92, miR-96, miR-155, miR-183 |
| Prognosis | miR-18a, miR-21, miR-20a, miR-31, miR-143, miR-145, miR-153, miR-181b, miR-200c, miR-203, miR-106a, miR-17-92, miR-135a, miR-335, miR-206, miR-10b, miR-146a/b, let7a/b |
| Treatment | miR-21, miR-17, miR-215, miR-125b, miR-137, miR-143, miR-145, miR-192, miR-622, miR-630 |

Healthy controls. In this analysis, the researchers found that they could not only discriminate CRC from controls (miR-29a yielded an area under the curve (AUC) of 0.844, and miR-92a yielded an AUC of 0.838), but also discriminate advanced adenomas from controls (the AUC was 0.769 for miR-29a and 0.749 for miR-92a). Furthermore, combined ROC analyses using these two miRNAs revealed an increased AUC with an 83.0% sensitivity and 84.7% specificity in discriminating CRC, and an AUC demonstrating 73.0% sensitivity and 79.7% specificity in discriminating advanced adenomas. These results suggested that plasma miR-29a and miR-92a have potential as novel noninvasive biomarkers for CRC detection and that a combination of different miRNAs may provide a higher sensitivity and specificity than a single miRNA.

More recently, miR-21, miR-601, miR-760 and miR-221 from plasma were also reported to be potential CRC biomarkers. In these studies, miR-221 and miR-21 were upregulated in the plasma of CRC patients compared to healthy controls[78,81,85], while miR-601 and miR-760 were down-regulated[78]. Moreover, a study conducted in two independent CRC cohorts suggested that high levels of plasma miR-141 could predict poor survival, and thus miR-141 may serve as an independent prognostic factor for advanced CRC patients[80].

**miRNA biomarkers in stool**

Stool-based miRNA detection has been widely studied as a noninvasive screening method for CRC (Table 4). Koga et al[87] conducted an miRNA expression analysis of exfoliated colonocytes isolated from the feces of 197 CRC patients and 119 healthy controls. They analyzed the miRNA expression of the miR-17-92 cluster (including miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a), miR-21, and miR-135 by quantitative real-time PCR and found that expression of the miR-17-92 cluster and miR-135 was much higher in CRC patients than in healthy controls ($P < 0.0001$). miR-21, on the other hand, could not discriminate between the two groups. The miR-17-92 cluster detected distal tumors better than proximal tumors, as the sensitivity of miRNA expression for these tumors was 81.5% and 52.9%, respectively.

In another study, Wu et al[88] evaluated the feasibility of miR-21 and miR-92a detection in stool samples from 88 patients with CRC, 57 patients with colorectal polyps and 101 healthy controls. These results showed that patients with CRC had significantly higher levels of miR-21 ($P < 0.001$) and miR-92a ($P < 0.0001$) in their stool compared with normal controls. miR-92a levels provided a higher sensitivity for distal rather than proximal CRC ($P < 0.05$). In addition, stool miR-21 and miR-92a levels decreased significantly ($P < 0.01$) after surgical resection of tumor, which suggests that miR-92a and miR-21 from stool samples could serve as screening biomarkers for colorectal cancer.

In addition, miR-144* and miR-106a were found to be significantly overexpressed in adenomas and in the stool of CRC patients compared with healthy individuals[90,90]. These studies have confirmed that miRNAs from stool samples require validation as diagnostic biomarkers for CRC.

**Brief summary**

miRNAs have been closely linked to colorectal cancer development. They can serve as screening and diagnosis markers for CRC and also as potential prognostic and predictive markers. As a rough outline for the reader, we provide here a table to display the relationship between currently identified miRNAs and screening, diagnosis, prognosis and treatment in colorectal cancer (Table 5). As research continues, more miRNAs correlated with CRC will be discovered, and the mechanism of miRNA regulation will be deciphered. Therefore, it is highly likely that more effective miRNA biomarkers for CRC patients will be found in the future.

