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SHORT COMMUNICATION

Circulating DNA markers for assessing antitumor therapy efficacy and monitoring metastatic colorectal cancer

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Abstract

Introduction: Colorectal cancer remains one of the leading causes of oncological morbidity and mortality. Aberrant methylation patterns of tumor-suppressor genes and oncogenes have been detected in circulating DNA isolated from the blood of cancer patients, suggesting their potential utility as diagnostic and prognostic markers.

Objective: To evaluate changes in the methylation levels of *VIM*, *SEPTIN9*, and *DCC* genes in cell-surface-bound cell-free DNA (csb-cfDNA) from the blood of metastatic colorectal cancer (mCRC) patients after antitumor treatment over a long-term follow-up period.

Methods: Blood samples were collected from mCRC patients (n = 21) at baseline, after three courses of preoperative chemotherapy (FOLFOXIRI regimen), 10–15 days post-surgery, and every three months during follow-up. The methylation levels of *VIM*, *SEPTIN9*, and *DCC* genes were quantified by methylation-specific polymerase chain reaction.

Results: Preoperative chemotherapy and tumor resection were each associated with a significant decrease in *SEPTIN9* and *VIM* methylation levels in csb-cfDNA compared to baseline (2.3-fold and 2.0-fold, respectively; $p \leq 0.05$). DCC methylation decreased 1.5-fold after surgery relative to pre-treatment levels ($p \leq 0.05$). During follow-up after completion of combined treatment, methylation levels of *SEPTIN9*, *VIM*, and *DCC* remained stable in mCRC patients without signs of disease progression (n = 8), showing no significant change from post-surgical values (days 10–15). In contrast, mCRC patients with disease progression (n = 13) exhibited increased methylation levels of *SEPTIN9*, *VIM* and *DCC* compared to post-resection values.

Conclusion: These findings indicate that monitoring *VIM*, *DCC*, and *SEPTIN9* methylation levels in csb-cfDNA has potential for evaluating the effectiveness of antitumor therapy and for early detection of relapses in mCRC patients during the follow-up period.

Keywords: Metastatic colorectal cancer; Relapse diagnostics; Monitoring; Methylation; Circulating DNA

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide. Most cases are treated with a combination of chemotherapy, radiotherapy, and surgery, although treatment is often accompanied by complications that can compromise overall survival rates.[1] Evidence from large clinical trials suggests that radiotherapy may be unnecessary for certain forms of rectal cancer, potentially sparing patients from radiation-induced side effects.[2,3] Nevertheless, any treatment regimen carries a risk of residual disease and early relapse, underscoring the need for sensitive, minimally invasive tests to facilitate the early detection of recurrence during post-treatment follow-up.

Several blood-based markers of rectal cancer have been identified.[4] Among these, nucleic acid-based alterations, such as somatic mutations and aberrant DNA methylation, have been detected in the plasma and serum of cancer patients and have been the subject of intensive research in the last few decades. Aberrant DNA methylation often precedes other molecular changes and may contribute to the initiation of cancer development.[5] Both hypo- and hypermethylation have been observed across early-stage cancer, tumor development, and disease progression.[6]

Hypermethylation status of single-copy genes in circulating DNA from blood plasma, including *RARB*, *RASSF1A*, *APC*, *MGMT*, *ITGA4*, *MAP3K14-AS1*, *IKZF1*, *CLDN1*, *MSC*, *INHBA*, *SLC30A10*, *BCAT1*, *SEPT9*, *GRIA4*, *SLC8A1*, *SYN3*, *T-UCRs*, *TMEM240*, *EYA4*, and *EHD3*, enables detection of CRC with 60–80% sensitivity and 70–90% specificity. However, neither individual markers nor their combinations have yet achieved the performance required for a clinically effective test.[7] Still, *SEPTIN9*, *IKZF1*, *BCAT1*, and *SDC2* have demonstrated the highest diagnostic value and, therefore, have been incorporated into commercial assays now used in clinical practice. Currently available tests include EpiproColon (Epigenomics, Germany), targeting *SEPTIN9*; ColoDefense (VersaBio, China), targeting *SEPTIN9*, *SDC2*; and the SpecColon stool test (China), targeting *SFRP2* and *SDC2*, all approved for CRC screening and diagnosis. The COLVERA™ assay (*IKZF1*, *BCAT1*) provides clinically relevant information regarding the completeness of surgical resection, relapse-free survival, and residual disease risk.[7]

