

Original Article

Hypermethylated *WNT10A* and its clinical significance in colorectal cancer

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Abstract: Colorectal cancer (CRC) is a heterogeneous disease in which unique subtypes are characterized by distinct genetic and epigenetic alterations. DNA methylation, a well-documented epigenetic modification, is a promising biomarker for the diagnosis and prognosis of cancers, including CRC. *WNT10A* is a member of the Wnt family. It belongs to the Wnt signaling pathway and is involved in CRC. However, studies regarding the methylation and expression of *WNT10A* in CRC are limited. In the current study, we analyzed the methylation status of *WNT10A* in 146 patients with CRC and normal controls. These samples were classified into two groups. The first group was an initial discovery set (i.e., fresh tissue samples from 40 patients with CRC and adjacent normal control samples). The second group was an independent validation set (i.e., formalin-fixed and paraffin-embedded [FFPE] samples from 106 patients with CRC and cutting edge tissues). The results showed a higher level of *WNT10A* hypermethylation of in CRC samples than in controls (Fresh tissue cohort: $P = 2.8E-5$; FFPE cohort: $P = 3.6E-4$). This finding was verified by *WNT10A* methylation data from The Cancer Genome Atlas portal (TCGA) ($P = 1.9E-83$). Subgroup analysis of clinical characteristics showed a higher *WNT10A* methylation level in elder patients (aged > 60 y) ($P = 0.037$) and, patients with distant metastasis ($P = 0.033$), rectal cancer ($P = 0.03$), and mucinous adenocarcinoma ($P = 0.02$). Furthermore, TCGA RNAseq data demonstrated lower *WNT10A* expression in patients with CRC than in controls ($P = 4.0E-3$) and showed a negative correlation between expression and methylation ($r = -0.37$, $P = 5.7E-13$). Moreover, the efficiency of *WNT10A* methylation for CRC diagnosis was analyzed in both cohorts of the present study and the TCGA cohorts, which indicated the potential use of *WNT10A* methylation as a tool for diagnosis of CRC.

Keywords: DNA methylation, *WNT10A*, CRC, TCGA, diagnosis

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer among both males and females in the United States of America (USA). The American Cancer Society provides (<https://www.cancer.org/research.html>) annual comprehensive overviews of the incidence of human cancers, (including CRC), reporting the numbers of new cases and mortality in the USA. Although, the reports show a decreased trend in the incidence of CRC from 1975 to 2014, it is estimated that 140,250 new cases and 50,630 new deaths will occur in 2018 in the USA [1]. Moreover, the incidence in individuals aged < 55 years increased by approximately 2% per year from 1990 to 2014 [2]. Nevertheless, the burden of CRC cases and deaths in China is much higher than that reported in the USA [3].

The etiology and pathology of CRC are well documented. Diet is a major etiological factor. A range of putative dietary carcinogens or daily behaviors, such as Western diet, a low level of physical activity, high consumption of red meat and processed food, are significantly correlated with the development of CRC [4, 5]. However, multiple theories have emerged following the rising incidence of CRC in younger individuals. One theory hypothesized that older generations were exposed to a lower-risk environment than the younger generation [6]. Hessami and his colleagues comprehensively analyzed the association of CRC incidence in younger and elderly patients with dietary risks. They concluded that the unique genetic pattern of different populations cannot be ignored [7]. In addition, previous studies demonstrated strong evidence for genetic susceptibility to CRC through, interaction with the environment, including diet [8, 9].

The multistage nature of carcinogenesis is defined by an accumulation of genetics and/or epigenetic events. Genome wide association studies (GWAS) revealed the significance of genes and pathways hitherto unsuspected of playing a role in CRC [10]. Genetic alterations involve mutations in oncogenes and tumor suppressor genes, such as the adenomatous polyposis coli (*APC*), Kirsten-ras (*K-ras*), and p53 [11-13]. It has been largely demonstrated that defects in these genes affect certain basic biological processes, including cell fate, cell survival, and genome maintenance [13, 14]. However, only 10% of CRC cases are characterized by the “classic” genotype [15, 16]. In addition, the initiation and promotion of cancer may be caused by epigenetic mechanisms, which are defined as heritable changes in gene function that are not due to changes in DNA sequence [15]; in particular, aberrant epigenetic modifications can result in transcriptional silencing [17, 18]. Epigenetic alterations-specifically DNA methylation-are an emerging biomarker that may be potential useful in diagnosis, prognosis and treatment, of various disease including cancer, type 1 diabetes [19, 20].

