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RESEARCH ARTICLE

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Significant association of *PRMT6* hypomethylation with colorectal cancer

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Background: Protein arginine N-methyltransferase 6 (PRMT6) was deemed to be indispensable in the variety of biological processes. Upregulated *PRMT6* was found in various human diseases including cancer. Herein, we investigated the performance of *PRMT6* methylation in the diagnosis for CRC.

Methods: A quantitative methylation-specific polymerase chain reaction (qMSP) method was used to measure *PRMT6* promoter methylation. The percentage of methylated reference (PMR) was applied to represent gene methylation level.

Results: Our data indicated that *PRMT6* promoter methylation levels were significantly lower in CRC tissues than those in paired nontumor tissues (median PMR: 36.93% vs 63.12%, P = 1E-6) and normal intestinal tissues (median PMR: 36.93% vs 506.55%, P = 8E-12). We further examined the potential role of *PRMT6* hypomethylation by the receiver operating characteristic (ROC) curve. Our results showed that the area under the curve (AUC) was 0.644 (95% CI = 0.596-0.733) between CRC tissues and paired nontumor tissues, 0.958 (95% CI = 0.919-0.998) between CRC tissues and normal intestinal tissues, and 0.899 (95% CI = 0.825-0.972) between paired nontumor tissues and normal intestinal tissues.

Conclusion: Our study firstly indicated that the hypomethylation of *PRMT6* promoter could be a novel diagnostic biomarker for CRC.

KEYWORDS
colorectal cancer, DNA methylation, protein arginine N-methyltransferase 6, quantitative methylation-specific polymerase chain reaction

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the fourth most universal cause of cancer-related death globally.[1,2] CRC is a complex disease influenced both by genetic factors and by environmental factors.[3] As for bridging factors of genetics and environment, epigenetic modifications modulate gene expression.[4,5] DNA methylation is one of the widely studied epigenetic mechanisms, and it often occurs in CpG dinucleotide-rich regions. Aberrant gene methylation [6-8] was deemed as one of the most promising diagnostic tools for cancer,[9-11] including CRC.[12]

PRMT6 encodes protein arginine methyltransferase 6 which could methylate protein on arginine residue.[13] The arginine N-methyltransferase family members often bind to chromatin and act as transcriptional coactivators or corepressors. PRMTs were often found to be aberrantly regulated in various cancer types, such as

Abbreviations: CpG, cytosine-phosphate-guanine; CRC, colorectal cancer; GEO, gene expression omnibus; PMR, percentage of methylated reference; *PRMT6*, protein arginine methyltransferases 6 genes; qMSP, quantitative methylation-specific polymerase chain reaction; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

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prostate cancer,[14] breast cancer,[15,16] and lung cancer.[17] However, no article was reported on whether *PRMT6* hypomethylation was associated with CRC.

Here, we assessed the association between *PRMT6* promoter methylation and CRC. The goal of our study was to determine whether *PRMT6* hypomethylation could be used as a diagnostic biomarker for CRC.

2 | MATERIALS AND METHODS

2.1 | Tissue samples

A total 121 CRC patients (mean age, 61.62 ± 11.55 years) were recruited from Zhejiang Tumor Hospital (Zhejiang, China), Shaoxing First People's Hospital (Zhejiang, China), and Third Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (Nanjing, China) between August 2011 and January 2015. Tumor tissues were obtained from the central parts of tumor, and paired nontumor tissues were recruited at least 5 cm away from tumor lesion. In addition, normal intestinal tissues from 22 healthy participants were collected at Zhejiang Tumor Hospital (Zhejiang, China) at the same time. All the individuals were diagnosed by colonoscopy and pathological examination. None of the participants had a history of preoperative chemotherapy or radiation therapy before sampling. Microscopic examination showed at least 80% of cancer cells in each tumor tissue sample and no tumor cells in paired nontumor tissues. Normal intestinal tissues were collected from healthy participants. Tumor tissues and their paired nontumor tissues were taken in the same block and layer.[18] All the clinical information was extracted from the medical records (Table 1). Written informed consent form was obtained from each participant. Permission for the study was given by Human Research Ethics Committees in Ningbo University and the above three hospitals.