Most studies developing cell-free DNA (cfDNA)-based assays for CRC have enrolled both localized and metastatic CRC (mCRC) patients, with only a few publications identifying markers specific to metastatic forms. Correspondingly, few cfDNA methylation markers have been studied for monitoring tumor response to multimodal treatment in mCRC patients. For instance, Vagionas *et al.*[8] analyzed methylation levels of *SOX17* and *WNT5a* in plasma cfDNA and reported an association between hypermethylation of these genes and both disease burden and poor survival in patients with mCRC.[8] Separate research groups have assessed chemotherapy and radiotherapy efficacy in mCRC based on methylation markers in plasma cfDNA, with hypermethylation of *NPY*, *WIF1*, *SFRP2*, and *SDC2* linked to unfavorable antitumor treatment outcomes.[9-11] Chen *et al.*[12] monitored disease progression in patients with inoperable CRC and liver metastases by quantifying *SEPTIN9* and *NPY* methylation levels.

However, to date, no studies have examined the combined methylation profiles of *SEPTIN9*, *VIM*, and *DCC* in cell-surface-bound cfDNA (csb-cfDNA) from mCRC patients across the full treatment trajectory (from baseline through post-treatment follow-up) in order to assess their utility for early detection of relapse and metastases. Here, we present results from a pilot exploratory study evaluating the significance of circulating methylated markers (*SEPTIN9*, *VIM*, and *DCC*) for the low-invasive follow-up of mCRC patients after treatment.

2. Materials and methods

2.1. Patient characteristics, treatment, and follow-up

This pilot study enrolled 21 patients aged 35–70 years with a morphologically verified diagnosis of mCRC and an isolated liver metastasis (stage IV), who were treated at the Tomsk Cancer Research Institute between 2021 and 2024. The study included patients with tumors of the colon (cecum, ascending, transverse, descending, and sigmoid colon, as well as the hepatic and splenic flexures), the rectosigmoid junction, and the upper ampullary rectum.

All procedures were carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Tomsk Cancer Research Institute. Clinico-morphological features of mCRC patients are presented in Table 1. This cohort of patients is completely different from the cohort of patients reported previously.[13]

In the first stage of combined treatment, patients received three courses of preoperative chemotherapy according to the FOLFOXIRI regimen: irinotecan 165 mg/m^2, intravenous (IV), 60-minute infusion, day 1; oxaliplatin 85 mg/m^2, IV, 120-minute infusion, day 1; leucovorin 200 mg/m^2, IV, 40-minute infusion, days 1–2; 5-fluorouracil 3,200 mg/m^2, IV, 48-hour infusion, days 1–2. Targeted therapy was added based on mutational status: cetuximab 500 mg/m^2 (IV, 60-minute infusion, day 1) for patients with wild-type *KRAS* and *BRAF*, or bevacizumab 5 mg/kg (IV, 60-minute infusion, day 1) for patients with

mutant *KRAS* and *BRAF*. Three weeks after completion of preoperative treatment, patients underwent surgery involving simultaneous resection of the primary tumor and hepatic metastatic lesions. All mCRC patients with isolated liver involvement received adjuvant chemotherapy according to the FOLFOXIRI regimen.

Table 1. Clinico-morphological features of metastatic colorectal cancer patients

Clinico-morphological features	Values (*n*, %)
Gender (*n* = 21)	
≤60 years	14 (67%)
>60 years	7 (33%)
Age (*n* = 21)	
Female	12 (57%)
Male	9 (43%)
Stage (*n* = 21)	
I–II	0
III	0
IV	21 (100%)
Histology type (*n* = 21)	
SCC	0
AD	21 (100%)
Degree of tumor cell differentiation (*n* = 21)	
High grade	4 (19%)
Moderate	6 (29%)
Low grade	11 (52%)
Outcome of disease (*n* = 21)	
Favorable prognosis[a]	8 (38%)
Unfavorable prognosis[b]	13 (62%)
KRAS-mutation status	
wtKRAS	19 (90%)
mtKRAS	2 (10%)
BRAF-mutation status	
wtBRAF	21 (100%)
mtBRAF	0

Notes: a: Without distant metastasis, b: With distant metastasis. Abbreviations: AC: Adenocarcinoma, mt: Mutant-type; *n*: Number of patients in group; SCC: Squamous cell carcinoma; wt: Wild-type.

