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Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Anil Mishra, PhD, Doctor, Department of Allergy and Immunology, Children’s Hospital Medical Center, Cincinnati, OH 45229, United States

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World Journal of Gastrointestinal Oncology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
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E-mail: editorialoffice@wjgnet.com
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Baishideng Publishing Group Inc
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Telephone: +1-925-2238242
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Advance in plasma *SEPT9* gene methylation assay for colorectal cancer early detection

Yu Wang, Pei-Min Chen, Rong-Bin Liu

Yu Wang, School of Public Health, Guangzhou Medical University, Guangzhou 510180, Guangdong Province, China

Pei-Min Chen, School of Public Health, Guangzhou Medical University, Guangzhou 510180, Guangdong Province, China

Rong-Bin Liu, Department of Ultrasound, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong Province, China

ORCID number: Yu Wang (0000-0002-0650-8126); Pei-Min Chen (0000-0002-5337-9910); Rong-Bin Liu (0000-0003-2170-7921).

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Correspondence to: Rong-Bin Liu, MD, Key Laboratory of Malignant Tumor Gene Regulation and Target Therapy of Guangdong Higher Education Institutes, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, No. 107, Yanjiang West Road, Yuexiu District, Guangzhou 510120, Guangdong Province, China. liurb3@mail2.sysu.edu.cn
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Abstract

This review article summarizes the research advances of the plasma-based *SEPT9* gene methylation assay for the clinical detection of colorectal cancer and its limitations. Colorectal cancer is a common malignancy with a poor prognosis and a high mortality, for which early detection and diagnosis are particularly crucial for the high-risk groups. Increasing evidence supported that *SEPT9* gene methylation is associated with the pathogenesis of colorectal cancer and that detecting the level of methylation of *SEPT9* in the peripheral blood can be used for screening of colorectal cancer in susceptible populations. In recent years, the data obtained in clinical studies demonstrated that the *SEPT9* gene methylation assay has a good diagnostic performance with regard to both sensitivity and specificity with the advantage of better acceptability, convenience and compliance with serological testing compared with fecal occult blood tests and carcinoembryonic antigen for colorectal cancer (CRC). Furthermore, the combination of multiple methods or markers has become a growing trend for CRC detection and screening. Nevertheless, the clinical availability of the methylated *SEPT9* assay is still limited because of the large degree of sample heterogeneity caused by demographic characteristics, pathological features, comorbidities and/or technique selection. Another factor is the cost-effectiveness of colorectal cancer screening strategies that hinders its large-scale application. In addition, improvements in its accuracy in detecting adenomas and premalignant polyps are required.

Key words: Plasma; *SEPT9*; Methylation; Colorectal cancer; Early detection

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Core tip: The methylated *SEPT9* gene has been implicated as a biomarker for colorectal cancer associated with the pathogenesis of colorectal cancer (CRC). In this article, we reviewed the literature on the correlation of *SEPT9* gene and colorectal cancer and the theoretical basis of the *SEPT9* gene methylation assay. Then, we focused on the diagnostic performance of the *SEPT9* gene methylation assay for CRC by analyzing the clinical trial studies and compared that assay with other methods. Finally, we discussed the limitations of the *SEPT9* gene methylation assay in clinical application. We hope that this article can provide a comprehensive overview of the progress achieved in the *SEPT9* methylation assay for both the basic and clinical sciences.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system and results in significant morbidity and mortality. As it is estimated, there were approximately 135430 new cases of colorectal cancer, including men and women, in 2017^[1]. The incidence is higher in men than women and markedly increases with age^[2]. CRC kills almost 700000 people every year, making it the world's fourth deadliest cancer (after lung, liver and stomach cancers)^[3]. As research has shown, the incidence and mortality rates of CRC vary up to 10-fold worldwide, with distinct gradients across human development, pointing towards widening disparities and an increasing burden in countries in transition^[4]. In general, its incidence and mortality rates are still rising rapidly in many low-income and middle-income countries.

The initial symptoms of colorectal cancer, however, are atypical, leading to a poor prognosis and high fatality rate. Therefore, screening of CRC in the population is of great significance for its early diagnosis and treatment. Currently, CRC screening approaches are divided into two categories: Invasive and noninvasive methods. The invasive methods, such as colonoscopy, remain the main screening tools due to their very good diagnostic performance, enabling the detection and removal of precancerous lesions^[5]. However, it requires thorough bowel preparations. Additionally, discomfort and privacy infringement contribute to poor compliance among patients. Non-invasive screening approaches, which include fecal occult blood tests (FOBT), fecal immunochemical tests (FITs) and

carcinoembryonic antigen (CEA), are more easily acceptable. However, their effectiveness may not be guaranteed. Although various guideline-recommended methods are available for CRC detection, patient compliance remains low. The data in 2013 showed that only approximately 57% of eligible adults adhered to the screening recommendations provided by the United States Preventive Services Task Force^[6]. Thus, it is very important to develop an efficient approach to enhance patient compliance that can be applied to screening the general population.

