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SCTR hypermethylation is a diagnostic biomarker in colorectal cancer

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Abstract

Diagnostic markers for both colorectal cancer (CRC) and its precursor lesions are lacking. Although aberrant methylation of the secretin receptor (*SCTR*) gene was observed in CRC, the diagnostic performance has not been evaluated. Therefore, this study aimed to assess and verify the diagnostic value of *SCTR* methylation of CRC and its precursor lesions through integrating the largest methylation data. The diagnostic performance of *SCTR* methylation was analyzed in the discovery set from The Cancer Genome Atlas (TCGA) CRC methylation data (N = 440), and verified in a large-scale test set (N = 938) from the Gene Expression Omnibus (GEO). Targeted bisulfite sequencing analysis was developed and applied to detect the methylation status of *SCTR* in our independent validation set (N = 374). Our findings revealed that the *SCTR* gene was frequently hypermethylated at its CpG islands in CRC. In the TCGA discovery set, the diagnostic score was constructed using 4 CpG sites (cg01013590, cg20505223, cg07176264, and cg26009192) and achieved high diagnostic performance (area under the ROC curve [AUC] = 0.964). In the GEO test set, the diagnostic score had robust diagnostic ability to distinguish CRC (AUC = 0.948) and its precursor lesions (AUC = 0.954) from normal samples. Moreover, hypermethylation of the *SCTR* gene was also found in cell-free DNA samples collected from CRC patients, but not in those from healthy controls. In the validation set, consistent results were observed using the targeted bisulfite sequencing array. Our study highlights that hypermethylation at CpG islands of the *SCTR* gene is a potential diagnostic biomarker in CRCs and its precursor lesions.

KEYWORDS

biomarker, colorectal cancer, diagnosis, DNA methylation, *SCTR*

Abbreviations: AJCC, American Joint Commission on Cancer; AUC, area under the ROC curve; cfDNA, cell-free DNA; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; FIT, fecal immunochemical test; FOBT, fecal occult blood testing; GEO, Gene Expression Omnibus; LRES, long-range epigenetic silencing; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

DaPeng Li and Lei Zhang are contributed equally to the article.

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1 | INTRODUCTION

Colorectal cancer (CRC) is currently the third most commonly diagnosed cancer and the second leading cause of mortality in the world based on GLOBOCAN 2018, with an estimate of 1.85 million diagnosed novel cases and 0.88 million deaths.[1] Both hereditary and non-hereditary factors have been linked to the development of CRC.[2,3] Hereditary and genetic factors, including a family history of CRC and inherited mutations, account for a small fraction of CRC cases, however more than half of CRC cases are attributable to non-hereditary factors. Despite improved medications, CRC is still the main cause of cancer death because of advanced-stage diagnosis and the few number of effective drugs. Survival rate of CRC sharply declines at an advanced tumor stage, whereas patients diagnosed at stage I or II disease have a 5-y survival rate of 91% and 82%, and those at stage IV disease have 5-y survival rate of 12%.[4] Early detection of CRC is crucial to reduction in CRC-related mortality. Currently, a various CRC screening tests are available, including colonoscopy, FOBT, and FIT, however the various limitations of these tests have led to poor patient compliance and low participation rates.[5,6] For instance, colonoscopy is invasive and expensive despite being the gold standard for CRC screening. By contrast, blood CRC screening tests may improve participation rates, given the advantage of less invasive procedure and complications.

DNA methylation alterations are involved in initiation and progression of cancers by reprogramming of the epigenetic landscape. DNA methylation has been proposed as a potential biomarker for clinical diagnosis, prognosis, and prediction of treatment responses in various types of cancers, including CRC.[7,8] With respect to CRC diagnosis, some aberrantly methylated genes, such as *SEPT9*, *SFRP2*, *NDRG4*, *BMP3*, and *IKZF1*, have been suggested as potential markers.[9-14] One of the most widely studied methylation-based markers for the diagnosis of CRC is hypermethylation of the *SEPT9* gene, which was commercialized (Epi proColon) and approved by the United States Food and Drug Administration (US FDA) in 2016.[15] The diagnostic performance of this methylation-based test varied in multiple studies, with sensitivity ranging from 48% to 90% and specificity from 73% to 97%, respectively.[7] One of the major limitations is its poor sensitivity for the detection of colorectal adenomas, as detection and removal of these precursor lesions are essential for reducing the incidence of CRC.[9,16-18] Therefore, methylation-based markers for the diagnosis of CRC and its precursor lesions are still required.