**HISTONE MODIFICATION**

Although DNA methylation has been the most extensively studied epigenetic alteration in CRC, increasing numbers of studies have also explored how histone modifications in tumor cells compared to normal colorectal cells. Only tissue samples can be used for histone profiling, so these biomarkers are most useful for the postoperative prognosis of CRC patients. Thus far, the best studies on histone modification have addressed post-translational methylation and acetylation by multiple enzymes. Tamagawa et al[90] created duplicate 2-mm-core tissue microarrays from 54 paraffin-embedded samples of primary colorectal adenocarcinomas and corresponding liver metastases to evaluate the methylation patterns of histone H3 lysine 27 (H3K27), H3 lysine 36 (H3K36) and the expression of H3K27 methylase EZH2. These microarrays were then probed in immunohistochemical assays to search for biomarkers that could identify these patients. These results revealed that H3K27me2 levels were lower in liver metastases than in the corresponding primary tumors, and these levels correlated with tumor size and poorer survival rates. H3K36me2 levels were higher in liver metastases than in the corresponding primary tumors and correlated with histological type and lymph node metastasis. In addition, this study conducted a multivariate survival analysis and suggested that the methylation level of H3K27me2 detected by immuno-

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**Table 5 Relationship between microRNAs and screening, diagnosis and prognosis in colorectal cancer**

| Screening | miR-17-92, miR-20a, miR-21, miR-92, miR-96, miR-106a, miR-135, miR-144, miR-203, miR-326, miR-181b, miR-601, miR-760 |
|-----------|---------------------------------------------------------------|
| Diagnosis | miR-133b, miR-143, miR-145, miR-17-92, miR-18a, miR-20a, miR-21, miR-31, miR-92, miR-96, miR-155, miR-183 |
| Prognosis | miR-18a, miR-21, miR-20a, miR-31, miR-143, miR-145, miR-153, miR-181b, miR-200c, miR-203, miR-106a, miR-17-92, miR-135a, miR-335, miR-206, miR-10b, miR-146a/b, let7a/b |
| Treatment | miR-21, miR-17, miR-215, miR-125b, miR-137, miR-143, miR-145, miR-192, miR-622, miR-630 |
histochemistry may be an independent prognostic factor for metachronous liver metastasis in colorectal cancer patients. In fact, prior to this study, this group used the same method to validate other histone patterns, including histone H3 lysine 4 (H3K4) dimethylation, histone H3 lysine 9 (H3K9) dimethylation and histone H3 lysine 9 (H3K9) acetylation. They found that dimethylation of H3K4 and acetylation of H3K9 correlated with tumor histological type, and lower levels of H3K4 dimethylation correlated with a poor survival rate. Multivariate survival analysis showed that H3K4 dimethylation status is an independent prognostic factor for colorectal cancer patients.[91]

Using chromatin immunoprecipitation (ChIP) coupled with quantitative PCR and high-throughput sequencing, Gezer et al.[92] observed reduced plasma levels for two histone methylation biomarkers, H3K9me3 and H4K20me3, in patients with CRC and characterized these modifications in the circulation. They found that lower H3K9me3 levels had potential as biomarkers for CRC. These studies have provided a good start for the examination of histone modification for the prognosis of CRC. Research is ongoing to find histone biomarkers useful for colorectal cancer patients.

**DISCUSSION**

As we have discussed above, a variety of DNA methylation, miRNA and histone biomarkers from stool, blood and tissue have been reported for CRC detection. Some of the markers identified are derived from tumor cells and others are derived from non-tumor cells in the tumor microenvironment or blood. DNA and miRNA biomarkers mostly shed from tumor cells, and so, theoretically, these molecules should be more specific than protein biomarkers such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA242 and CA724, which are currently applied widely in the clinic. This is because nucleic acid-based markers can be amplified and thus produce a stronger signal, thereby permitting a greater sensitivity. In addition to the superior assay performance of DNA and miRNA, these samples are easier to store because effective preservation buffers that can prevent DNA and miRNA degradation in stool samples are available. In contrast, no preservation buffer for proteins in stool samples has been reported so far. In comparison with DNA and miRNA, protein biomarkers show lower specificity because tumors often induce inflammatory reactions, and some of the biomarkers that initially showed promise for cancer detection now appear to also detect a wide range of bowel diseases, such as ulcerative colitis and Crohn's disease. Moreover, protein biomarkers have often been altered in more than one type of cancer. For example, CEA has been reported as a biomarker for various malignancies, including colorectal, pancreatic, lung, renal and breast cancers.[93-96] In spite of these issues, protein biomarkers may still be useful for large-scale screening for CRC because proteins can be observed through assays in small sample volumes with relatively simple and cheap assays.