Studies^[7-9] have shown that the DNA methylation of certain genes is closely related to the development of colorectal cancer. Beggs *et al.*^[10] verified that methylation changes contribute substantially to the progression from normal mucosa to adenoma and to carcinoma; for instance, GRASP, which encodes the general receptor for phosphoinositide 1-associated scaffold protein, was differentially methylated in colorectal cancer. Aberrant DNA methylation in the genome may contribute to malignant transformation by silencing multiple tumor-suppressor genes. This type of epigenetic alteration is believed to occur early in tumor development and may precede genetic changes^[11]. In recent years, *SEPT9* gene methylation has been recognized as a hotspot and is considered to be a specific biomarker of the early stages of colorectal cancer. It may be a reliable indicator for screening CRC among high-risk individuals. This paper reviews the progress in the plasma-based *SEPT9* gene methylation assay for the detection of colorectal cancer.

SEPT9

As we know, there are 14 members (SEPT1-SEPT14) in the SEPT gene family, whose protein products Septins are a series of highly conserved GTP binding protein family. In humans, there are 13 genes, respectively named SEPT1 to SEPT13; the *SEPT9* gene is located on the human chromosome 17q25. 3^[12], contains 17 exons, and spans 240×10^3 bp. The 5'-end regulatory regions of the *SEPT9* gene have a -C- phosphor -G- site (CpG island), which is the main site of DNA methylation. In mammals, 60%-90% of CpG sites are methylated, and most of the remaining unmethylated residues are clustered in CpG islands within functional gene promoters^[13]. It has been shown^[12,14,15] that SEPT 9 has 18 distinct transcripts encoding 15 polypeptides, with two transcripts (SEPT9_v4 and v4*) encoding the same polypeptide.

SEPT9 GENE AND COLORECTAL CANCER

In recent years, growing evidence has shown that the *SEPT9* gene is associated with malignant tumors. Peterson *et al.*^[16] used immunoprecipitation and immunofluorescence studies to analyze SEPT9_i1 and found that it interacts with both α and γ tubulin.

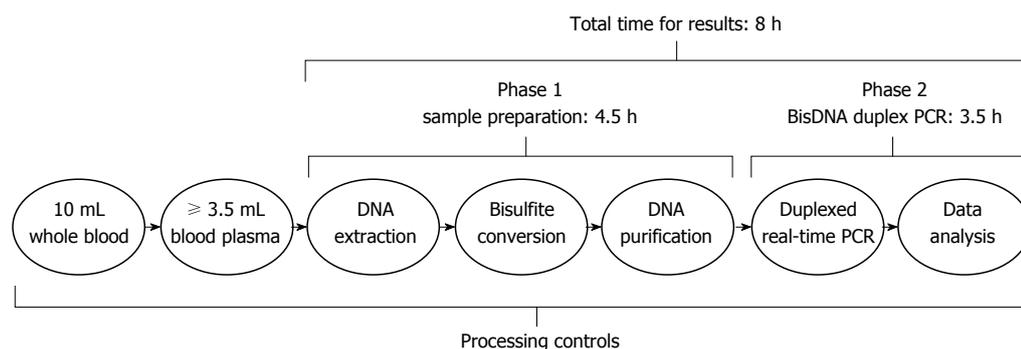


Figure 1 The outline of the Epi proColon work flow. The test consists of the Epi proColon Plasma Quick kit, PCR kit, and Control kit. The total assay time is approximately 8 h. For the Plasma Quick kit, 3.5 mL of plasma was mixed with an equal volume of lysis buffer; after incubating for 10 min, magnetic beads and absolute ethanol were added. After 45 min, impurities were removed from the magnetic beads by centrifugation; the purified DNA was then released from the beads in the elution buffer and treated at 80 °C with a solution of ammonium bisulfite for deamination of cytosine^[34]. After a series of washing steps, the converted DNA (bisulfite-modified DNA, bisDNA) was captured by magnetic beads. The bisDNA was assayed with the PCR kit on a Duplexed Real-Time PCR device. Finally, methylated SEPT9 and PCR results were recorded by the instrument software. In the whole working flow, the processing controls were included to monitor the execution of the procedure and ensure the validity of the test result and model^[34].

SEPT9_i1-expressing cells demonstrated dramatic chromosome segregation defects, centrosome amplification and cytokinesis defects, which indicates that SEPT9_i1 increases genomic instability in the process of tumorigenesis through two potential molecular mechanisms: defective chromosome segregation and cytokinesis failure. Additionally, expression of HIF^[17], JNK^[18] and Rho signaling pathways^[19] may also be potential mechanisms of colorectal cancer development in which the *SEPT9* gene is involved.

SEPT9 gene encodes a protein called septin-9, which is part of a group of proteins called septins. Septins are involved in various biological processes such as division of cytoplasm, cell polarization, vesicle transport and membrane reconstruction. The septin-9 protein also appears to act as a tumor suppressor, which means that it regulates cell growth and prevents cells from dividing too fast or in an uncontrolled way^[20]. When the methylation occurs at a CpG island, genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent^[21]; DNA methylation gradually accumulates on long-term silenced gene and may result in the inactivation of cancer suppressor genes. Tóth *et al.*^[22] have found that *SEPT9* mRNA expression decreased from adenoma to dysplasia to carcinoma in the progression of colon neoplastic disease, which presents a strong significant correlation of *SEPT9* methylation with the mRNA's low expression in CRC. Thus, downregulation of *SEPT9* mRNA and the decrease in *SEPT9* expression may account for the pathological progression from benign to malignant lesions in colon tissues.