Pre-treatment assessment included general clinical tests, video colonoscopy, tumor biopsy with histological examination, contrast-enhanced spiral computed tomography (CT) of the chest and abdomen, contrast-enhanced magnetic resonance imaging (MRI) of the pelvic organs, mutational analysis of *KRAS*, *NRAS*, *BRAF*, and *EGFR*, and measurement of serum tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9).

The clinical and radiological response to preoperative treatment was evaluated using RECIST 1.1 criteria. Adverse events were graded according to NCI CTCAE (v.4.03). Post-operative complications were classified according to the Clavien–Dindo classification. The tumor pathomorphological response to chemotherapy and targeted therapy was assessed using the Mandard tumor regression grading system.

Venous blood samples were collected at the following time points: before treatment (baseline), 15–30 days after the final course of preoperative chemotherapy, on days 10–15 post-surgery, and every three months during follow-up (up to one year).

Patient follow-up was performed every three months and included IV contrast-enhanced spiral CT scans of the abdominal and thoracic cavities, contrast-enhanced MRI scans of the pelvic organs, video colonoscopy, and monthly determination of CEA levels. In patients with disease progression, distant metastases were detected in the retroperitoneum, liver, or lungs. The progression-free follow-up period ranged from 58 to 65 months.

2.2. Circulating DNA extraction and bisulfite conversion

Venous blood samples were stabilized and fractionated into plasma and blood cells as described previously.[14] csb-cfDNA was isolated using a previously established protocol.[14] Briefly, blood cells were incubated with nine volumes of phosphate-buffered saline (PBS) containing 5 mM ethylenediaminetetraacetic acid (EDTA) for 5 min at room temperature. Cells were pelleted by centrifugation, incubated with an equal volume of 0.25% trypsin solution, and then inactivated with a trypsin inhibitor for 4 min at room temperature. Plasma, PBS/EDTA, and trypsin eluates were centrifuged for an additional 20 min at 800× g and stored at −40 °C in aliquots. cfDNA was isolated from the csb-fraction eluates using the QIAamp DNA Micro kit (Cat. No. 56304, Qiagen, United States of America [USA]) and subsequently subjected to sodium bisulfite modification and purification using the EZ DNA Methylation-Direct kit (Cat. No. D5020, Zymo Research, USA).

2.3. Methylation-specific quantitative polymerase chain reaction

Methylated fragments of *SEPTIN9* were quantified by TaqMan-based quantitative polymerase chain reaction

(qPCR). Reactions were performed in a final volume of 25 µL containing 2.5 µL of DNA, 60 nM of each specific primer, 30 nM of a sequence-specific fluorescent probe, 1 U DNA polymerase, 1× polymerase chain reaction (PCR) buffer, 200 µM dNTP, and 2.5 mM $MgCl_2$. The PCR cycling program included initial denaturation at 95 °C for 10 minutes, followed by 40 amplification cycles: 95 °C for 15 seconds, then annealing and elongation at 56 °C for 1 minute, with fluorescence acquisition during the latter step.

Methylated fragments of *VIM* and *DCC* were quantified by EvaGreen-based qPCR (Biotium, USA). Reactions were performed in a final volume of 25 µL containing 2.5 µL of DNA, 2.5 mM $MgCl_2$, 1× PCR buffer, 200 µM dNTP, 60 nM of each gene-specific primer, 1× EvaGreen dye, and 1 U Taq DNA polymerase. The PCR program consisted of an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles: 95 °C for 15 seconds, gene-specific annealing (60 °C for *VIM*; 58.5 °C for DCC) for 10 seconds, and extension at 72 °C for 20 seconds with fluorescence acquisition at this step.

Unmethylated *ACTB* was used as a reference gene and quantified using the same TaqMan-based protocol as *SEPTIN9*. A fully methylated DNA sample (Zymo Research, USA) was used as a control. Amplification was performed using a CFX96 instrument (Bio-Rad, USA). Primer and fluorescent probe sequences are provided in Table 2.