2.2 | DNA extraction, bisulfite conversion, and qMSP

The details of DNA extraction from tissue samples and bisulfite conversion were as previously described.[6] The qMSP was applied to measure the methylation level, and the details of qMSP were as shown in our previous publications.[7,19] The detail of PCR was conducted as before [6]and the qMSP primer sequences were shown in Table 1. Percentage of methylated reference (PMR) was calculated by $2^{-\Delta\Delta Ct}$ quantification approach, in which $\Delta\Delta Ct$ = sample DNA(Ct_{PRMT6} - $Ct_{ACTBcontrol}$) - fully methylated DNA(Ct_{PRMT6} - $Ct_{ACTBcontrol}$) to represent *PRMT6* methylation level.

2.3 | GEO and TCGA datasets

To evaluate the association between *PRMT6* methylation and *PRMT6* expression, the data of 372 samples in TCGA colorectal adenocarcinoma cohort was downloaded from cBioPortal (http://www.cbioportal.org/). Meanwhile, data with *PRMT6* expression changes in several cancer cell lines before and after 5′-AZA-deoxycytidine (5-AZA) treatment was retrieved from GEO DataSets (GSE38823, https://www.ncbi.nlm.nih.gov/pubmed).

2.4 | Statistical analysis

Nonparametric rank test was used to assess the methylation differences between two groups. Spearman correlation test was used to assess the correlation between *PRMT6* methylation and clinical characteristics. ROC curve analysis was used to assess the diagnostic value of *PRMT6* promoter methylation for CRC. A two-sided $P < .05$ was defined to be statistically significant.

3 | RESULTS

The target fragment in promoter was used to represent the methylation level of *PRMT6* gene (GRCh37/hg19 assembly, chr1:107599926-107599989, Figure 1A). In addition, Sanger sequencing manifested that the amplified fragment matched the target sequence and the bisulfite conversion was complete (Figure 1B). Capillary electrophoresis showed that the length of *PRMT6* target fragment was 64 bp as expected (Figure 1C).

To evaluate the value of *PRMT6* methylation in the diagnosis of CRC, we recruited tumor tissues and paired nontumor tissues of 121 CRC patients and normal intestinal tissues of 22 healthy participations. Our study showed that the methylation levels of *PRMT6* promoter were significantly lower in CRC tissues than those in the paired nontumor tissues (P = 1E-6, Figure 2), and than those in the normal intestinal tissues (P = 8E-12, Figure 2). In addition, we also observed significantly lower *PRMT6* methylation levels in the paired nontumor tissues compared to those in normal intestinal tissues (P = 3E-9, Figure 2).

Further ROC curve analysis showed that the AUC was 0.644 (95% CI = 0.596-0.733) with a specificity of 53.7% and a sensitivity of 74.4% between tumor tissues and paired nontumor tissues (Figure 3A). Between tumor tissues and normal intestinal tissues, *PRMT6* hypomethylation yielded a significant AUC of 0.958 (95% CI = 0.919-0.998) with a specificity of 78.5% and a sensitivity of 95.5% (Figure 3B). Furthermore, *PRMT6* hypomethylation yielded

TABLE 1 The qMSP primer sequences

Gene (product length)	Forward primer sequences (5′→3′)	Reverse primer sequences (5′→3′)
PRMT6 (64 bp)	AGCGATTAGATGTTGGAATG	CCACACCATAATACTACTTCAC
ACTB (133 bp)	TGGTGATGGAGGAGGTTTAGTAAGT	AACCAATAAAACCTACTCCTCCCTTAA