Regarding the comparison between stool and blood biomarkers, we know that both of these sample types have been under investigation and improvements continue to be made. In a biomarker search, sample collection, storage and handling have a significant impact on the performance of a specific test. Indeed, using stool samples to detect new biomarkers is not standardized; for example, the buffers used to collect and store stool samples were different in each study, and the methods of DNA or RNA isolation also varied. Therefore, it is difficult to compare the performance of different biomarkers based on the current research. By contrast, blood detection is more standardized and readily accepted by the general population. Moreover, biomarkers in blood are more stable than in stool because of the absence of microflora. DNA, miRNA and proteins have all been shown to be stable in unprocessed EDTA tubes or non-centrifuged clotted blood for 24 h or longer at room temperature. This is particularly true for miRNA, which, as a result of its short length, is more stable in blood than other types of nucleic acids. So, at a practical level, degradation problems for biomarkers during storage and transport should be taken into account, and it is important to standardize detection procedures. For example, the bias will be enormous and the results will not be interpretable if comparing the data from newly collected samples of CRC patients with those from archived samples of adenoma patients or healthy persons. Thus, various samples should be collected in the very same manner at the very same time according to the REMARK guidelines to improve the comparability between various results. Studies specifically addressing these questions are highly desirable.

**CONCLUSION**

Epigenetic biomarkers and the use of blood and stool samples each have their own advantages and disadvantages for clinical screening, diagnosis and prognosis. Although many studies on these biomarkers are preliminary, some markers have demonstrated better performance than the current FOBT test. No biomarker-based assay is ready for large-scale population screening, however.

The standardization of sample preparation and testing protocols is very important for the widespread deployment of techniques and the comparison of results from different studies. Moreover, large well-controlled studies are urgently needed to identify the accuracy of epigenetic biomarkers for CRC detection in asymptomatic populations. Much work remains before such observations can be translated into routine clinical practice.

**REFERENCES**

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
DNA methylation markers - 2003; - 2005; - 2011; - 2012

Schuebel KE, Ching W, Cope L, Glöckner SC, Suzuki H, Yi JM, Chan TA, Van Neste L, Van Crieekinge W, Van den Bosch S, van Engeland M, Ting AH, Jair K, Yu W, Toyoda M, Imai K, Ahuja N, Herman JG, Baylin SB. Comparing the DNA hypermethylation with gene mutations in human colorectal cancer. PLoS Genet 2007; 3: 1709-1722 [PMID: 17892325]

Yi JM, Dhir M, Guzzetta AA, Iacobuzio-Donahue CA, Heo K, Yang KM, Suzuki H, Jair KW, van Engeland M, Weijenberg MP. Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clin Cancer Res 2010; 16: 5107-5114 [PMID: 20753311 DOI: 10.1158/1078-0432.CCR-10-0217]

Leung WK, To KF, Man EP, Chan MW, Bai AH, Hui AJ, Chan FK, Sung JJ. Quantitative detection of promoter hypermethylation in multiple genes in the serum patients with colorectal cancer. Am J Gastroenterol 2005; 100: 2274-2279 [PMID: 16183180]

Wallner M, Herbst A, Behrens A, Crispin A, Stieber P, Göke B, Lamerz R, Kolligs FT. Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clin Cancer Res 2006; 12: 7347-7352 [PMID: 17189406]

Ebert MP, Model F, Mooney S, Hale K, Lograsso J, Tunes-Priddy L, Hoffmann J, Csepregi A, Röcken C, Molinar B, Schulz HU, Malfertheiner P, Lofton-Day C. Aristless-like homebox-4 gene methylation is a potential marker for colorectal adenocarcinomas. Gastroenterology 2006; 131: 1418-1430 [PMID: 17101318]