The methylation index (MI) was calculated as follows:

$$\text{MI (\%)} = 100 \times (\text{methylated target gene fragments}/(\text{methylated target gene fragments} + \text{unmethylated } \textit{ACTB} \text{ fragments}) \quad (1)$$

Statistical analysis was performed using Statistica 8.0 and MedCalc software version 23.6.1 (Ostend, Belgium). The Mann–Whitney *U* test was used to assess the significance of differences between comparison groups. Relapse-free survival rate (RFS) were evaluated using the Kaplan–Meier method. Correlations between methylation levels, protein marker levels, and mutational status were analyzed using Spearman's rank correlation. Diagnostic significance was assessed via receiver operating characteristic analysis.

3. Results

In this study, we analyzed dynamic changes in the methylation levels of *SEPTIN9*, *VIM*, and *DCC* in csb-cfDNA from the blood of mCRC patients throughout the follow-up period after neoadjuvant chemotherapy and surgery.

Methylation levels of all three markers changed significantly over the course of treatment (Figure 1). Reductions in *SEPTIN9* and *VIM* methylation were observed in 68% (14/20) and 60% (12/20) of mCRC patients, respectively, and were associated with the response to chemotherapy (baseline versus post-chemotherapy: $p = 0.03$ and $p = 0.05$, Mann–Whitney *U* test). *DCC* methylation, however, did not change in response to chemotherapy ($p = 0.215$).

Table 2. Oligonucleotide sequences for quantitative methyl-specific polymerase chain reaction

Primers and probes	Sequences 5'–3'
SEPT9-F	AAATAATCCCATCCAACTA
SEPT9-Rev	GATT-TEG-GTTGTTTATTAGTTATTATGT
SEPT9-Blocker	GTTATTATGTTGGATTTTGTGGTTAATGTGTAG-SpC3
SEPT9-Probe	FAM-TTAACCGCGAAATCCGAC-BHQ1
VIM-F	TCGTTTCGAGGTTTTCGCGTTAGAGAC
VIM-Rev	CGACTAAAACTCGACCGACTCGCGA
DCC-F	TTGTTCGCGGATTTTGGTTTC
DCC-Rev	ACCGATTACTTAAAAATACGCG
ACTB-F	TGGTGATGGAGGAGGTTTAGTAAGT
ACTB-Rev	AACCAATAAAACCTACTCCTCCCTTAA
ACTB-Probe	FAM-ACCACCACCCAACACACAATAACAACAAACACA-BHQ1

Following surgery, *SEPTIN9* and *VIM* methylation levels decreased further, with post-operative values being significantly lower than pre-treatment levels in nearly all patients (2.3-fold and 2.0-fold reductions, respectively; $p = 0.02$ and $p = 0.03$, paired t-test; Figure 1). In contrast, *DCC* methylation was 1.5-fold higher post-surgery than at baseline ($p = 0.05$, paired t-test). No significant associations were found between changes in *VIM*, *SEPTIN9*, or *DCC* methylation levels and patient age, sex, *KRAS* or *BRAF* mutation status, or serum CEA levels ($p \geq 0.05$, Spearman's test). Notably, a positive correlation between the *SEPTIN9* methylation level and serum CA 19-9 level was demonstrated ($R = 0.471$, $p < 0.05$, Spearman's test).

It was shown that in 88% of cases ($n = 7/8$), decreased methylation levels for *VIM* (less than 55%), *DCC* (less than 38%), and *SEPTIN9* (less than 58%) were associated with disease-free survival in patients with mCRC (hazard ratio = 0.56; 95% confidence interval: 0.34–0.92; $p = 0.02$; median follow-up interval was 2 years; Figure 2). The average methylation levels of the *VIM*, *DCC*, and *SEPTIN9* genes in csb-cfDNA from mCRC patients were used as the threshold values.

Next, we analyzed changes in *SEPTIN9*, *VIM*, and *DCC* methylation levels in csb-cfDNA from the blood of mCRC patients during the follow-up period after the completion of combined treatment. In mCRC patients without signs of disease progression ($n = 8$), methylation levels of all three markers remained stable throughout follow-up, showing no significant change from values recorded on days 10–15 post-surgery. Conversely, patients with disease progression ($n = 13$) showed increased methylation of *SEPTIN9*, *VIM*, and *DCC* relative to post-resection levels (Figure 3). Notably, according to the results of instrumental examination methods, there were no signs of disease progression at that same time point. Due to detected changes in methylation levels of circulating markers in the blood of these patients, additional control examinations using instrumental methods were performed after 1–2 months (median: 1.5 months), which provided clinical confirmation of mCRC progression.