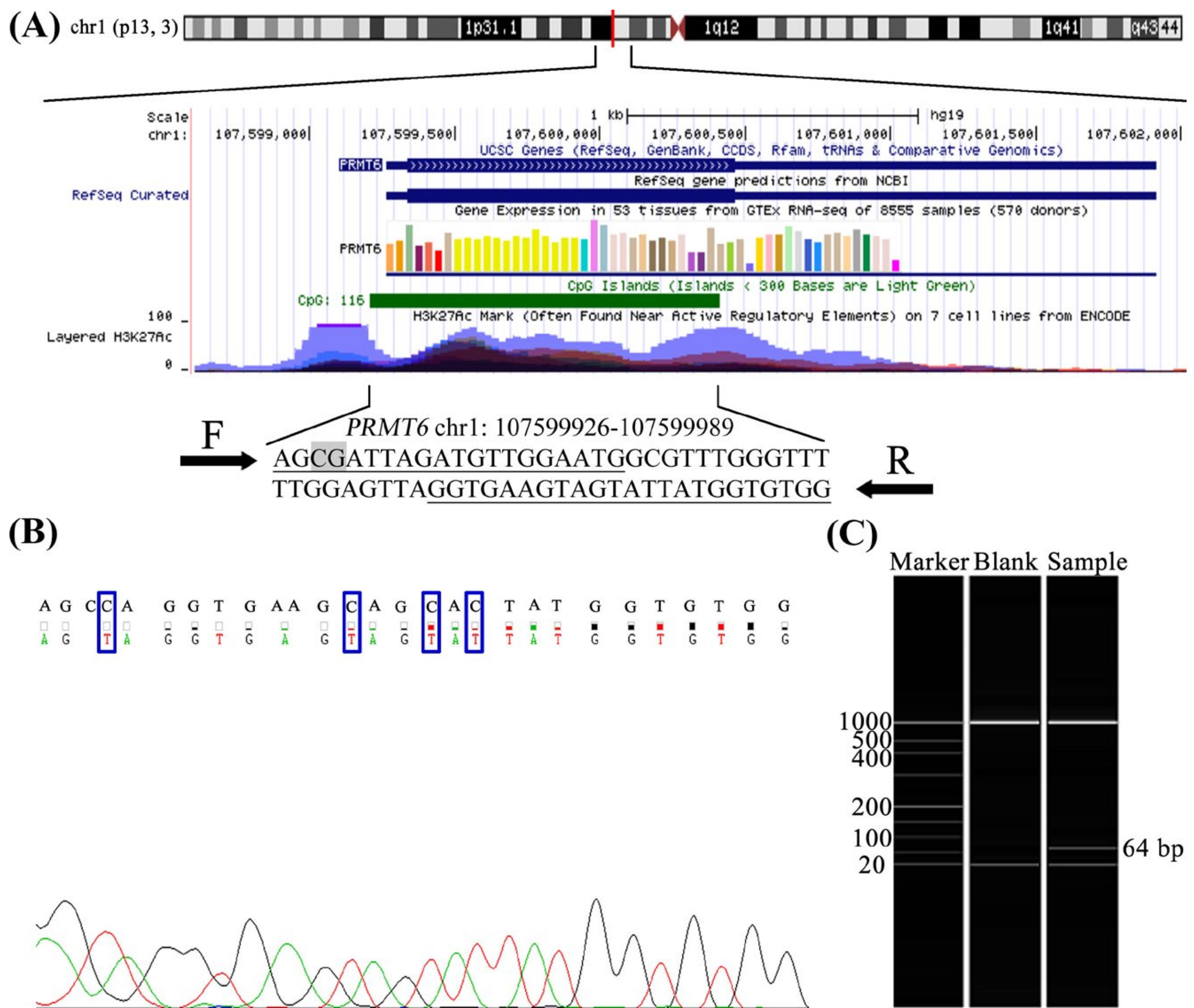

FIGURE 1 The target sequence of *PRMT6* methylation assay. A, The genomic position and functional annotation of amplified fragment were based on UCSC genome browser GRCh37 assembly. The qMSP primers are underlined, and one CpG sites are in gray. F denotes forward primer; R denotes reverse primer. B, The picture on the left is the sequencing result of *PRMT6* qMSP product. The top row of the sequence is the original DNA sequence; the second row of the sequence is the converted sequence. C, The results of capillary electrophoresis. The first column is the marker band; the second column is the blank control; the third column is 64-bp qMSP product as expected

a significant AUC of 0.899 (95% CI = 0.825-0.972) with a specificity of 78.5% and a sensitivity of 81.8% to distinguish the difference between adjacent nontumor tissues and normal intestinal tissues (Figure 3C). To further evaluate the diagnostic value of *PRMT6* hypomethylation in CRC, we compared the positive predictive value (PPV) of *PRMT6* hypomethylation and CEA in 39 samples. Then, we used ROC best value (73.0%) as cutoff value to divide the *PRMT6* methylation levels into either hypermethylation or hypomethylation, and we found that the PPV of *PRMT6* hypomethylation in tumor was 64.1%, which was higher than the PPV of CEA (33.3%).

Subsequently, we examined the correlation between *PRMT6* methylation and the clinicopathological features of CRC patients. Our results showed that *PRMT6* methylation in tumor was not significantly associated with gender, age, clinical stage, differentiation, tumor size or lymph node metastasis of the patients (all $P > .05$, Table 1). There was a significant correlation between *PRMT6* hypomethylation and differentiation in paired nontumor tissues ($P = .032$, Table 1), and there was no significant correlation of *PRMT6* hypomethylation with other clinical features (all $P > .05$, Table 2).