The secretin receptor (*SCTR*), which was the first to be discovered in duodenal mucosa, is a G protein-coupled receptor and a member of the glucagon-VIP-secretin receptor.[19] The *SCTR* gene is located on chromosome 2q14.2, which contains 3 hypermethylation genes (*EN1*, *SCTR*, and *INHBB*) in correlation with LRES in CRC.[20] Previous studies have shown that hypermethylated *EN1* in stool DNA may have application as a non-invasive biomarker of CRC.[21] *SCTR* has been found to be hypermethylated in CRC, and was associated with downregulation of gene expression.[20-22] However, the potential of *SCTR* methylation in the diagnosis of CRC has not been previously evaluated. We therefore investigated whether hypermethylation of *SCTR* is a promising early detection molecular marker in CRC and its precursor lesions.

2 | MATERIALS AND METHODS

2.1 | Public data collection

To identify the methylation profile of the *SCTR* gene in CRCs, 10 public methylation datasets were obtained from TCGA and the GEO, https://www.ncbi.nlm.nih.gov/geo/), totaling 1378 samples (Table 1). TCGA CRC methylation dataset (N = 440) of the Illumina 450k array was download from the UCSC Xena Browser (https://xena.ucsc.edu/), and was used as the discovery set. The methylation level of CpG site was expressed as a β value, and calculated as $M/(M+U)$, where M and U represent methylated intensity and unmethylated intensity, respectively. Ten CpG sites throughout the *SCTR* gene were available after removing 12 CpG sites with missing data larger than 20% (Table S1). Furthermore, TCGA CRC gene expression profile was also downloaded from the UCSC Xena Browser. By a systematic search in the GEO database, 8 methylation datasets of the Illumina 450k array were obtained as test set (N = 930): GSE42752, GSE48684, GSE68060, GSE77718, GSE77954, GSE101764, GSE107352, and GSE129364.[23-29] An additional methylation dataset for the Illumina EPIC array with 4 cell-free DNA (cfDNA) samples from CRC and 4 samples from healthy controls were obtained from GSE122126.[30]

2.2 | Construction and evaluation of methylation-based diagnostic score

The workflow is described in Figure 1. Differential methylation analyses between CRC tissues and normal tissues were performed for 10 CpG sites at *SCTR* in the TCGA discovery set. The diagnostic performance of individual CpG site for distinguishing CRC tissues and normal tissues was assessed by ROC curves. Candidate CpG sites were selected based on an AUC of >0.90. Using the logistic regression model, a 4-CpG diagnostic score was constructed in the discovery set. As the test set, 8 GEO methylation datasets were used to evaluate the robustness and generalizability of the 4-CpG diagnostic score. The GEO test set was divided into 2 parts: test set A (CRC tissues and normal tissues) and test set B (adenoma tissues and normal tissues).

2.3 | Inhouse validation study

To further assess the *SCTR* methylation, we performed a quantitative methylation analysis in an independent validation set (N = 374). Briefly, our study collected 10 tissues of hyperplastic polyps, 8 tissues of adenomas, 275 tissues of CRC, 23 adjacent normal tissues,

TABLE 1 Overview of the datasets used in this study

Dataset	Source	Assay	Sample type	Number of samples
Discovery set (N = 440)				
	TCGA CRC	Infinium 450K	Tissue	Normal = 45, CRC = 395
Test set (N = 938)				
Test set A	GSE42752	Infinium 450K	Tissue	Normal = 41, CRC = 22
	GSE48684[a]	Infinium 450K	Tissue	Normal = 41, CRC = 64
	GSE68060[a]	Infinium 450K	Tissue	Normal = 36, CRC = 82
	GSE77718	Infinium 450K	Tissue	Normal = 96, CRC = 96
	GSE77954	Infinium 450K	Tissue	Normal = 11, CRC = 13
	GSE101764	Infinium 450K	Tissue	Normal = 149, CRC = 112
	GSE107352	Infinium 450K	Tissue	Normal = 21, CRC = 30
Test set B	GSE48684[a]	Infinium 450K	Tissue	Normal = 41, Adenoma = 42
	GSE77954[a]	Infinium 450K	Tissue	Normal = 11, Adenoma = 12
	GSE129364	Infinium 450K	Tissue	Normal = 3, Adenoma = 59
Test set C	GSE122126	Infinium EPIC	cfDNA	Normal = 4, CRC = 4
Validation set (N = 374)				
	Inhouse study	Targeted bisulfite sequencing	Tissue	Normal = 23, Polyp = 10, Adenoma = 8, CRC = 275
	Inhouse study	Targeted bisulfite sequencing	WBC	Normal = 29, CRC = 29

Abbreviations: cfDNA, cell-free DNA; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas; WBC, white blood cell.

[a]Normal tissues from GSE48684 and GSE77954 were used in both test set A and test set B.