Receiver operating characteristic analysis demonstrated

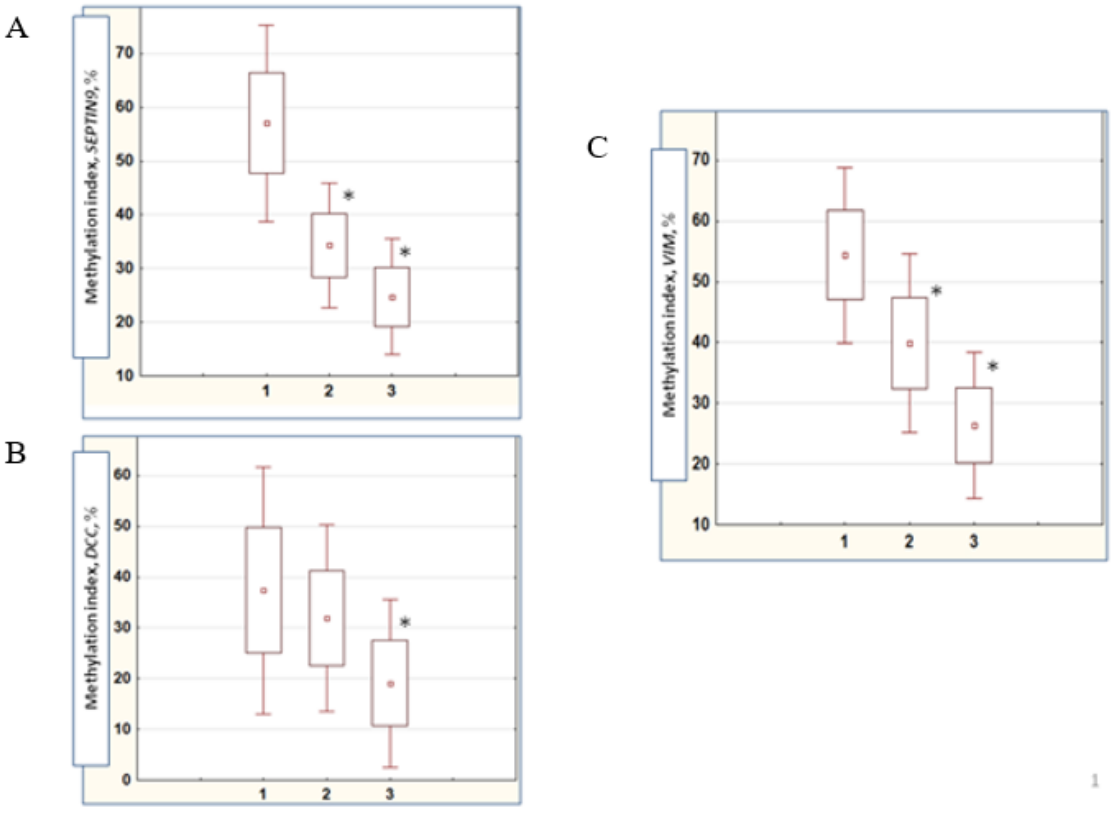


Figure 1. Methylation levels of circulating markers in blood from metastatic colorectal cancer patients at different stages of treatment. (A) *SEPTIN9*, (B) *DCC*, (C) *VIM*.
Notes: 1: Before treatment; 2: after chemotherapy; 3: after surgery; * $p \leq 0.05$.