Lofton-Day C, Model F, Devos T, Tetzner R, Distler J, Schuster M, Song X, Lesche R, Liebenberg V, Ebert B, Molinar B, Grützmann R, Pilarsky C, Sledziewski A. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem 2008; 54: 414-423 [PMID: 18089654]

Grützmann R, Molinar B, Pilarsky C, Habermann JK, Schlag PM, Saeger HD, Mielchke S, Stolz T, Model F, Roblick UJ, Bruch HP, Koch R, Liebenberg V, Devos T, Song X, Day RH, Sledziewski AZ, Lofton-Day C. Sensitive detection of colorectal cancer in peripheral blood by sepnt 9 DNA methylation assay. PLoS One 2008; 3: e3759 [PMID: 19018228 DOI: 10.1371/journal.pone.0003759]

Tänzer M, Balluff B, Distler J, Hale K, Leodolter A, Röcken C, Molinar B, Schmid R, Lofton-Day C, Schuster T, Ebert MP. Performance of epigenetic markers SEPT9 and ALX4 in plasma for detection of colorectal precancerous lesions. PLoS One 2010; 5: e9061 [PMID: 20410221 DOI: 10.1371/journal.pone.0009061]

Ahlquist DA, Taylor WR, Mahoney DW, Zou H, Domanico M, Thibodeau SN, Boardman LA, Berger BM, Lidgard GP. The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. Clin Gastroenterol Hepatol 2012; 10: 272-277.e1 [PMID: 22019796 DOI: 10.1016/j.cgh.2011.10.008]

Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, Fang JC, Samowitz WS, Heichman KA. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med 2011; 9: 153 [PMID: 21268215 DOI: 10.1186/1741-7015-9-153]

Herbst A, Rahmig K, Stieber P, Philipp A, Jung A, Ofner A, Crispin A, Neumann J, Lamerz R, Kolligs FT. Methylation of NEUROG1 in serum is a sensitive marker for the detection of early colorectal cancer. Am J Gastroenterol 2011; 106: 1110-1118 [PMID: 21362623 DOI: 10.1038/ajg.2011.6]

Tang D, Liu J, Wang DR, Yu HF, Li YK, Zhang JQ. Diagnos-
Wang X et al. Epigenetic biomarker in colorectal cancer

tic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. Clin Invest Med 2011; 34: E88-E95 [PMID: 21463549]

Tan SH, Ida H, Lau QC, Goh BC, Chiang WS, Loh M, Ito Y. Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3. Oncol Rep 2007; 18: 1225-1230 [PMID: 17914577]

He Q, Chen HY, Bai EQ, Luo YX, Fu RJ, He YS, Jiang J, Wang HQ. Development of a multiplex MethLight assay for the detection of multigene methylation in human colorectal cancer. Cancer Genet Cytoflogen 2010; 202: 1-10 [PMID: 20804913 DOI: 10.1016/j.cancergen.2010.01.018]

Herbst A, Wallner M, Rahimig K, Steiber P, Crispin A, Lamerz R, Kolligs FT. Methylation of helicase-like transcription factor in serum of patients with colorectal cancer is an independent predictor of disease recurrence. Eur J Gastroenterol Hepatol 2009; 21: 565-569 [PMID: 19282772 DOI: 10.1097/MEG.0b013e32832a6ec2]

Carmona FJ, Azuara D, Berenguer-Llargo A, Fernandez AF, Biendo S, de Oca J, Rodriguez-Moranta F, Salazar R, Villanueva A, Fraga MF, Guardiola J, Capella G, Esteller M, Moreno V. DNA methylation biomarkers for noninvasive diagnosis of colorectal cancer. Cancer Prev Res (Phil) 2013; 6: 656-665 [PMID: 23694962 DOI: 10.1158/1940-6207.CAPR-12-10501]

Kisiel JB, Yab TC, Nazer Hussain FT, Taylor WR, Garrity-Park MM, Sandborn WJ. Lofthus EV, Wolff BG, Smyrk TC, Itzkowitz SH, Rubin DT, Zou H. Mahoney DW, Ahlquist DA. Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2013; 37: 546-554 [PMID: 23347191 DOI: 10.1111/apt.12218]