In addition, an inverse correlation between *PRMT6* methylation and *PRMT6* expression was observed according to our analysis of data from 372 TCGA colorectal adenocarcinoma samples ($r = -.378$, $P = 4E\text{-}14$, Figure 4A). In addition, GEO data analysis showed that *PRMT6* expression was significantly increased when cell lines (SAS and HCS3) were treated with demethylation agent (5-AZA, fold changes (FC) = 1.30 and 1.16, Figure 4B).

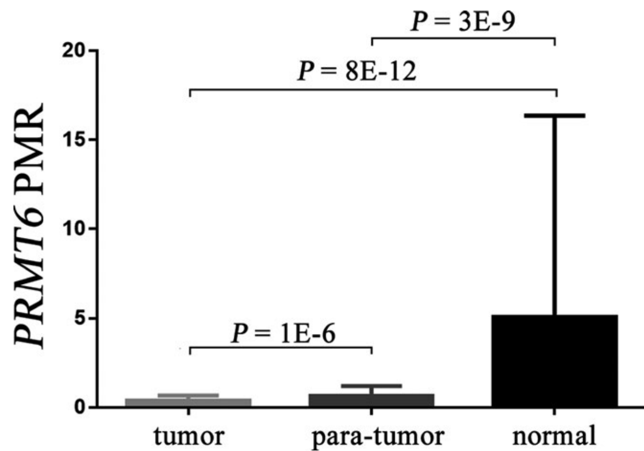

FIGURE 2 Comparisons of percentage of methylated reference (PMR) of *PRMT6* between tumor tissues, paired nontumor tissues, and normal intestinal tissues. PMR denotes the percentage of methylated reference, and data are presented as median (interquartile range). *P* value is calculated by a nonparametric test

4 | DISCUSSION

In this study, we analyzed *PRMT6* methylation levels of tumor tissues with those in the paired nontumor tissues and normal intestinal tissues. Moreover, we evaluated the correlations between *PRMT6* methylation and a series of clinical features. Our results showed that *PRMT6* methylation levels were significantly lower in tumor tissues than those in nontumor and normal intestinal tissues. These findings led us to speculate that *PRMT6* hypomethylation might be used as a diagnostic biomarker of CRC.

PRMTs can methylate the arginine residues of proteins,[13] including p16 and p21 which are tumor suppressor proteins.[16,20,21] Methylation of p16 usually resulted in decreased protein function and promoted tumorigenesis.[22] The p21 gene encodes a cyclin-dependent kinase inhibitory protein with tumor suppressor activity.[23] The p21 plays an important role in the development and progression of cancer,[24] and hypermethylation of p21 leads to downregulation of p21 gene expression.[24] Downregulated expression of *PRMT6* in CRC cells can induce p21 overexpression and inhibit cell growth and colony formation capacity.[25] *PRMT6* is often found to be overexpressed in cancers, including CRC,[12,25] bladder cancer,[17] lung cancer,[17] prostate cancer,[15,26] and breast cancer.[15] In the present study, we found *PRMT6* hypomethylation was significantly associated with CRC. Meanwhile, TCGA and GEO data analyses showed that *PRMT6* methylation was inversely correlated with mRNA expression. We hypothesized that *PRMT6* hypomethylation might increase the expression of PRMT6 protein and thus promotes cell proliferation by downregulating tumor suppressor genes.[27]

As a noninvasive method widely used in the detection of CRC, fecal occult blood test (FOBT) comprises guaiac (gFOBT) and immunochemical (iFOBT). However, the gFOBT test was easy to be effected by other factors, resulting in false-positive results. The detection rate of iFOBT decreased rapidly once the time from collection to laboratory inspection was deferred for over 5 days.[28–30] As for colonoscopy, despite its high sensitivity and specificity, there were still some shortcomings, such as complications (intestinal gastrointestinal bleeding, intestinal perforation,) and low compliance.[31] Carcinoembryonic antigen (CEA) in serum is one of the biomarkers widely used in the diagnosis of cancer;[32] however, it is still defective because increased CEA levels were not only found in cancer patients but also in smokers, which reduced its specificity in cancer diagnosis.[33,34] Meanwhile, CEA lacks ideal sensitivity in the early diagnosis of CRC.[35,36] A review based on 9834 CEA test results indicated that the sensitivity of CEA ranged from 50% to 80%.[37] In addition, the sensitivity of serum CEA in detecting the risk of CRC recurrence was only 64%.[38] Here, our study found that *PRMT6* hypomethylation had a specificity of 78.5% and a sensitivity of 95.5% for the detection of CRC, and we also found the PPV of *PRMT6* hypomethylation was higher than that of serum CEA. However, future investigation is needed to access the combined diagnosis of *PRMT6* methylation and CEA levels for CRC.