PLASMA SEPT9 GENE METHYLATION ASSAY

Methods of the SEPT9 gene methylation assay

Due to epigenetic silencing of the *SEPT9* gene by promoter methylation in plasma, the company

Epigenomics AG first studied SEPT9 methylation based on the SEPT9 biomarker available in Europe in 2008^[23]. After one year, a commercial kit was finalized and the first generation of the CE-marked Epi proColon real-time PCR kit was launched. This CE-marked IVD (*In vitro* Diagnostic) kit became publicly available in Europe in 2010. Currently, the second generation of the assay is commercially available as the Epi proColon 2.0 assay^[24].

In general, The Epi proColon test is an *in vitro* diagnostic PCR method for the qualitative detection of SEPT9 DNA methylation levels in plasma derived from patients' whole blood specimens (Figure 1). To perform the test, approximately 10 mL of whole blood is a source of sufficient plasma for the analysis. The testing cycle performed with the current manual workflow takes approximately 8 h. As a first step, a minimum of 3.5 mL of blood plasma is isolated from the blood sample. Then, the Epi proColon 2.0 test consists of two phases. In Phase I, DNA is extracted from the plasma fraction and treated with bisulfite-conversion reagents and purified to obtain highly purified DNA^[25]. In Phase II, the test detects the hyper-methylated v2 region of the SEPT 9 gene and a region of the ACTB (β -actin) gene as an internal control by duplex real-time PCR^[26]. Finally, the Epi proColon 2.0 test only reports qualitative positive and negative results. A positive test is indicative of an increased likelihood for having CRC and a colonoscopy is recommended as a follow-up for diagnostic evaluation.

Diagnostic performance of plasma SEPT9 gene methylation

Increasingly, studies^[27-30] are suggesting that the methylation status of SEPT9 is a reliable index for screening CRC. For evaluating its diagnostic performance, we have collected several research results in which the sensitivity and specificity are key indicators. Table 1

Table 1 Sensitivity and specificity of the *SEPT9* gene methylation assay for colorectal cancer detection

Publications	Number of cases	Sensitivity	Specificity	Algorithm	Assay used	Ref.
Tóth <i>et al</i> (2012)	184 (92 CRC, 92 no evidence of disease)	95.6%	84.8%	1/3	Epi proColon 2.0	[27]
		(95%CI: 89.2%-98.8%)	(95%CI: 75.8%-91.4%)			
Church <i>et al</i> (2014)	1516 (53 CRC, 1457 without CRC)	79.3%	98.9%	2/3	Epi proColon 1.0	[31]
		(95%CI: 69.6%-87.1%)	(95%CI: 94.1%-100%)			
Potter <i>et al</i> (2014)	1544 (44 CRC, 1500 non-CRC)	48.2%	91.5%	1/3	Epi proColon 1.0	[34]
Su <i>et al</i> (2014)	234 (172 CRC, 62 controls)	68.0%	80.0%	-	MSP-DHPLC	[28]
		(95%CI: 53%-80%)	(95%CI: 78%-82%)			
Johnson <i>et al</i> (2014)	301 (101 CRC, 200 non-CRC)	88.4%	93.5%	-	Epi proColon 1.0	[32]
		73.3%	81.5%	-		
Jin <i>et al</i> (2014)	476 (135 CRC, 341 non-CRC)	(95%CI: 63.9%-80.9%)	(95%CI: 75.5%-86.3%)	2/3	Epi proColon 2.0	[29]
		74.8%	87.4%			
Ørntoft <i>et al</i> (2015)	300 (150 CRC, 150 controls)	(95%CI: 67.0%-81.6%)	(95%CI: 83.5%-90.6%)	1/3	Epi proColon 1.0	[33]
		73.0%	82.0%			
Sharif <i>et al</i> (2016)	90 (45 CRC, 45 controls)	(95%CI: 64%-80%)	(95%CI: 75%-88%)	-	MS-HRM assay	[52]
		84.4%	99.0%			
Wu <i>et al</i> (2016)	1031 (291 CRC, 740 non-CRC)	73.0%	97.5%	-	Epi proColon 2.0	[30]
		76.6%	95.9%	-		
Nian <i>et al</i> (2016)	25 studies, 9927 samples (2975 CRC, 6952 non-CRC)	(95%CI: 71.3%-81.4%)	92.0%	2/3	Epi proColon 2.0	[35]
		71.0%	(95%CI: 89%-94%)			

CRC: Colorectal cancer.

shows the data from clinical trials using the *SEPT9* gene methylation assay published since 2012.

From the table, it can be seen that the plasma *SEPT9* gene methylation assay exhibited a high overall sensitivity and specificity for CRC detection. Moreover, with the improved method used in the subsequent studies, especially after the application of the second-generation *SEPT9* methylation assay (Epi proColon 2.0, Epigenomics AG, Germany), the detection sensitivity increased from approximately 48.2%-73.3%^[31-34] to approximately 71.0%-95.6%^[27,29,30,35], while the specificity improved from 80.0%-91.5% to 84.8%-98.9%. Meanwhile, Wu *et al*^[30] reported that the new *SEPT9* assay, with enhanced technical simplicity and a lower cost, presented a sensitivity of 76.6% and a specificity of 95.9%, which not only did not differ in performance compared with Epi proColon 2.0 but also reduced the complexity of the testing process and appeared to be a simpler, cheaper, more efficient, convenient, and user-friendly alternative for CRC screening. Additionally, methylation of *SEPT9* detected by MSP-DHPLC (methylation-specific polymerase chain reaction (PCR)-denaturing high-performance liquid chromatography)^[28] shows that the sensitivity and specificity are as high as 88.4% and 93.5%, respectively, which also appears to be a useful biomarker in a clinical laboratory setting. Tóth *et al*^[36] measured the positive predictive value and negative predictive value, which reached up to 93.8% (30/32) and 84.6% (22/26), respectively, supporting the reliability of this assay for CRC detection. Nian *et al*^[35] also estimated an area under the curve (AUC) of 0.88 and diagnostic odds ratio of 27 (95%CI: 18-42) using a bivariate mixed effect model. Furthermore, Ørntoft *et al*^[33] found that the clinical sensitivity for