29 white blood cell samples from CRC, and 29 white blood cell samples from healthy controls. These samples were collected at the Third Affiliated Hospital of Harbin Medical University. All patients with CRC were newly diagnosed and underwent surgery without neoadjuvant chemotherapy, and were confirmed by postoperative pathology. Clinicopathological information including age, gender, tumor location, and tumor stage, were obtained from clinical records and pathological report.[31] Tumor stage was determined using the AJCC staging system for patients with CRC. Written informed consent was obtained from all study participants and the study protocol was approved by the Medical Ethics Committee of Harbin Medical University.

2.4 | *SCTR* quantitative methylation analysis

Quantitative methylation analysis of *SCTR* was evaluated using MethylTarget sequencing (Genesky Biotechnologies Inc). As previously described, this targeted bisulfite sequencing assay is a next-generation sequencing technology for methylation profiling of targeted genomic regions.[32] Genomic DNA from tissues and white blood cells were extracted using the classic phenol-chloroform procedure and a QIAamp DNA Blood Mini Kit (Qiagen). DNA samples were bisulfite-converted with a EpiTect Fast DNA Bisulfite Kit (QIAGEN GmbH) using the manufacturer's protocol. Corresponding primers for *SCTR* were designed using Primer3 software (Figure S1).[33] A two-step PCR approach was performed for each bisulfite-converted DNA sample, with the first PCR for amplifying the targeted DNA sequence and the second PCR for adding barcodes. Sequencing was performed on an Illumina HiSeq 2000 system using a 150-bp paired-end mode.

2.5 | Statistical analysis

All statistical analyses were performed in R version 3.5.1 software, and a *P*-value < .05 was considered significant. All genomic coordinates referred to the human genome version GRCh37/hg19. Student *t* test was used to evaluate the significant difference of methylation levels and expression levels of the *SCTR* gene between 2 groups. Spearman correlation method was applied to assess coordinated methylation (co-methylation) of adjacent CpG sites. The association between *SCTR* methylation and expression was confirmed by Pearson correlation coefficients in 391 samples from TCGA CRC dataset. ROC and AUC were used to verify the discriminative performance of *SCTR* methylation. An optimized cut-off value was selected at the maximal Youden index in ROC. The binary logistic regression model was fitted to construct the diagnostic score using multiple CpG sites. The trend test for *SCTR*