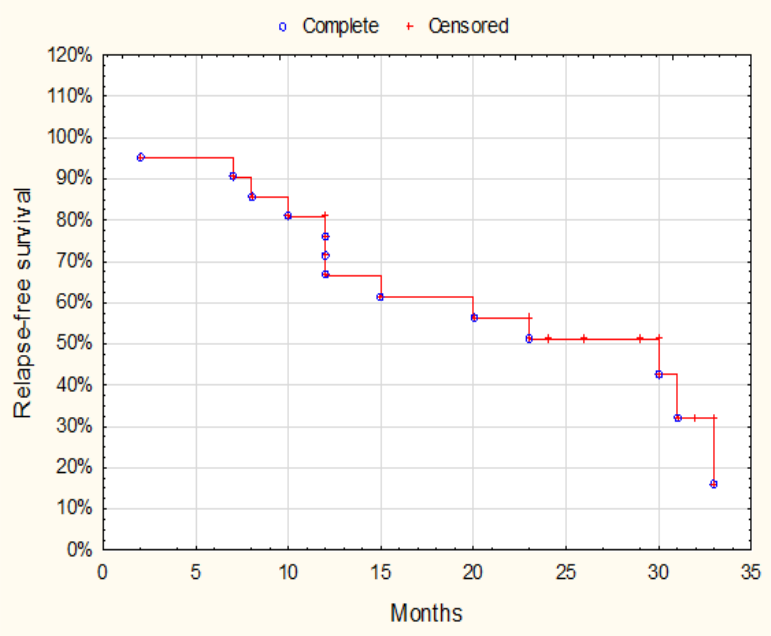


Figure 2. Relapse-free survival of metastatic colorectal cancer patients

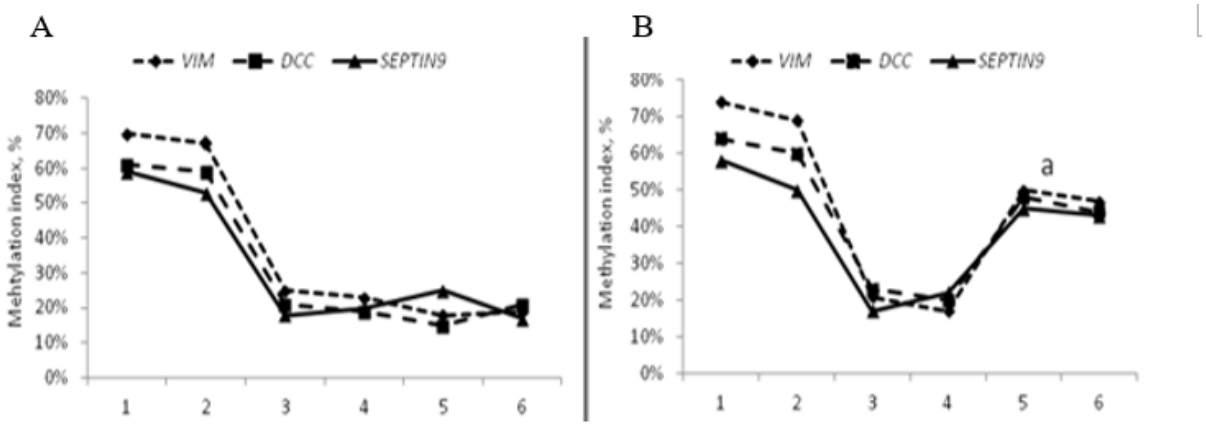


Figure 3. Dynamic changes in methylation levels of circulating markers in blood from metastatic colorectal cancer patients during the follow-up period
Notes: A: Patient with signs of disease progression; B: patient without signs of disease progression; 1: before treatment; 2: after chemotherapy; 3: 10–15 days after surgery; 4: 6 months after surgery; 5: 9 months after surgery; 6: 1 year after surgery; a: time of clinical relapse confirmation by instrumental examination.

that the individual markers enabled effective differentiation between mCRC patients with and without disease progression. Specifically, *SEPTIN9* methylation differentiated these groups with 80% sensitivity and 74% specificity (threshold: 58%, area under the curve [AUC]: 0.83). *VIM* methylation yielded a sensitivity and specificity of 81% and 70%, respectively (threshold: 55%, AUC: 0.78), while *DCC* provided 81% sensitivity and 70% specificity (threshold: 38%, AUC: 0.76). Remarkably, the combination of all three markers (*SEPTIN9+VIM+DCC*) improved the diagnostic performance, achieving a sensitivity of 81% and a specificity of 80% (AUC: 0.87; Figure 4).

4. Discussion

In recent years, research into circulating methylated markers in CRC has expanded rapidly, with studies focusing on early diagnosis, prognosis, and evaluation of treatment effectiveness.[8-11] However, there is a lack of