CRC stages I-IV was 37%, 91%, 77%, and 89%, respectively. In comparison, Jin *et al*^[29] described that methylated *SEPT9* was positive in 66.7% of stage I (12/18), 82.6% of stage II (19/23), 84.1% of stage III (37/44), and 100% of stage IV (5/5) cases in 90 cases of CRC whose stages were identified based on the surgically resected specimens. The results indicate that advanced stage CRCs are more easily detected by *SEPT9* methylation than the early stage. Although the sensitivity and specificity reported in Table 1 come from different studies, leading to the variation in the ability to detect CRC, these results are still comparable because the majority of studies used Epi proColon products as the commercialized tests, and multiple PCR reactions are performed in all of these studies, which determine the final test result.

As for the test performance of other non-invasive CRC detection approaches, according to retrospective case control studies^[27,31,37,38], the FOBT identifies individuals with CRC with a sensitivity between 33% and 79% and a specificity between 87% and 98%. Another recent case control study by Tóth *et al*^[27] showed that the FOBT was positive in 29.4% (5/17) of NED (no evidence of disease) and 68.2% (15/22) of CRC and that elevated CEA levels were detected in 14.8% (4/27) of NED and 51.8% (14/27) of CRC. Both the FOBT and CEA showed a lower sensitivity and specificity than *SEPT9* (95.6% and 84.8%). In addition, Lee *et al*^[39] reported that the sensitivity was as high as 79% (95%CI: 69%-86%) for FIT for CRC with a specificity of 94% (95%CI: 92%-95%) by meta-analysis, which is at the same level as *SEPT9*^[27]. Johnson *et al*^[32] obtained estimates of 68.0% (95%CI: 58.2-76.5%) for the sensitivity and 97.4% (95%CI: 94.1%-98.9%) for the specificity of FIT, and drew the

conclusion that the sensitivity of the Epi proColon test was statistically comparable to FIT by analyzing the paired samples. A study by Song *et al.*^[40] also showed that the SEPT9 assay exhibited significantly higher sensitivity than the FIT test (75.6% vs 67.1%, $P < 0.05$) in pooled data of the symptomatic population. In general, compared with these other CRC detection tests, the SEPT9 gene methylation assay shows a good diagnostic performance in both sensitivity and specificity with the advantage of better acceptability and compliance of serological testing.

Hence, the promoter hyper-methylation analysis of plasma SEPT9 DNA has the potential to serve as a non-invasive screening method for the identification of specific biomarkers, enabling early detection of CRC in a large population. This approach holds promise for increased accuracy, safety, affordability, and patient compliance^[41].

Combined detection of the SEPT9 assay with other colorectal cancer detection tests

The combination of multiple methods or markers has become an increasing trend in CRC detection and screening. A recent study conducted by Wu *et al.*^[30] demonstrated that the combination of SEPT9 + FIT had a high sensitivity for CRC detection (94.4%), and the sensitivity of combined examination of SEPT9 + FIT + CEA was 97.2% (76.6%, SEPT9 alone). Another study^[42] found that the sensitivity of joint examination of SEPT9 and FIT in CRC diagnosis was 97.8% (80.0%, SEPT9 alone) and that the specificity was 52.9%, whereas the advanced adenoma diagnosis was 67.6% (10.8%, SEPT9 alone) and 47.4%, respectively, which suggested that the combination of the SEPT9 and FIT assays not only significantly enhanced the sensitivity for CRC detection but also increased the positive detection rate for advanced adenoma. In the study of Yu *et al.*^[43], it was seen that the under-ROC curve area of SEPT9 with CEA and FOBT for CRC detection reached 0.935. Furthermore, other than the tests mentioned above, SEPT9 may be combined with other existing biomarkers for CRC detection, such as glycoprotein markers or other methylation markers^[12]. A study published by Tänzer *et al.*^[44] demonstrated the combined analysis of methylation status of SEPT9 and ALX4 to be highly significant in the detection of colorectal polyps with a sensitivity and specificity reaching 71% and 95%, respectively, indicating the potential use of the combined methods in detecting advanced precancerous colorectal lesions. However, further studies are still required to evaluate the effect of combined biomarker assays on CRC detection and screening.