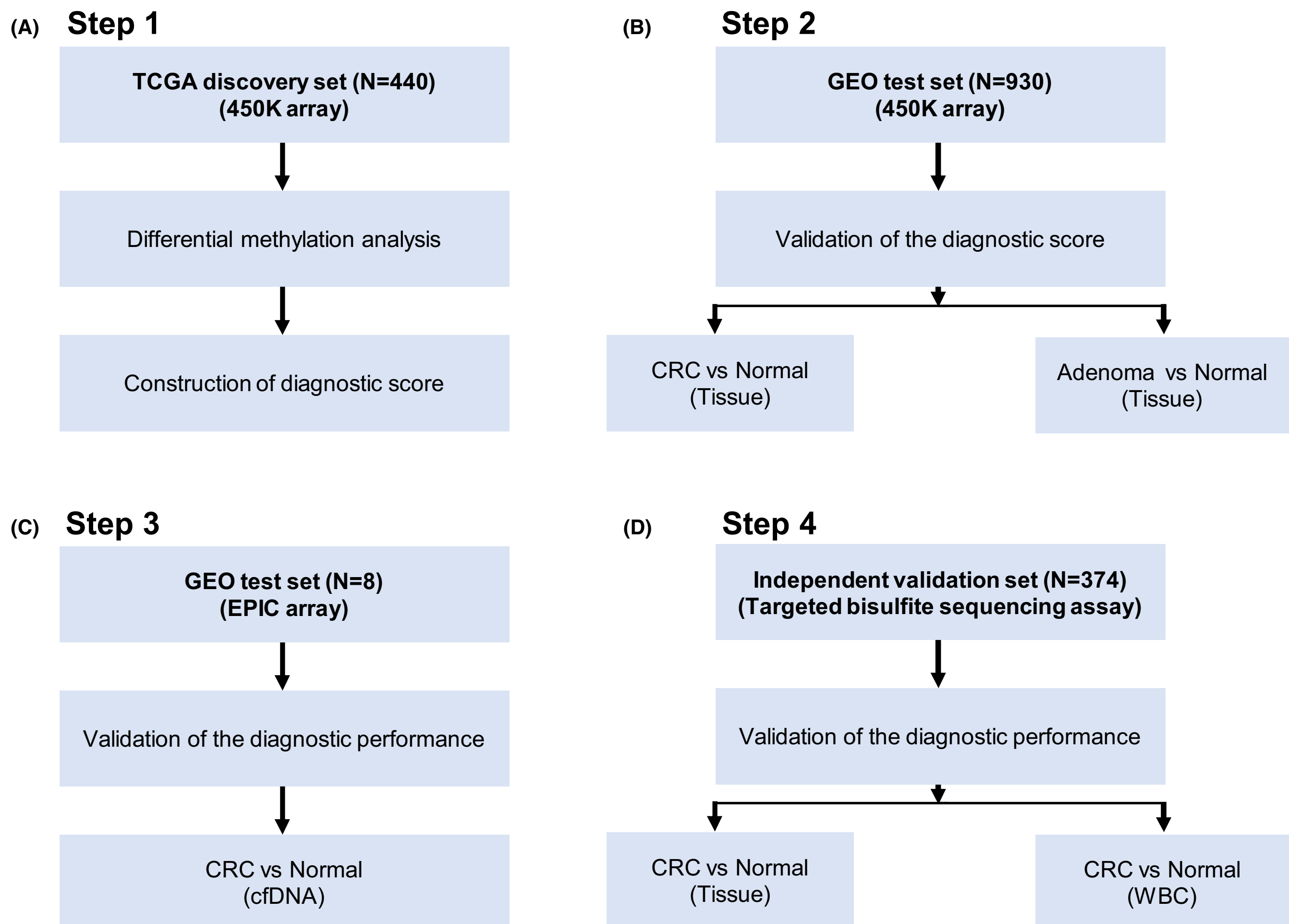

FIGURE 1 Overall workflow of the various analyses in different methylation datasets

methylation at different disease stages during neoplastic progression was determined using a linear regression model.

3 | RESULTS

3.1 | *SCTR* methylation and expression in colorectal cancer tissues from the TCGA discovery set

In the TCGA discovery set, differential methylation analyses were performed between 395 CRC and 45 normal tissues in 10 CpG sites of the *SCTR* gene. Based on location relative to CpG islands, 4 CpG sites were located in the Open Sea (cg19706534, cg13880193, cg10944063, and cg19576736), one in North Shelf (cg24549161), one in North Shore (cg16943697) and 4 in CpG islands (cg01013590, cg20505223, cg07176264, and cg26009192). Using Student *t* test, methylation levels for 4 CpG sites at CpG islands were significantly higher in CRC than those in normal samples (Figure 2). Four (cg13880193, cg10944063, cg24549161, and cg16943697) out of 6 residual CpG sites were significantly lower in CRC than those in normal samples. Spearman correlation coefficients were calculated to investigate the methylation patterns of 10 CpG sites on *SCTR* in 395 CRC samples. Strong correlations in 4 CpG sites at CpG islands indicated that these CpG sites shared a similar methylation status, which was termed co-methylation (Figure S2). In TCGA dataset, *SCTR* expression was downregulated in 380 CRC samples compared with 51 normal samples (Figure S3). Moreover, methylation levels of 4 CpG sites at CpG islands were negatively correlated with its gene expression in 391 paired methylation and expression data (Figure S4). Collectively, CpG islands of *SCTR* were significantly hypermethylated in CRC and were associated with downregulation of gene expression.

3.2 | *SCTR* methylation as detection biomarker for colorectal cancer

We investigated the diagnostic performance of 10 CpG sites as detection markers for CRC. Therefore, we calculated the AUC values of individual CpG sites to discriminate CRC (N = 395) from normal samples (N = 45). Sensitivities and specificities under the different cut-off value for methylation levels are shown in Figure S5. Four CpG sites at CpG islands showed high discriminative ability, with AUC values in the range 0.919-0.953.

These 4 CpG sites with high AUC values (>0.90) were then selected to construct the diagnostic score using a logistic regression method. The 4-CpG diagnostic score was a sum of the methylation levels of 4 CpG sites with weights given by the corresponding coefficient from the logistic regression model (Figure S6): (7.501 × cg01013590) + (1.522 × cg20505223) + (3.454 × cg07176264) + (3.682 × cg26009192). ROC analyses showed that the diagnostic score yielded an AUC of 0.964 in TCGA discovery set (Figure 3A). Using a fixed cut-off value of 6.154, the diagnostic score yielded an accuracy of 94.5% (416 of 440) in the discovery set; the sensitivity and specificity were 93.9% and 100% (Table 2), respectively.

We first applied the diagnostic score of TCGA discovery set to the GEO test set A. The diagnostic score had robust performance (AUC = 0.948) to distinguish 419 CRC tissues from 395 normal tissues in the GEO test set A (Figure 3B). Using the same cut-off value of 6.154 in the GEO test set A, the accuracy was 91.4% (744 of 814); the sensitivity and specificity were 88.8% and 94.2%, respectively.