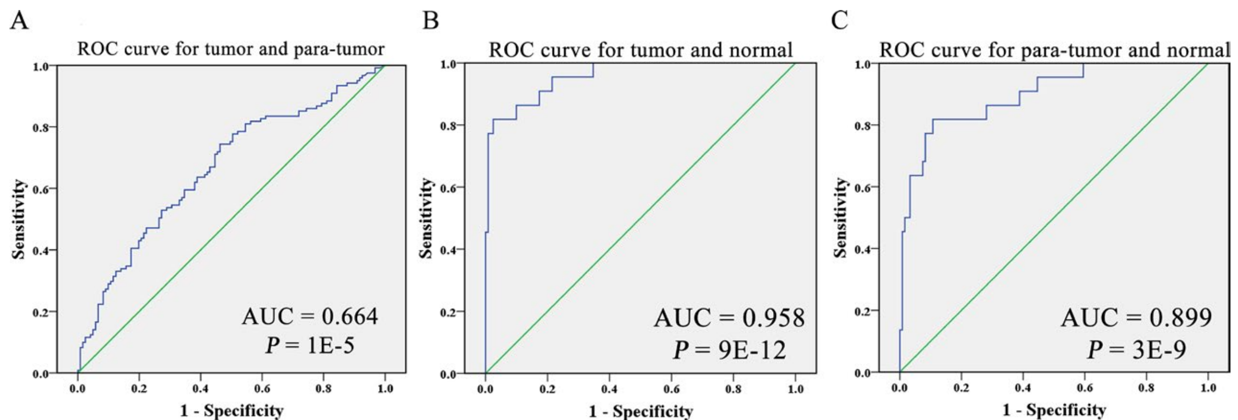

FIGURE 3 ROC curves of *PRMT6* hypomethylation as a diagnostic biomarker for colorectal cancer (CRC). ROC curve for the diagnostic value of *PRMT6* hypomethylation between CRC tumor tissues and para-tumor tissues A, between CRC tumor tissues and normal intestinal tissues B, between para-tumor tissues, and normal intestinal tissues C, ROC: receiver operating characteristic. AUC: area under the curve

TABLE 2 Association of *PRMT6* methylation with clinical characteristics in CRC patients

Variables	N	Tumor PMR (%)	*P* value	Para-tumor PMR (%)	*P* value
Total	121	36.93 (19.89, 65.95)		63.12 (37.01, 119.50)	
Gender[a]					
Male	80	41.37 (20.43, 70.80)	.553	67.08 (41.24, 120.30)	.198
Female	39	34.97 (18.96, 56.38)		61.56 (16.52, 120.50)	
Age (y)[a]					
≤65	78	33.15 (19.38, 59.63)	.097	64.33 (41.41, 146.58)	.114
>65	41	51.77 (21.84, 92.10)		55.05 (20.19, 101.24)	
Differentiation[a]					
poorly	17	40.55 (12.73, 66.66)	.653	51.97 (38.80, 108.48)	**.032**
moderate + well	101	36.93 (21.81, 69.09)		64.12 (36.81, 120.70)	
Tumor size[a]					
≤5 cm	80	36.92 (23.52, 67.80)	.563	55.29 (41.24, 106.53)	.210
>5 cm	40	38.52 (14.6, 65.04)		87.96 (33.9, 128.32)	
Lymphatic metastasis[a]					
Yes	56	31.49 (13.95, 56.61)	.235	67.53 (41.80, 146.12)	.442
No	63	47.24 (21.96, 77.17)		57.21 (35.77, 111.7)	

PMR, the percentage of methylated reference.
Data are presented as median (interquartile range).
P value is calculated by Spearman test. Two-sided *P* value < .05 (in bold).
[a]A small amount (<1%) of patients did not have the information.