Elliott GO, Johnson JT, Scarli J, Dainty J, Williams EA, Garg D, Coupe A, Bradburn DM, Mathers JC, Belshaw NJ. Quantitative profiling of CpG island methylation in human stool for colorectal cancer detection. Int J Colorectal Dis 2013; 28: 35-42 [PMID: 22291128 DOI: 10.1007/s00384-012-1532-5]

Azurza D, Rodríguez-Moranta F, de Oca J, Sanjuan X, Guardiola J, Lobaton T, Wang A, Boadas J, Piqueras M, Monfort D, Galter S, Esteller M, Moreno V, Capella G. Novel methylation panel for the early detection of neoplasia in high-risk ulcerative colitis and Crohn’s colitis patients. Inflamm Bowel Dis 2013; 19: 165-173 [PMID: 22532293 DOI: 10.1007/s10592-012-1994-0]

Bosch LJ, Oort FA, Neerincx M, Khalid-de Bakker CA, Terhaar LJ, Oort FA, van Dinteren J, Melotte V, Jonkers DM, Maselke LG, Dreyfuss HM, Grootelaar C, Louwagie J, van Kriekinge W, Coupé VM, Mulder CJ, van Engeland M, Carvalho B, Meijer GA. DNA methylation of phosphatase and actin regulator 3 (PACAPR3) as a diagnostic and therapeutic marker in colon cancer. PLoS One 2009; 4: e6555 [PMID: 19662690 DOI: 10.1371/journal.pone.0006555]

Li M, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laursen B, Levin B, Juhi A, Harner N, Mooinova H, Durkee K, Schmidt K, He Y, Diehl F, Velucesco VE, Zhou S, Diaz LA, Kinzler KW, Markowitz SD, Vogelstein B. Sensitieve digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009; 27: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]

Baek YH, Chang E, Kim YJ, Kim BK, Sohn JH, Park DJ. Stool methylation-specific polymerase chain reaction assay for the detection of colorectal neoplasia in Korean patients. Dis Colon Rectum 2009; 52: 1452-1459; discussion 1459-1463 [PMID: 19617759 DOI: 10.1007/s00106-008-97953]

Ausch C, Kim YH, Tsuchiya KD, Dzieciatkowski S, Washington MK, Park JN, Maselke LG, Dreyfuss HM, Brüne AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. Mol Cancer 2009; 8: 1244-1258 [PMID: 19700653 DOI: 10.1039/p1jc00526e]

Kim MS, Louwagie J, Carvalho B, Terhaar Sive Droste JS, Melotte V, Jonkers DM, Herman JG, de Bruijn AP, van Engeland M, GA. DNA methylation of checkpoint kinase 1 as a novel diagnostic and therapeutic marker in colon cancer. PLoS One 2009; 4: e6555 [PMID: 19662690 DOI: 10.1371/journal.pone.0006555]

Li M, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laursen B, Levin B, Juhi A, Harner N, Mooinova H, Durkee K, Schmidt K, He Y, Diehl F, Velucesco VE, Zhou S, Diaz LA, Kinzler KW, Markowitz SD, Vogelstein B. Sensitieve digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009; 27: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]

Melotte V, Lentjes MH, van den Bosch SM, Hellebrekers DM, de Hoon JP, Wouters KA, Daenen KL, Partouw-Hendriks IE, Stessels F, Louwagie J, Smits KM, Weijenberg MP, Sanduleanu S, Khalid-de Bakker CA, Oort FA, Meijer GA, Jonkers DM, Herman JG, de Bruijn AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. J Natl Cancer Inst 2009; 101: 916-927 [PMID: 19535783 DOI: 10.1093/jnci/djp131]

Hellebrekers DM, Lentjes MH, van den Bosch SM, Melotte V, Wouters KA, Daenen KL, Smits KM, Akiyama Y, Yuasa Y, Sanduleanu S, Khalid-de Bakker CA, Jonkers D, Weijenberg MP, Louwagie J, van Kriekinge W, Carvalho B, Meijer GA, Baylin SB, Herman JG, de Bruijn AP, van Engeland M. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. Clin Cancer Res 2009; 15: 3990-3997 [PMID: 19509152 DOI: 10.1186/1078-0432-CCR-09-0055]