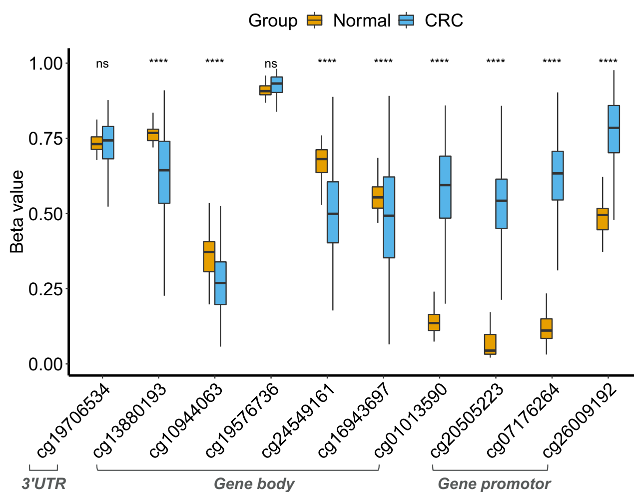

FIGURE 2 Differential methylation analysis for 10 CpG sites of the *SCTR* gene between colorectal cancer tissues and normal tissues in TCGA discovery set. Gene promoter was defined as the region containing TSS200, TSS1500, 5′UTR, and the first exon. Symbols indicated statistical significance for the *t* test: ns, $P > .05$; **** $P \leq .0001$

3.3 | *SCTR* methylation as detection biomarker for precursor lesions of colorectal cancer

Next, we assessed the performance of the diagnostic score in the GEO test set B for differentiating between 113 adenomas and 55 normal samples. The diagnostic score could effectively differentiate adenoma from normal samples (AUC = 0.954, Figure 3C). Applying the cut-off value of 6.154, the accuracy was 91.7% (154 of 168); the sensitivity and specificity were 90.3% and 94.6%, respectively. Unsupervised hierarchical clustering of 4 CpG site could separate adenoma from normal samples (Figure S7).

3.4 | *SCTR* methylation in cell-free DNA samples from colorectal cancer

Moreover, we investigated whether CpG islands of the *SCTR* gene were hypermethylated in cfDNA from CRC patients. Three CpG

TABLE 2 Performance metrics of 4-CpG diagnostic score in TCGA discovery set and GEO test sets

	TCGA discovery set CRC vs Normal	GEO test set A CRC vs Normal	GEO test set B Adenoma vs Normal
Accuracy (95% CI)	0.945 (0.920, 0.965)	0.914 (0.893, 0.932)	0.917 (0.864, 0.954)
Sensitivity	0.939	0.888	0.903
Specificity	1.000	0.942	0.946
Kappa	0.760	0.828	0.818

Abbreviations: CRC, colorectal cancer; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas.

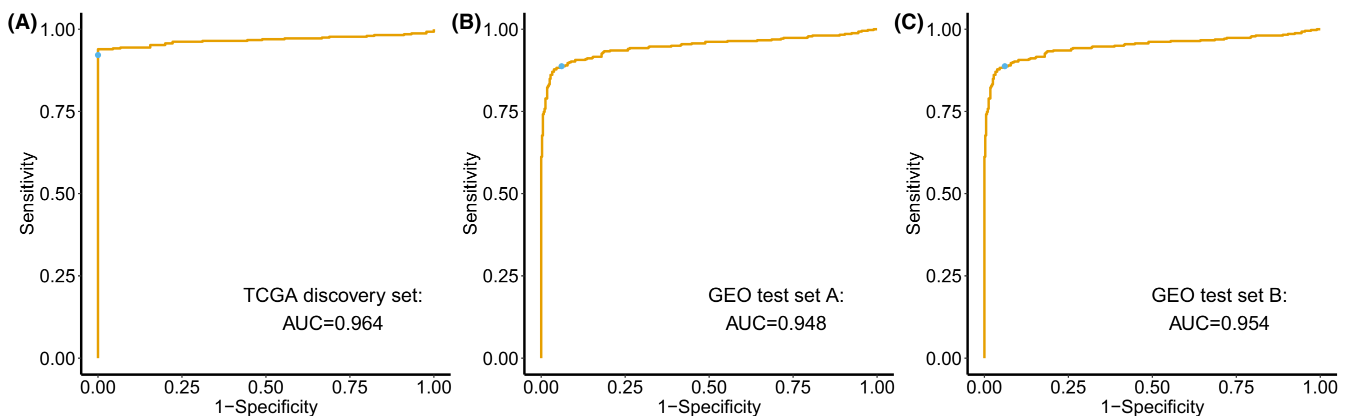

FIGURE 3 Diagnostic performance of the 4-CpG diagnostic score for colorectal cancer and its precursors. ROC curves of the diagnostic score constructed by the logistic regression model in (A) the TCGA discovery set, (B) the GEO test set A, and (C) the GEO test set B. The blue point indicates sensitivity and specificity at the fixed cut-off value of 6.154