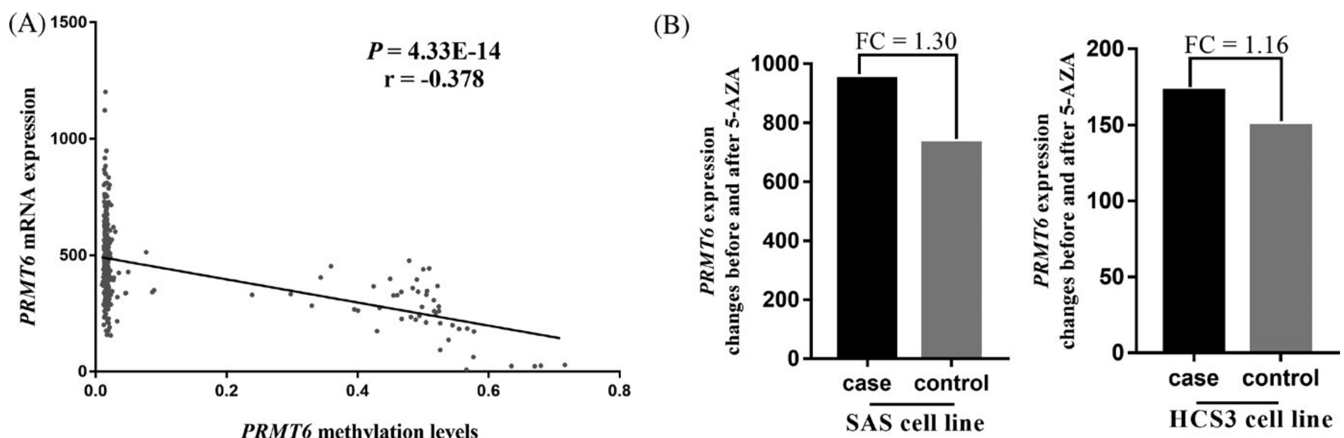

FIGURE 4 Association between *PRMT6* methylation status and expression. A, There was an inverse correlation between DNA methylation level and mRNA expression in TCGA colorectal adenocarcinoma cohort (http://www.cbioportal.org/); B, expression status of several cell lines before and after 5-AZA treated in GEO database. *P* value is calculated by Spearman rank correlation test. FC: fold change

The benign colorectal disease can gradually progress to advanced adenoma and invasive adenocarcinoma.[39–41] Abnormal gene methylation was found in hyperplastic polyps with cancerous potential.[42] Previous studies identified that there was aberrant methylation of multiple genes between CRC tissues and colorectal benign polyp tissues and between colorectal benign polyp tissues and healthy intestinal tissues.[43–48] In the present study, we did not perform the similar comparison due to a lack of

colorectal benign polyp tissues. Future study is needed to investigate the role of *PRMT6* methylation in colorectal benign polyp tissues.

However, our study has some limitations. Firstly, although this is the first study to investigate the methylation of *PRMT6* in CRC, we did not evaluate the role of *PRMT6* hypomethylation in the benign colorectal tissues, which usually have a high risk of CRC. Secondly, we did not collect serum protein markers, such as CEA, in healthy individuals to compare the diagnostic value of *PRMT6* methylation with the conventional biomarkers. Future analysis is needed to evaluate the joint role of methylation biomarkers and protein biomarkers for the diagnosis of CRC. Lastly but not least, our study did not have enough amount of samples to evaluate the correlation of *PRMT6* methylation with *PRMT6* expression. However, our TCGA data analysis showed an inverse correlation between *PRMT6* methylation and *PRMT6* expression.

In summary, we found a significant association of *PRMT6* hypomethylation with CRC. Our results suggested that *PRMT6* hypomethylation might be used as a diagnostic biomarker for CRC, although further validation with large samples is needed in the future.

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AUTHORS CONTRIBUTIONS

SD and RP conceived and designed the experiments. HY, XY, CZ, JZ, JD, YZ, BW, and YM performed the experiments. RP analyzed the data. RP and SD contributed to completion of figures, tables, and the writing of this manuscript.