Wang DR, Tong D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. World J Gastroenterol 2009; 14: 524-531

CG, Cao GW. [Gene methylation in stool for the screening of colorectal cancer and pre-malignant lesions]. Zhonghua Weishang Za Zhi 2011; 14: 52-56 [PMID: 21271362]
Dysregulation of microRNA-34a expression causes drug-resistance to 5-FU in human colon cancer DLD-1 cells. Cancer Lett 2011; 300: 197-204 [PMID: 21067862 DOI: 10.1016/j.canlet.2010.10.006]

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Vissone R, Iorio M, Roldo C, Ferracin M, Peltre RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negri M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006; 103: 2257-2262 [PMID: 16461460]

Arndt GM, Ossele L, Cullen LM, Lai A, Druker R, Eisbacher M, Zhang C, Tran N, Fan H, Retzafi K, Bittner A, Raponi M. Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. BMC Cancer 2009; 9: 574 [PMID: 19843336 DOI: 10.1186/1471-2407-9-374]

Ma Y, Bao-Han W, Ly X, Su Y, Zhao X, Yin Y, Zhang X, Zhou Z, MacNaughton WK, Wang H. MicroRNA-34a mediates the autocrine signaling of PAR2-activating proteinase and its role in colon cancer cell proliferation. PLoS One 2013; 8: e22836 [PMID: 23991105 DOI: 10.1371/journal.pone.0022836]

Zhou J, Zhou Y, Yin B, Hao W, Zhao L, Ju W, Bai C. 5-Fluorouracil and oxaliplatin modify the expression profiles of microRNAs in human colon cancer cells in vitro. Oncol Rep 2010; 23: 121-128 [PMID: 19568767]

Wang YX, Zhang YX, Zhang BF, Yang CQ, Chen XM, Gao HJ. Initial study of microRNA expression profiles of colorectal cancer without lymph node metastasis. J Dig Dis 2010; 11: 50-54 [PMID: 20324321 DOI: 10.1111/j.1751-2980.2009.00413.x]

Toiyama Y, Takahashi M, Hur K, Nagasaki T, Tanaka K, Iwroue Y, Kusunoki T, Sahi J, Myeon J. Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. J Natl Cancer Inst 2013; 105: 849-859 [PMID: 23704278 DOI: 10.1093/jnci/djt101]

Wang Q, Huang Z, Ni S, Xiao X, Xu Q, Wang L, Huang D, Tan C, Sheng W, Du X. Plasma miR-601 and miR-760 are novel biomarkers for the early detection of colorectal cancer. PLoS One 2012; 7: e4398 [PMID: 22970209 DOI: 10.1371/journal.pone.0043989]

Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J, Galandiuk S. Plasma miR-21: a potential diagnostic marker of colorectal cancer. Ann Surg 2012; 256: 544-551 [PMID: 22863872 DOI: 10.1097/SLA.0b013e318265bd6f]

Wang LG, Gu J. Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. Cancer Epidemiol 2012; 36: e61-e67 [PMID: 22018950 DOI: 10.1016/j.canep.2011.05.002]

Cheng H, Zhang L, Cogdoll DE, Zheng H, Schetter AJ, Nykter M, Harris CC, Chen K, Hamilton SR, Zhang W. Circulating plasma MiR-141 is a novel biomarker for metastatic colorectal cancer and predicts poor prognosis. PLoS One 2011; 6: e17745 [PMID: 21445232 DOI: 10.1371/journal.pone.0017745]

Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 2010; 127: 118-126 [PMID: 20698283]
19876917 DOI: 10.1002/ijc.25007

83 Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Ji-ang L, Tian Y, Lin TY. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol 2010; 25: 1674-1680 [PMID: 20880178 DOI: 10.1111/j.1440-1746.2010.06417.x]

84 Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS, Sung JJ. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009; 58: 1375-1381 [PMID: 19201770 DOI: 10.1136/gut.2008.167817]