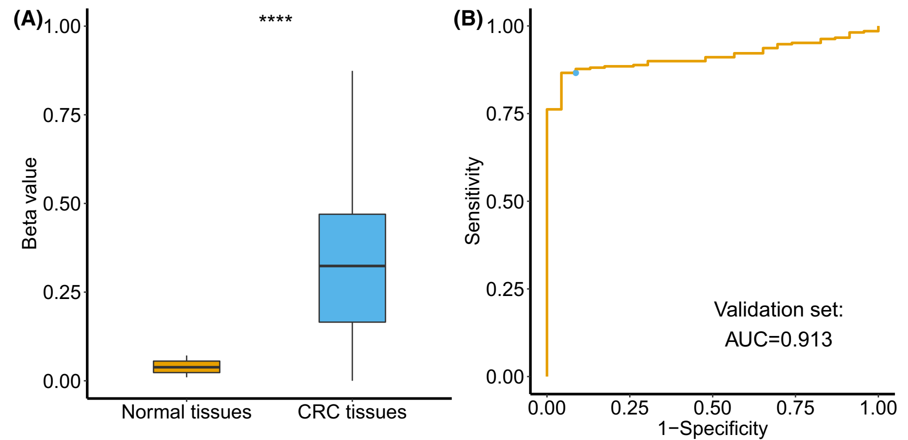

FIGURE 4 Methylation status of cg20505223 in the validation set. A, Differential methylation analysis of cg20505223 between 272 colorectal cancer tissues and 23 normal tissues, **** $P \le .0001$. B, Receiver operating characteristic curve analysis and Youden index analysis of cg20505223 for discriminating colorectal cancer tissues and normal tissues. The blue point indicates sensitivity and specificity at the cut-off value of 0.071

sites were available in a dataset with 8 cfDNA samples (Figure S8). Hypermethylation of 3 CpG sites was found in cfDNA samples collected from CRC (3 out of 4), but not in those from healthy individuals (0 of 4).

3.5 | *SCTR*-specific sequencing array in colorectal tissue samples of the validation set

For clinical application, a cost-effective detection technology is needed to detect the methylation level of the *SCTR* gene. Thus, the diagnostic performance of *SCTR* methylation was further assessed in our independent validation set using target bisulfite sequencing, which is a reliable technology and is less costly than Infinium 450K array. Based on the location of cg20505223 from the Infinium 450K array, we designed a *SCTR*-specific sequencing array to detect methylation status at CpG islands. The target region covered 27 CpG sites. Genomic locations of the 27 CpG sites are described in Table S2. Three CRC tissue samples with a bisulfite conversion rate below 98% were filtered out after quality control. Using *t* test, differential methylation analyses were performed for cg20505223, as well as 26 adjacent CpG sites. Methylation levels of cg20505223 (mean β value = 0.331) were significantly higher in 272 CRC tissues compared with those (mean β value = 0.043) in 23 normal tissues (Figure 4A). Similar results from differential methylation analyses were also observed in 26 adjacent CpG sites (Figure S9 and Table S2).

With regards to diagnostic performance, the AUC value was 0.913 using cg20505223 to discriminate between CRC and normal tissues (Figure 4B), which was slightly lower compared with that (AUC = 0.919) in TCGA discovery set. Under a cut-off value of 0.071 at the maximal Youden index, the sensitivity and specificity were 86.6% and 95.7% (Table S3). When subgroup analyses were performed by clinicopathological variables, methylation levels (Table S4) and AUC values (Figure S10) for cg20505223 were consistent in different subgroups. In particular, no significant differences ($P = .880$) of the diagnostic performance were found between the early-stage group (AJCC stage I/II) and the advanced-stage group (AJCC stage III/IV). Similar diagnostic performance was also observed in 26 adjacent CpG sites, with AUC values ranging from 0.907 to 0.948 (Table S3). Therefore, results from the validation set confirmed that *SCTR* was hypermethylated at its CpG islands region, which could distinguish CRC tissues from normal tissues using a cost-effective method.

3.6 | *SCTR*-specific sequencing array in white blood cell samples of the validation set

As white blood cells contribute the largest proportion of background DNA in cfDNA samples, methylation difference in white blood cells samples between CRC patients and healthy controls may confound the methylation profile in cfDNA. We applied *SCTR*-specific sequencing array in white blood cell samples from 29 CRC patients and 29 healthy controls. White blood cell samples had low methylation levels for cg20505223 for both CRC patients (mean β value = 0.024) and healthy controls (mean β value = 0.038). Although a significant difference was found in 2 groups ($P = .011$), the direction of differences was such that methylation levels in CRC patients were lower than those in healthy controls (Figure 5). Twenty-six adjacent CpG sites showed low methylation levels in white blood cell samples from CRC patients and healthy controls (Table S5). Therefore, *SCTR* hypermethylation in cfDNA samples from CRC patients was not derived from white blood cells.