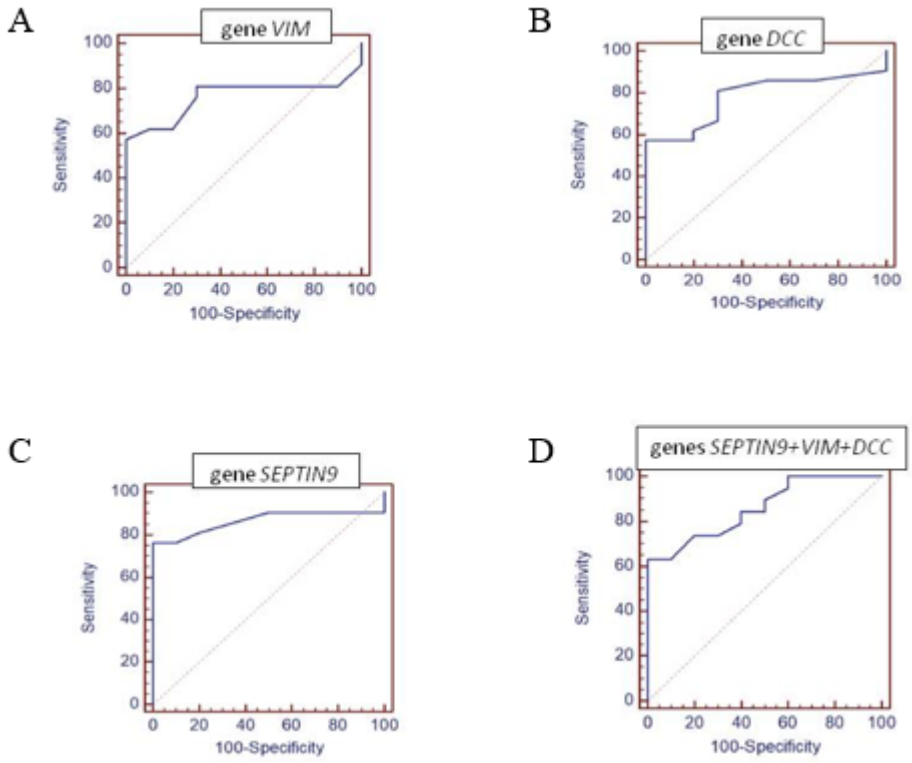


Figure 4. Receiver operating characteristic curve of diagnostic significance of the circulating methylated markers for discrimination between metastatic colorectal cancer (mCRC) patients with disease progression and mCRC patients without disease progression. (A) *VIM* gene, (B) *DCC* gene, (C) *SEPTIN9* gene, (D) *VIM+DCC+SEPTIN9* genes.

comprehensive data on cfDNA methylation monitoring in CRC, particularly in metastatic disease. It remains unclear which aberrantly methylated genes are most informative and at what time intervals they should be assessed. This gap likely reflects a combination of technical and methodological challenges, including variability in the sensitivity of methylation detection assays, the quality of DNA bisulfite conversion, differences in sample processing and extracellular DNA extraction protocols, and heterogeneity in patient cohort composition due to the underestimation of pathogenetically relevant features.

In our study, marker genes were selected according to two criteria: first, the presence of aberrantly methylated forms in cfDNA from blood plasma has been previously established; and second, methylation levels in tumor tissue have been shown to be significantly higher in CRC patients compared with healthy subjects.[15,16] Accordingly, we selected *SEPTIN9*, *VIM*, and *DCC* for analysis. All three genes are involved in regulating the cell cycle, epithelial–mesenchymal transition, cell division, and mitosis, and are hypermethylated in malignant diseases.[17] In the blood plasma of healthy individuals, methylation of the *SEPTIN9*, *VIM*, and *DCC* genes was less than 5%; however, in malignant diseases, particularly gastrointestinal tumors, a significant increase in the methylation level of these genes was detected (for *SEPTIN9* [50–80%]; for *VIM* [53–91%]; for *DCC* [44–83%]).[18-20] According to our results, mCRC patients included in the present study had a high level of methylation of the *SEPTIN9*, *VIM*, and *DCC* genes before treatment, consistent with the above-mentioned reports (pre-treatment methylation levels: *SEPTIN9* mean value 58%; *DCC* mean value *38%*; *VIM* mean value 55% (Figure 1).

The diagnostic potential of methylated markers (*SEPTIN9*, *VIM*) has been demonstrated in several studies. Yuan *et al.*[18] developed a three-gene methylation signature (*SEPTIN9*, *SDC2*, and *VIM*) for CRC diagnosis using tumor tissue samples, achieving high diagnostic accuracy (sensitivity = 96%, specificity = 100%, AUC = 0.997). Liu *et al.*[19] identified a 29-locus methylation panel spanning *SDC2*, *SEPTIN9*, and *VIM* for CRC diagnosis in tissue and stool samples. This panel demonstrated strong and reliable performance in stool-based testing, with a sensitivity of 91.43%, specificity of 100%, and an AUC of 99.31%. The

diagnostic accuracy of this model for stage I and stage II CRC was 100% (11/11) and 91.3% (21/23), respectively.