85 Wu CW, Ng SS, Dong YJ, Ng SC, Leung WW, Lee CW, Wong YN, Chan FK, Yu J, Sung J. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut 2012; 61: 739-745 [PMID: 21930727 DOI: 10.1136/gut.2011.239236]

86 Kalimutho M, Del Vecchio Blanco G, Di Cecilia S, Sileri P, Cretella M, Pallone F, Federici G, Bernardini S. Differential expression of miR-144* as a novel fecal-based diagnostic marker for colorectal cancer. J Gastroenterol Hepatol 2011; 46: 1391-1402 [PMID: 21863218 DOI: 10.1111/j.1440-1746.2011.06358.x]

87 Koga Y, Yasunaga M, Takahashi A, Kuroda J, Moriyama Y, Akasu T, Fujita S, Yamamoto S, Baba H, Matsumura Y. MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. Cancer Prev Res (Phila) 2010; 3: 1435-1442 [PMID: 20959518 DOI: 10.1158/1940-6207.CAPR-10-0036]

88 Wu CW, Ng SS, Leung WW, Lee CW, Wong YN, Chan FK, Yu J, Sung J. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut 2012; 61: 739-745 [PMID: 21930727 DOI: 10.1136/gut.2011.239236]

89 Link A, Balaguier F, Shen Y, Nagasaka T, Lozano J, Boland CR, Goel A. Fecal MicroRNAs as novel biomarkers for colon cancer screening. Cancer Epidemiol Biomarkers Prev 2010; 19: 1766-1774 [PMID: 20551304 DOI: 10.1158/1055-9965.EPI-10-0027]

90 Tamagawa H, Oshima T, Numata M, Yamamoto N, Shiozawa M, Morinaga S, Nakamura Y, Yoshihara M, Sakuma Y, Kameda Y, Akaike M, Yuka N, Rino Y, Masuda M, Miyagi Y. Global histone modification of H3K27 correlates with the outcomes in patients with metachronous liver metastasis of colorectal cancer. Eur J Surg Oncol 2013; 39: 655-661 [PMID: 23523318 DOI: 10.1016/j.ejso.2013.02.023]

91 Tamagawa H, Oshima T, Shiozawa M, Morinaga S, Nakamura Y, Yoshihara M, Sakuma Y, Kameda Y, Akaike M, Masuda M, Imada T, Miyagi Y. The global histone modification pattern correlates with overall survival in metachronous liver metastasis of colorectal cancer. Oncol Rep 2012; 27: 637-642 [PMID: 22076537 DOI: 10.3892/or.2011.1547]

92 Gezer U, Ustek D, Yörüker EE, Cakiris A, Abaci N, Leszinski G, Dalay N, Holdenrieder S. Characterization of H3K9me3- and H4K20me3-associated circulating nucleosomal DNA by high-throughput sequencing in colorectal cancer. Tu- mour Biol 2013; 34: 329-336 [PMID: 23086575 DOI: 10.1007/s13277-012-0554-5]

93 Chung HW, Lim JB, Jang S, Lee KJ, Park KH, Song SY. Serum high mobility group box 1 is a powerful diagnostic and prognostic biomarker for pancreatic ductal adenocarcinoma. Cancer Sci 2012; 103: 1714-1721 [PMID: 22703527 DOI: 10.1111/j.1349-7006.2012.02358.x]

94 Grunnet M, Sorensen JB. Carcinoembryonic antigen (CEA) as tumor marker in lung cancer. Lung Cancer 2012; 76: 138-143 [PMID: 22153832 DOI: 10.1016/j.lungcan.2011.11.012]

95 Ye YL, Bian J, Huang YP, Guo Y, Li ZX, Deng CH, Dai YP, Sun XZ. Primary mucinous adenocarcinoma of the renal pelvis with elevated CEA and CA19-9. Urol Int 2011; 87: 484-488 [PMID: 21893942 DOI: 10.11159/00329767]

96 Marie P, Ozretic P, Levanat S, Oreskovic S, Antunac K, Bekeći-Oresković L. Tumor markers in breast cancer—evaluation of their clinical usefulness. Coll Antropol 2011; 35: 241-247 [PMID: 21661578]

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