3.7 | *SCTR* methylation in neoplastic progression in the validation set

To determine the methylation status of the *SCTR* gene at different disease stages during neoplastic progression, the methylation status of 313 colorectal tissues, including 23 normal tissues, 10 hyperplastic polyps, 8 adenomas, and 272 CRCs, were evaluated in the validation set. As shown in Figure 6, the medians of average methylation level of targeted regions in different disease stages were as follows: normal tissues, 0.063; hyperplastic polyps, 0.080; adenomas, 0.141; and CRCs, 0.370. The trend test showed that methylation levels in CpG islands of *SCTR* increased sequentially from normal to hyperplastic polyp, to adenoma, and to CRC (trend test $P < .001$).

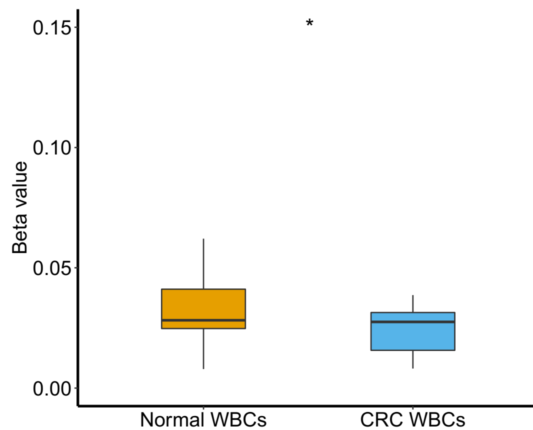

FIGURE 5 Methylation levels of cg20505223 in white blood cell samples from 29 colorectal cancer patients and 29 healthy controls. * $P \le .05$

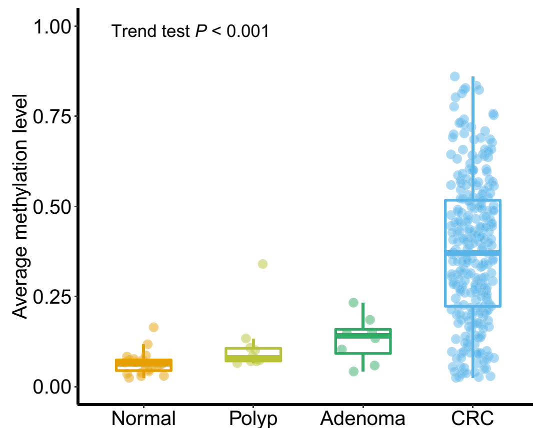

FIGURE 6 Average methylation levels of the *SCTR* gene at different disease stages during neoplastic progression * $P \le .05$

4 | DISCUSSION

In this present study, we investigated the potential use of *SCTR* methylation as a diagnostic marker for CRC and its precursor lesions by integrating the largest methylation data (N = 1752). Our study indicated that methylation levels of 4 CpG sites at CpG islands of the *SCTR* gene were significantly higher in CRC samples than those in normal samples. Overall, the 4-CpG diagnostic score had an excellent performance to distinguish CRC and adenomas from normal samples, with AUC values over 0.948. Hypermethylation of the *SCTR* gene was also found in cfDNA samples from CRC patients and could be detected by a cheaper PCR-based technology. These results highlighted that *SCTR* methylation had a strong potential as a novel marker for the detection of CRC and its precursor lesions in clinical applications.

Our study validated findings of previous mechanism studies that found that the *SCTR* gene was hypermethylated at CpG sites in its CpG island in CRC, and therefore was negatively associated with gene expression. A study showed that *SCTR* was hypermethylated in 88% (23 of 26) of CRC, and was associated with gene silencing.[20] Another study reported similar results of *SCTR* methylation in CRC, but a lower frequency (48 of 90, 53%) of *SCTR* methylation in CRC.[21] Currently, greater numbers of genome-wide methylation data could be accessible from publicly available datasets such as TCGA and GEO; this provides the opportunity to quantitatively evaluate methylation change of the *SCTR* gene in CRC. Integrating the largest genome-wide methylation data, our study demonstrated that 4 CpG sites at CpG islands of the *SCTR* gene were highly hypermethylated in both CRC and adenomas.