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REFERENCES

1. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014;383:1490-1502.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359-E386.
3. Bishak YK, Payahoo L, Osatdrahimi A, et al. Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. *Asian Pac J Cancer Prev*. 2015;16:9-21.
4. Toporcov TN, Znaor A, Zhang ZF, et al. Risk factors for head and neck cancer in young adults: a pooled analysis in the INHANCE consortium. *Int J Epidemiol*. 2015;44:169-185.
5. Matsubara N. Epigenetic regulation and colorectal cancer. *Dis Colon Rectum*. 2012;55:96-104.
6. Li J, Chen C, Bi X, et al. DNA methylation of CMTM3, SSTR2, and MDFI genes in colorectal cancer. *Gene*. 2017;630:1-7.
7. Li B, Chen X, Jiang Y, et al. CCL2 promoter hypomethylation is associated with gout risk in Chinese Han male population. *Immunol Lett*. 2017;190:15-19.
8. Wu D, Chen X, Xu Y, et al. Prognostic value of MLH1 promoter methylation in male patients with esophageal squamous cell carcinoma. *Oncol Lett*. 2017;13:2745-5.
9. Goel A, Boland CR. Epigenetics of colorectal cancer. *Gastroenterology*. 2012;143:e1.
10. Chen X, Hu H, Liu J, et al. FOXF2 promoter methylation is associated with prognosis in esophageal squamous cell carcinoma. *Tumour Biol*. 2017;39:1010428317692230.
11. Chen X, Yang Y, Liu J, et al. NDRG4 hypermethylation is a potential biomarker for diagnosis and prognosis of gastric cancer in Chinese population. *Oncotarget*. 2017;8:8105-8119.
12. Ravegnini G, Zolezzi Moraga JM, Maffei F, et al. Simultaneous analysis of SEPT9 promoter methylation status, micronuclei frequency, and folate-related gene polymorphisms: the potential for a novel blood-based colorectal cancer biomarker. *Int J Mol Sci*. 2015;16:28486-28497.
13. Migliori V, Phalke S, Bezzi M, et al. Arginine/lysine-methyl/methyl switches: biochemical role of histone arginine methylation in transcriptional regulation. *Epigenomics*. 2010;2:119-137.
14. Almeida-Rios D, Graca I, Vieira FQ, et al. Histone methyltransferase PRMT6 plays an oncogenic role of in prostate cancer. *Oncotarget*. 2016;7:53018-53028.
15. Kim NH, Kim SN, Seo DW, et al. PRMT6 overexpression upregulates TSP-1 and downregulates MMPs: its implication in motility and invasion. *Biochem Biophys Res Comm*. 2013;432:60-65.
16. Phalke S, Mzoughi S, Bezzi M, et al. p53-Independent regulation of p21Waf1/Cip1 expression and senescence by PRMT6. *Nucleic Acids Res*. 2012;40:9534-9542.
17. Yoshimatsu M, Toyokawa G, Hayami S, et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. *Int J Cancer*. 2011;128:562-573.
18. Rauscher GH, Kresovich JK, Poulin M, et al. Exploring DNA methylation changes in promoter, intragenic, and intergenic regions as early and late events in breast cancer formation. *BMC Cancer*. 2015;15:816.
19. Yang Y, Chen X, Hu H, et al. Elevated UMOD methylation level in peripheral blood is associated with gout risk. *Sci Rep*. 2017;7:11196.
20. Wang X, Huang Y, Zhao J, et al. Suppression of PRMT6-mediated arginine methylation of p16 protein potentiates its ability to arrest A549 cell proliferation. *Int J Biochem Cell Biol*. 2012;44:2333-2341.
21. Kleinschmidt MA, de Graaf P, van Teeffelen HA, et al. Cell cycle regulation by the PRMT6 arginine methyltransferase through repression of cyclin-dependent kinase inhibitors. *PLoS One*. 2012;7:e41446.
22. Ma WL, Wang L, Liu LX, et al. Effect of phosphorylation and methylation on the function of the p16INK4a protein in non-small cell lung cancer A549 cells. *Oncol Lett*. 2015;10:2277-2282.
23. Li YJ, Hoang-Xuan K, Zhou XP, et al. Analysis of the p21 gene in gliomas. *J Neurooncol*. 1998;40:107-111.
24. Akhter N, Akhtar MS, Ahmad MM, et al. Association of mutation and hypermethylation of p21 gene with susceptibility to breast cancer: a study from north India. *Mol Biol Rep*. 2014;41:2999-3007.
25. Lim Y, Yu S, Yun JA, et al. The prognostic significance of protein arginine methyltransferase 6 expression in colon cancer. *Oncotarget*. 2018;9:9010-9020.
26. Vieira FQ, Costa-Pinheiro P, Ramalho-Carvalho J, et al. Deregulated expression of selected histone methylases and demethylases in prostate carcinoma. *Endocr Relat Cancer*. 2014;21:51-61.
27. Stein C, Riedl S, Ruthnick D, et al. The arginine methyltransferase PRMT6 regulates cell proliferation and senescence through transcriptional repression of tumor suppressor genes. *Nucleic Acids Res*. 2012;40:9522-9533.
28. Oono Y, Iriguchi Y, Doi Y, et al. A retrospective study of immunochemical fecal occult blood testing for colorectal cancer detection. *Clin Chim Acta*. 2010;411:802-805.