Limitations of the SEPT9 methylation assay

Although the plasma-based SEPT9 methylation assay performs well with regard to both sensitivity and specificity, its clinical availability is still limited. As we can see in Table 1, there is a large degree of

heterogeneity among studies, which may be due to many causes, especially the impacts of non-tumor-related factors on DNA methylation, such as aging, sex, race, hormone levels, dietary factors^[45], lifestyle factors (smoking and alcohol consumption)^[46], and other environmental exposure factors. Song *et al.*^[47] found a high PDR (positive detection rate) of SEPT9 methylation in normal subjects and cancer patients over 60 years, which may reflect increased SEPT9 gene methylation levels with age. Additionally, the increased false negative rate of the SEPT9 assay is associated with diabetes, arthritis and arteriosclerosis ($P < 0.05$)^[33], which can explain why the diagnostic performance of the SEPT9 assay varies compared to previous retrospective case-control studies. Nevertheless, not enough is known to approximate the effect of demographic characteristics, pathological features and/or comorbidities on the results of the SEPT9 methylation assay. Moreover, using a 2/3 algorithm test has a high true negative rate, although its sensitivity was higher with a 1/3 algorithm test^[35]. On account of the capability of excluding non-cancer samples and avoiding the rate of misdiagnosis, the 2/3 algorithm is recommended for CRC detection. Therefore, the technique and method selection could also affect the laboratory results and lead to heterogeneity. Further studies should pay more attention to examining the variation in diagnostic accuracy and validating potential confounding factors affecting DNA methylation status, in the design of future experimental studies. These non-neoplastic factors should be taken into consideration when evaluating DNA methylation to avoid the influence those caused on the testing results.

The cost-effectiveness is another limitation that limited large-scale application of the SEPT9 methylation assay. It was reported^[48] that the methylated SEPT9-based strategies were not a cost-saving with the costs of \$8400 to \$11500 per quality-adjusted life-year gained in comparison with established screening strategies including FOBT, FIT, and colonoscopy. The current cost of the methylated SEPT9 test in Europe is approximately 150 Euros, considerably more than fecal tests^[31]. In brief, FIT dominated methylated SEPT9 and was preferred among all of the alternatives^[49,50]. Even so, the biomarker for colorectal cancer screening still offers potential benefits over current methods, but in order to realize its full potential, the plasma-based assay will need to be acceptable to clinicians and patients compared to current technologies and the medical environment. As the emerging SEPT9 methylation assay becomes available clinically, the decision over whether to adopt it will require weighing its costs, utilization and longitudinal adherence against the alternative of putting efforts into improving current screening strategies. At the population level, methylated SEPT9 yielded incremental benefit at acceptable costs when it increased the fraction of the population screened more than it was substituted for other strategies^[48]. Thus, screening costs, utilization,

adherence, and follow-up are the influential determinants of the cost-effectiveness of colorectal cancer screening strategies.

Moreover, the capability of the *SEPT9* gene methylation assay for detecting adenomas, which is the most common precancerous lesion of CRC, is limited. For early stage CRC (Stage I), polyps or adenomas, methylated *SEPT9* alone presented quite low sensitivity with approximately 35%^[25], 20%^[51] and 11.2%^[31], respectively, indicating that this biomarker may be far from sufficient and effective at screening asymptomatic CRC patients, despite the diagnostic value of detecting advanced stage CRCs (III-IV). With the transformation of the medical pattern, the focus of hygiene work is switching to prevention rather than curing. Thus, the detection of precancerous or early stage colorectal cancer is very crucial for the health workers to identify high-risk groups and to provide an accurate early diagnosis. Still, this assay faces significant challenges nowadays when introduced for detecting early pre-invasive pathological changes, such as adenomas and premalignant polyps. On the one hand, there is plenty of room for improvement in the method of the methylated *SEPT9* assay itself, such as amelioration of DNA isolation or enhancement of PCR efficiency. On the other hand, the combination of the *SEPT9* assay with other markers in CRC detection is at its initial stage, in spite of the detection rate increasing to 37%^[44] by applying an additional methylation marker like ALX4, but further research is still needed to evaluate the effect of joint detection and to explore its possibility, for the sake of improving the sensitivity for detection of early cancers and advanced adenomas. More studies on early-stage CRC are expected in the future.

FUTURE PERSPECTIVES

Taken together, the use of the plasma-based methylated biomarker *SEPT9* gene should be the alternative approach for CRC screening due to greater diagnostic performance, convenience, and compliance in comparison with non-serological methods. The methylated *SEPT9* assay showed relatively high pooled sensitivity, whereas it was also affected by many factors, leading to the high level of heterogeneity. Future clinical diagnostic studies of methylation in blood should consider the impacts of these factors, especially non-neoplastic factors (*e.g.*, aging, sex, lifestyle, coexistent disease, methodology) on diagnostic accuracy. Moreover, the cost of the *SEPT9* methylation assay is still much higher than the FOBT and FIT. And further investigation of early CRC is still required, as a result of its sensitivity for the asymptomatic population in the screening setting still not being satisfactory, but improvements in accuracy can be expected as the diagnostic technology evolves.

In the future, deciphering epigenetic information including DNA methylation and applying it to the

selection of appropriate detection methods and the development of relevant therapy is likely to transform the diagnosis and treatment of colorectal cancer, consequently decreasing mortality.