Yet, few studies have examined the clinical significance of these markers specifically for CRC monitoring, and data on metastatic CRC remain particularly scarce. Gopal and coauthors[21] investigated the utility of circulating methylated markers for CRC monitoring and for predicting response to antitumor therapy. The authors studied the methylation status of *SEPTIN9* and *VIM* promoter regions in a cohort of Indian CRC patients before and after radiotherapy and chemotherapy. However, hypermethylation in cfDNA was detected in only 7% of serum samples from the CRC patients under study, precluding a meaningful analysis of methylation changes in response to antitumor therapy.

The present pilot study addresses this gap by analyzing methylation of *VIM*, *DCC*, and *SEPTIN9* in mCRC patients within a clinically relevant timeframe, i.e., across both the immediate post-treatment period and long-term follow-up. Importantly, we assessed gene methylation in csb-cfDNA from blood. According to our earlier results, the efficiency of gene methylation level detection could be improved by using csb-cfDNA fractions. Tumor-derived cfDNA has been shown to be not only detected in blood plasma but also bound to the surface of blood cells (csb-cfDNA), including both erythrocytes and leukocytes.[22-24] It should be noted that tumor-related DNA typically constitutes a minor fraction of cfDNA and is found at low concentrations, thus requiring high PCR specificity.[25] Therefore, csb-cfDNA can be a valuable source of tumor-derived DNA, which can increase the sensitivity of the methylation marker assay.[26-28] Monitoring of *SEPTIN9*, *VIM*, and *DCC* methylation levels in the blood of mCRC patients during post-treatment follow-up suggested their potential utility for the timely detection of disease recurrence at preclinical or early stages, before tumors become detectable by standard imaging or manifest clinically. Specifically, a methylation level at least two-fold higher than the values recorded on days 10–15 after surgery may serve as a threshold for potential recurrence. When mCRC recurrence is suspected based on changes in these circulating markers, a comprehensive clinical and instrumental evaluation can be recommended to inform further antitumor treatment decisions. Notably, circulating protein markers (CEA, CA 19-9) demonstrated lower sensitivity when compared with methylated DNA markers (*SEPTIN9*, *VIM*, *DCC*) for the monitoring of post-treatment mCRC patients in our study (for the *SEPTIN9+VIM+DCC* combination, sensitivity and specificity were 81% and 80%; for the CEA+CA 19-9 combination, sensitivity and specificity were 60% and 70%, respectively).

This pilot study provides original data, offering fundamental insights into the significant association between methylation level changes of the selected genes in the cfDNA of mCRC patients, therapeutic intervention, and disease recurrence. Due to the limited size of the cohort of mCRC patients included in the present study, the obtained results are explorative but encouraging. Despite the limitations, this study outlines key directions for future research. It is necessary to validate these results in independent groups of mCRC patients and non-metastatic CRC patients, as well as healthy donors and patients with non-oncological gastrointestinal diseases. Our findings expand the potential of these selected markers not only for post-therapeutic monitoring but also for CRC diagnosis. Further efforts should focus on improving the performance of this marker panel by combining it with other circulating nucleic acid-based markers.

5. Conclusion

To summarize, the circulating methylated marker panel comprising *SEPTIN9*, *VIM*, and *DCC* demonstrated potential for evaluating the effectiveness of antitumor therapy and for early detection of relapses in patients with mCRC during follow-up. Notably, all patients who experienced a relapse in this cohort showed elevations of all three methylated circulating markers approximately one month before clinical manifestation, whereas no such elevations were observed in patients who remained relapse-free. To definitively establish the diagnostic and prognostic significance of this three-marker panel, it must be validated in larger, representative cohorts of mCRC patients, healthy subjects, and non-metastatic CRC patients, accounting for patient age, response to preoperative therapy, and individual disease course.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All patients signed informed consent for voluntary participation. The study was approved by the institutional review board of the Tomsk Cancer Research Institute on December 10, 2022 (approval no. 16).

Consent for publication

Written informed consent has been obtained from the participant(s) to publish this paper.

Availability of data

Data will be made available upon reasonable request to the corresponding author.

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