29. van Rossum LG, van Rijn AF, van Oijen MG, et al. False negative fecal occult blood tests due to delayed sample return in colorectal cancer screening. *Int J Cancer*. 2009;125:746-750.
30. Li Y, Song L, Gong Y, et al. Detection of colorectal cancer by DNA methylation biomarker SEPT9: past, present and future. *Biomark Med*. 2014;8:755-769.
31. Committee ASoP, Fisher DA, Maple JT, et al. Complications of colonoscopy. *Gastrointest Endosc*. 2011;74:745-752.
32. Jessup JM, Thomas P. Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. *Cancer Metastasis Rev*. 1989;8:263-280.
33. Lee H, Song M, Shin N, et al. Diagnostic significance of serum HMGB1 in colorectal carcinomas. *PLoS One*. 2012;7:e34318.
34. Qi J, Qian C, Shi W, et al. Alu-based cell-free DNA: a potential complementary biomarker for diagnosis of colorectal cancer. *Clin Biochem*. 2013;46:64-69.
35. Fletcher RH. Carcinoembryonic antigen. *Ann Intern Med*. 1986;104:66-73.
36. Peng HX, Yang L, He BS, et al. Combination of preoperative NLR, PLR and CEA could increase the diagnostic efficacy for I-III stage CRC. *J Clin Lab Anal*. 2017;31. https://doi.org/10.1002/jcla.22075
37. Sorensen CG, Karlsson WK, Pommergaard HC, et al. The diagnostic accuracy of carcinoembryonic antigen to detect colorectal cancer recurrence – a systematic review. *Int J Surg*. 2016;25:134-144.
38. Tan E, Gouvas N, Nicholls RJ, et al. Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer. *Surg Oncol*. 2009;18:15-24.
39. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell*. 1996;87:159-170.
40. Stella S, Bruzzese A, Chiarini S. [Biological bases of the colorectal adenoma-carcinoma sequence]. *Il Giornale di Chirurgia*. 1996;17:473-476.
41. Takami K, Yana I, Kurahashi H, et al. Multistep carcinogenesis in colorectal cancers. *Southeast Asian J Trop Med Public Health*. 1995;26(Suppl 1):190-196.
42. Wynter CV, Walsh MD, Higuchi T, et al. Methylation patterns define two types of hyperplastic polyp associated with colorectal cancer. *Gut*. 2004;53:573-580.
43. Wang HL, Liu P, Zhou PY, et al. Promoter methylation of the RASSF1A gene may contribute to colorectal cancer susceptibility: a meta-analysis of cohort studies. *Ann Hum Genet*. 2014;78:208-216.
44. Wang YC, Yu ZH, Liu C, et al. Detection of RASSF1A promoter hypermethylation in serum from gastric and colorectal adenocarcinoma patients. *World J Gastroenterol*. 2008;14:3074-3080.
45. Li YW, Kong FM, Zhou JP, et al. Aberrant promoter methylation of the vimentin gene may contribute to colorectal carcinogenesis: a meta-analysis. *Tumour Biol*. 2014;35:6783-6790.
46. Suzumura K, Okada T, Satake M, et al. Laparoscopic splenectomy for inflammatory pseudotumor of the spleen. *Hepatogastroenterology*. 2011;58:1909-1911.
47. Abouzeid HE, Kassem AM, Abdel Wahab AH, et al. Promoter hypermethylation of RASSF1A, MGMT, and HIC-1 genes in benign and malignant colorectal tumors. *Tumour Biol*. 2011;32:845-852.
48. Wu PP, Zou JH, Tang RN, et al. Detection and clinical significance of DLC1 gene methylation in serum DNA from colorectal cancer patients. *Chin J Cancer Res*. 2011;23:283-287.

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