REFERENCES

- 1 Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 177-193 [PMID: 28248415 DOI: 10.3322/caac.21395]
- 2 Brenner H, Kloor M, Pox CP. Colorectal cancer. *The Lancet* 2014; **383**: 1490-1502 [DOI: 10.1016/s0140-6736(13)61649-9]
- 3 Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodríguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. *Int J Mol Sci* 2017; **18**: pii E197 [PMID: 28106826 DOI: 10.3390/ijms18010197]
- 4 Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; **66**: 683-691 [PMID: 26818619 DOI: 10.1136/gutjnl-2015-310912]
- 5 Li B, Gan A, Chen X, Wang X, He W, Zhang X, Huang R, Zhou S, Song X, Xu A. Diagnostic Performance of DNA Hypermethylation Markers in Peripheral Blood for the Detection of Colorectal Cancer: A Meta-Analysis and Systematic Review. *PLoS One* 2016; **11**: e0155095 [PMID: 27158984 DOI: 10.1371/journal.pone.0155095]
- 6 Fedewa SA, Sauer AG, Siegel RL, Jemal A. Prevalence of major risk factors and use of screening tests for cancer in the United States. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 637-652 [PMID: 25834147 DOI: 10.1158/1055-9965.EPI-15-0134]
- 7 Kondo Y, Issa JP. DNA methylation profiling in cancer. *Expert Rev Mol Med* 2010; **12**: e23 [PMID: 20663272 DOI: 10.1017/S1462399410001559]
- 8 Lessi F, Beggs A, de Palo M, Anti M, Macarone Palmieri R, Francesconi S, Gomes V, Bevilacqua G, Tomlinson I, Segditsas S. Down-regulation of serum/glucocorticoid regulated kinase 1 in colorectal tumours is largely independent of promoter hypermethylation. *PLoS One* 2010; **5**: e13840 [PMID: 21079778 DOI: 10.1371/journal.pone.0013840]
- 9 Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, Midorikawa Y, Nishimura Y, Sakamoto H, Seto Y, Aburatani H, Kaneda A. Three DNA methylation epigenotypes in human colorectal cancer. *Clin Cancer Res* 2010; **16**: 21-33 [PMID: 20028768 DOI: 10.1158/1078-0432.CCR-09-2006]
- 10 Beggs AD, Jones A, El-Bahrawy M, Abulafi M, Hodgson SV, Tomlinson IP. Whole-genome methylation analysis of benign and malignant colorectal tumours. *J Pathol* 2013; **229**: 697-704 [PMID: 23096130 DOI: 10.1002/path.4132]
- 11 Toiyama Y, Okugawa Y, Goel A. DNA methylation and microRNA biomarkers for noninvasive detection of gastric and colorectal cancer. *Biochem Biophys Res Commun* 2014; **455**: 43-57 [PMID: 25128828 DOI: 10.1016/j.bbrc.2014.08.001]
- 12 Song L, Li Y. *SEPT9*: A Specific Circulating Biomarker for Colorectal Cancer. *Adv Clin Chem* 2015; **72**: 171-204 [PMID: 26471083 DOI: 10.1016/bs.acc.2015.07.004]
- 13 Tucker KL. Methylated cytosine and the brain: a new base for neuroscience. *Neuron* 2001; **30**: 649-652 [PMID: 11430798 DOI: 10.1016/S0896-6273(01)00325-7]
- 14 McIlhatton MA, Burrows JF, Donaghy PG, Chanduloy S, Johnston PG, Russell SE. Genomic organization, complex splicing pattern and expression of a human septin gene on chromosome 17q25.3. *Oncogene* 2001; **20**: 5930-5939 [PMID: 11593400 DOI: 10.1038/sj.onc.1204752]
- 15 McDade SS, Hall PA, Russell SE. Translational control of *SEPT9* isoforms is perturbed in disease. *Hum Mol Genet* 2007; **16**: 742-752 [PMID: 17468182 DOI: 10.1093/hmg/ddm003]
- 16 Peterson EA, Stanbery L, Li C, Kocak H, Makarova O, Petty EM. *SEPT9_i1* and genomic instability: mechanistic insights and relevance to tumorigenesis. *Genes Chromosomes Cancer* 2011; **50**: 940-949 [PMID: 21910160 DOI: 10.1002/gcc.20916]