The main finding of this study was the identification of *SCTR* methylation as a promising marker for detection of CRC; this achieved sufficient discrimination accuracy to distinguish CRC from normal samples. The diagnostic score with 4 CpG sites achieved an AUC of 0.964 in TCGA discovery set. Moreover, the diagnostic score was externally verified in large-scale data from the GEO database. The AUC for discriminating CRC and normal tissues was 0.948 in the GEO test set, and proved generalizability of the 4-CpG diagnostic markers across different settings. Our study of *SCTR* methylation was extended to biological fluids of CRC patients by analyzing a GEO dataset. Hypermethylation of the *SCTR* gene was also found in cfDNA samples collected from CRC patients. Due to complex technology and cost-effectiveness, candidate gene method is more suitable for clinical application than a genome-wide method. Therefore, targeted bisulfite sequencing was used to verify *SCTR* methylation in our inhouse study, which had the advantages of being highly specific for targeted CpG sites with small genomic DNA amounts, and accurate methylation profiling of single CpG sites in targeted genomic regions. Our study found that the diagnostic performance of *SCTR* methylation in the validation set was similar to that of the discovery set, suggesting that it had potential for clinical transformation. Our inhouse study also ruled out the possible noise effects from white blood cells on the observed hypermethylation of cfDNA samples from CRC. Altogether, our study revealed that *SCTR* methylation had a strong potential as a diagnostic biomarker for CRC.

In screening settings, the detection of precursor lesions, which would allow earlier intervention by removal of these diseases, had significant importance in the prevention of CRC. Previous studies have indicated that various methylated genes were potential biomarkers for detection of CRC, however methylation-based markers that could diagnose both CRC and its precursor lesions are lacking.[7] As an example, the plasma-based *SEPT9* methylation marker has been commercialized for clinical use. Although the *SEPT9* methylation marker in plasma displayed high specificities in large studies, it showed poor sensitivities (7.9%-38.7%) for the detection of precursor lesions (adenomas).[9,16-18] Our study found that *SCTR* methylation achieved high accuracy in the detection of both CRC and adenomas.

As with most epithelial cancers, CRC results from an accumulative change of genetics and epigenetics that transforms precursor lesions in the colon and rectum into carcinomas. However, the role of DNA methylation in CRC formation is still not well understood. Previous studies have identified that methylated genes (*ITGA4*,

MGMT, *SLC5A8*, *SFRP2*, and *MINT1*) are involved in this progression sequence.[34] In this study, methylation levels at CpG islands of the *SCTR* gene increased sequentially in the polyp to a carcinoma progression sequence. In addition, hypermethylation of the *SCTR* gene was observed in even the earliest lesions of CRC, suggesting that it plays a role in the initiation of tumorigenesis.

SCTR methylation has been described in the development and progression of CRC. *SCTR* gene locations at the boundaries of chromosome 2q14.2, where gene suppression commonly occurs in CRC through a mechanism of LRES. Downregulation of genes at the chromosome 2q14.2 region has been associated with coordinated hypermethylation of 3 CpG islands of *EN1*, *SCTR*, and *INHBB*.[20,22] Of these, the methylated *EN1* CpG island in stool DNA has been shown to have application as a non-invasive biomarker of CRC.[21] Although there is a consistent finding for the association between cg20505223 and overall survival of CRC, this was not observed in TCGA discovery set and our inhouse validation set (Figure S11); the significant methylation difference of the *SCTR* gene may contribute to the specific characteristics of the CIMP and its clinicopathologic features in CRC patients. A previous study reported that *SCTR* hypermethylation had potential influence on specific characteristics of CIMP-positive CRC.[35] Further mechanism studies are warranted to provide a better understanding of the biological role of *SCTR* methylation on the development and progression of CRC.

Our study has some advantages. *SCTR* methylation was assessed in the largest study with different genome-wide methylation datasets that provided promising preliminary data on its diagnostic utility. *SCTR* methylation was further validated by targeted bisulfite sequencing, which would be a clinically applicable method given the advantages of competitive pricing over genome-wide approaches and accurate methylation profiling. Our study also has several limitations. First, although hypermethylation of the *SCTR* gene was observed in cfDNA samples from CRC patients, the number of samples of both CRC and heathy control was limited. However, we believe that *SCTR* methylation is worthy of further evaluation in prospective studies due to the excellent performance for discriminating CRC and its precursor lesions from normal tissues. Second, all available samples for the validation set were collected from a single center.

In conclusion, our study discovered and validated that hypermethylation at CpG islands of the *SCTR* gene holds great promise as a diagnostic biomarker for CRC and its precursor lesions based on integration analysis of large-scale methylation data from TCGA, GEO, and an inhouse database. Although *SCTR* hypermethylation was observed in the cfDNA samples from CRC patients, whether *SCTR* methylation can be applied as a non-invasive biomarker should be further determined in larger studies.

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DISCLOSURE

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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