- 17 **Kurosu T**, Tsuji K, Ohki M, Miki T, Yamamoto M, Kakihana K, Koyama T, Taniguchi S, Miura O. A variant-type MLL/SEPT9 fusion transcript in adult de novo acute monocytic leukemia (M5b) with t(11;17)(q23;q25). *Int J Hematol* 2008; **88**: 192-196 [PMID: 18642054 DOI: 10.1007/s12185-008-0133-0]
- 18 **Gonzalez ME**, Makarova O, Peterson EA, Privette LM, Petty EM. Up-regulation of SEPT9_v1 stabilizes c-Jun-N-terminal kinase and contributes to its pro-proliferative activity in mammary epithelial cells. *Cell Signal* 2009; **21**: 477-487 [PMID: 19071215 DOI: 10.1016/j.cellsig.2008.11.007]
- 19 **Ito H**, Iwamoto I, Morishita R, Nozawa Y, Narumiya S, Asano T, Nagata K. Possible role of Rho/Rhotekin signaling in mammalian septin organization. *Oncogene* 2005; **24**: 7064-7072 [PMID: 16007136 DOI: 10.1038/sj.onc.1208862]
- 20 **Burrows JF**, Chanduloy S, McIlhatton MA, Nagar H, Yeates K, Donaghy P, Price J, Godwin AK, Johnston PG, Russell SE. Altered expression of the septin gene, SEPT9, in ovarian neoplasia. *J Pathol* 2003; **201**: 581-588 [PMID: 14648661 DOI: 10.1002/path.1484]
- 21 **Banister CE**. Review of Epigenetics: A Reference Manual: A book edited by Jeffrey M. Craig and Nicholas C. Wong. *Epigenetics* 2014; **7**: 963-964 [DOI: 10.4161/epi.21137]
- 22 **Tóth K**, Galamb O, Spisák S, Wichmann B, Sipos F, Valcz G, Leiszter K, Molnár B, Tulassay Z. The influence of methylated septin 9 gene on RNA and protein level in colorectal cancer. *Pathol Oncol Res* 2011; **17**: 503-509 [PMID: 21267688 DOI: 10.1007/s12253-010-9338-7]
- 23 **Payne SR**. From discovery to the clinic: the novel DNA methylation biomarker (m)SEPT9 for the detection of colorectal cancer in blood. *Epigenomics* 2010; **2**: 575-585 [PMID: 22121975 DOI: 10.2217/epi.10.35]
- 24 **Issa IA**, Noureddine M. Colorectal cancer screening: An updated review of the available options. *World J Gastroenterol* 2017; **23**: 5086-5096 [PMID: 28811705 DOI: 10.3748/wjg.v23.i28.5086]
- 25 **Molnár B**, Tóth K, Barták BK, Tulassay Z. Plasma methylated septin 9: a colorectal cancer screening marker. *Expert Rev Mol Diagn* 2015; **15**: 171-184 [PMID: 25429690 DOI: 10.1586/14737159.2015.975212]
- 26 **Lamb YN**, Dhillon S. Epi proColon® 2.0 CE: A Blood-Based Screening Test for Colorectal Cancer. *Mol Diagn Ther* 2017; **21**: 225-232 [PMID: 28155091 DOI: 10.1007/s40291-017-0259-y]
- 27 **Tóth K**, Sipos F, Kalmár A, Patai AV, Wichmann B, Stoehr R, Golcher H, Schellerer V, Tulassay Z, Molnár B. Detection of methylated SEPT9 in plasma is a reliable screening method for both left- and right-sided colon cancers. *PLoS One* 2012; **7**: e46000 [PMID: 23049919 DOI: 10.1371/journal.pone.0046000]
- 28 **Su XL**, Wang YF, Li SJ, Zhang F, Cui HW. High methylation of the SEPT9 gene in Chinese colorectal cancer patients. *Genet Mol Res* 2014; **13**: 2513-2520 [PMID: 24535900 DOI: 10.4238/2014.January.17.5]
- 29 **Jin P**, Kang Q, Wang X, Yang L, Yu Y, Li N, He YQ, Han X, Hang J, Zhang J, Song L, Han Y, Sheng JQ. Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm. *J Gastroenterol Hepatol* 2015; **30**: 830-833 [PMID: 25471329 DOI: 10.1111/jgh.12855]
- 30 **Wu D**, Zhou G, Jin P, Zhu J, Li S, Wu Q, Wang G, Sheng J, Wang J, Song L, Han X, Qian J. Detection of Colorectal Cancer Using a Simplified SEPT9 Gene Methylation Assay Is a Reliable Method for Opportunistic Screening. *J Mol Diagn* 2016; **18**: 535-545 [PMID: 27133379 DOI: 10.1016/j.jmoldx.2016.02.005]
- 31 **Church TR**, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, Castaños-Vélez E, Blumenstein BA, Rösch T, Osborn N, Snover D, Day RW, Ransohoff DF; PRESEPT Clinical Study Steering Committee, Investigators and Study Team. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 2014; **63**: 317-325 [PMID: 23408352 DOI: 10.1136/gutjnl-2012-304149]
- 32 **Johnson DA**, Barclay RL, Mergener K, Weiss G, König T, Beck J, Potter NT. Plasma Septin9 versus fecal immunochemical testing for colorectal cancer screening: a prospective multicenter study. *PLoS One* 2014; **9**: e98238 [PMID: 24901436 DOI: 10.1371/journal.pone.0098238]
- 33 **Ørntoft MB**, Nielsen HJ, Ørntoft TF, Andersen CL; Danish Study Group on Early Detection of Colorectal Cancer. Performance of the colorectal cancer screening marker Sept9 is influenced by age, diabetes and arthritis: a nested case-control study. *BMC Cancer* 2015; **15**: 819 [PMID: 26514170 DOI: 10.1186/s12885-015-1832-6]
- 34 **Potter NT**, Hurban P, White MN, Whitlock KD, Lofton-Day CE, Tetzner R, Koenig T, Quigley NB, Weiss G. Validation of a real-time PCR-based qualitative assay for the detection of methylated SEPT9 DNA in human plasma. *Clin Chem* 2014; **60**: 1183-1191 [PMID: 24938752 DOI: 10.1373/clinchem.2013.221044]
- 35 **Nian J**, Sun X, Ming S, Yan C, Ma Y, Feng Y, Yang L, Yu M, Zhang G, Wang X. Diagnostic Accuracy of Methylated SEPT9 for Blood-based Colorectal Cancer Detection: A Systematic Review and Meta-Analysis. *Clin Transl Gastroenterol* 2017; **8**: e216 [PMID: 28102859 DOI: 10.1038/ctg.2016.66]
- 36 **Tóth K**, Wasserkort R, Sipos F, Kalmár A, Wichmann B, Leiszter K, Valcz G, Juhász M, Miheller P, Patai AV, Tulassay Z, Molnár B. Detection of methylated septin 9 in tissue and plasma of colorectal patients with neoplasia and the relationship to the amount of circulating cell-free DNA. *PLoS One* 2014; **9**: e115415 [PMID: 25526039 DOI: 10.1371/journal.pone.0115415]
- 37 **Lieberman DA**. Clinical practice. Screening for colorectal cancer. *N Engl J Med* 2009; **361**: 1179-1187 [PMID: 19759380 DOI: 10.1056/NEJMc0902176]
- 38 **Whitlock EP**, Lin JS, Liles E, Beil TL, Fu R. Screening for colorectal cancer: a targeted, updated systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2008; **149**: 638-658 [PMID: 18838718 DOI: 10.7326/0003-4819-149-9-200811040-00245]
- 39 **Lee JK**, Liles EG, Bent S, Levin TR, Corley DA. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. *Ann Intern Med* 2014; **160**: 171 [PMID: 24658694 DOI: 10.7326/M13-1484]
- 40 **Song L**, Jia J, Peng X, Xiao W, Li Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. *Sci Rep* 2017; **7**: 3032 [PMID: 28596563 DOI: 10.1038/s41598-017-03321-8]
- 41 **Herbst A**, Kolligs FT. Detection of DNA hypermethylation in remote media of patients with colorectal cancer: new biomarkers for colorectal carcinoma. *Tumour Biol* 2012; **33**: 297-305 [PMID: 22362383 DOI: 10.1007/s13277-012-0346-y]
- 42 **Dong W**. Detecting plasma methylated Septin9 gene combined with fecal immunochemical test in screening colorectal cancer and adenoma in outpatients(in Chinese). *Zhongguo Yixue Wenzhai* 2016; **(2)**: 44
- 43 **Yu D**, Zhang XH, Lu XX. Study on diagnostic value of SEPT9 gene methylation in serum for colorectal cancer (in Chinese). *Linchuang Jiannan Zazhi* 2015; **33**: 687 [DOI: 10.13602/j.cnki.jcls.2015.09.12]
- 44 **Tänzer M**, Balluff B, Distler J, Hale K, Leodolter A, Röcken C, Molnar 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: 20140221 DOI: 10.1371/journal.pone.0009061]
- 45 **Li L**, Choi JY, Lee KM, Sung H, Park SK, Oze I, Pan KF, You WC, Chen YX, Fang JY, Matsuo K, Kim WH, Yuasa Y, Kang D. DNA methylation in peripheral blood: a potential biomarker for cancer molecular epidemiology. *J Epidemiol* 2012; **22**: 384-394 [PMID: 22863985 DOI: 10.2188/jea.JE20120003]
- 46 **Ogino S**, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, Meyerhardt JA, Meissner A, Schernhammer ES, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol* 2013; **26**: 465-484 [PMID: 23307060 DOI: 10.1038/modpathol.2012.214]
- 47 **Song L**, Jia J, Yu H, Peng X, Xiao W, Gong Y, Zhou G, Han X, Li Y. The performance of the mSEPT9 assay is influenced by algorithm, cancer stage and age, but not sex and cancer location. *J Cancer Res Clin Oncol* 2017; **143**: 1093-1101 [PMID: 28224298 DOI: 10.1007/s00432-017-2363-0]
- 48 **Ladabaum U**, Allen J, Wandell M, Ramsey S. Colorectal cancer screening with blood-based biomarkers: cost-effectiveness of

- methyated septin 9 DNA versus current strategies. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 1567-1576 [PMID: 23796793 DOI: 10.1158/1055-9965.EPI-13-0204]
- 49 **Ladabaum U**, Alvarez-Osorio L, Rösch T, Brueggenjuergen B. Cost-effectiveness of colorectal cancer screening in Germany: current endoscopic and fecal testing strategies versus plasma methylated Septin 9 DNA. *Endosc Int Open* 2014; **2**: E96-E104 [PMID: 26135268 DOI: 10.1055/s-0034-1377182]
- 50 **Sharaf RN**, Ladabaum U. Comparative effectiveness and cost-effectiveness of screening colonoscopy vs. sigmoidoscopy and alternative strategies. *Am J Gastroenterol* 2013; **108**: 120-132 [PMID: 23247579 DOI: 10.1038/ajg.2012.380]
- 51 **Grützmann R**, Molnar B, Pilarsky C, Habermann JK, Schlag PM, Saeger HD, Miehke 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 septin 9 DNA methylation assay. *PLoS One* 2008; **3**: e3759 [PMID: 19018278 DOI: 10.1371/journal.pone.0003759]
- 52 **Behrouz Sharif S**, Hashemzadeh S, Mousavi Ardehaie R, Eftekharsadat A, Ghojazadeh M, Mehrtash AH, Estiar MA, Teimoori-Toolabi L, Sakhinia E. Detection of aberrant methylated SEPT9 and NTRK3 genes in sporadic colorectal cancer patients as a potential diagnostic biomarker. *Oncol Lett* 2016; **12**: 5335-5343 [PMID: 28105243 DOI: 10.3892/ol.2016.5327]

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