Aberrant methylation of tumor suppressor genes (TSGs) involved in multiple biological processes, such as DNA repair and apoptosis, such as *MLH1*, *MGMT*, *CDKN2A* and *RAFF1A* [21-23], may be detrimental to health of patients and lead to progression of cancer [24-27]. Although the development of human CRCs is considered a consequence of the accumulation of multiple genetic and epigenetic alterations/interactions, the Wnt signaling pathway appears to play a critical role in the etiology of this disease [14, 28]. Wnts in vertebrates are divided into canonical signaling and noncanonical members [29]. The most extensively characterized Wnt signaling pathway-the canonical Wnt/ β -catenin pathway-regulates the stability of transcription co-activator β -catenin. Consequently, this activates the expression of a set of target genes, regulating cell proliferation, behavior, and survival [30, 31]. As a member of the Wnt family, *WNT10A* is located on 2q35. Notably, the methylation and expression level of *WNT10A* in CRC is inconsistent [32-34].

The primary objective of the present study was to analyze the methylation status of *WNT10A* in

146 paired samples obtained from patients and corresponding normal controls, and investigate the associations between methylated *WNT10A* and clinical features. Moreover, the diagnostic strength of *WNT10A* hypermethylation in recognizing of CRC was analyzed. Subsequently, methylation and expression data along with clinical characteristics from a public database included large numbers of patients were accessed. This analysis was performed to verify the results and analyze the expression status of *WNT10A* in patients with CRC.

Materials and methods

Patient characteristics and collection of tissue specimens

An initial discovery set of 40 CRC fresh tissues and paired adjacent normal tissues were collected from CRC surgical specimens at the Department of Gastrointestinal Surgery in Ningbo First Hospital between April 2011 and April 2015. None of the patients underwent chemotherapy or radiotherapy prior to surgery. Following surgical excision, all specimens were immediately preserved in RNA-fixer reagent and stored in liquid nitrogen. Pathological diagnosis was performed by two experienced pathologists. Among the samples, there were 30 moderately differentiated cases, and 10 poorly differentiated cases. Of note, there were seven Stage I cases, 12 Stage II cases, 13 Stage III cases and eight Stage IV cases. All patients provided written informed consent for the collection of tissue samples prior to their participation in this study. The study was approved by the Human Research Ethical Committee of Ningbo First Hospital.

An independent validation set of 106 paired formalin-fixed and paraffin-embedded (FFPE) CRC and cutting edge tissues were collected from Diagnostic Pathology Central of Ningbo. Among those, there were two well differentiated, 70 moderately differentiated cases, and 34 poorly differentiated cases. Of note, there were nine Stage I cases, 43 Stage II cases, 50 Stage III cases and four Stage IV cases. The clinical characteristics of all the patients were available. All of tumors were staged by experienced surgeons according to the eighth edition (2016) of the tumor-node-metastasis staging system

Hypermethylated WNT10A in CRC

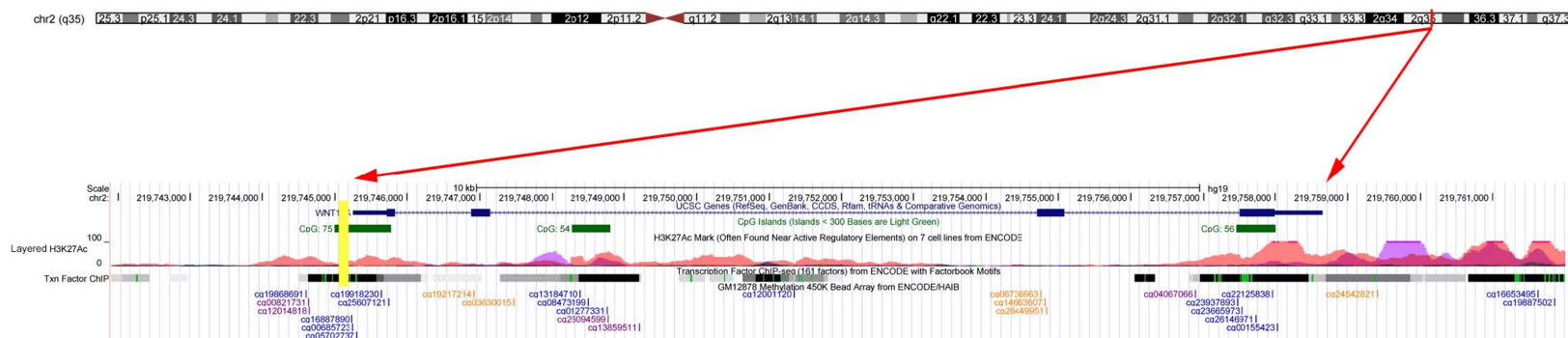


Figure 1. The detail information of the *WNT10A* gene. Each of the cg loci of *WNT10A* is labeled in the last layer according to its position; the yellow bar is the amplified segment of *WNT10A* in the current study; the green bands in the second layer indicate the CpG islands of *WNT10A*; the information of the layered H3K27Ac and the Txn factor chip are shown in the third and fourth layer. This information was downloaded from the UCSC Genome Browser (<http://genome.ucsc.edu/>).

Hypermethylated WNT10A in CRC

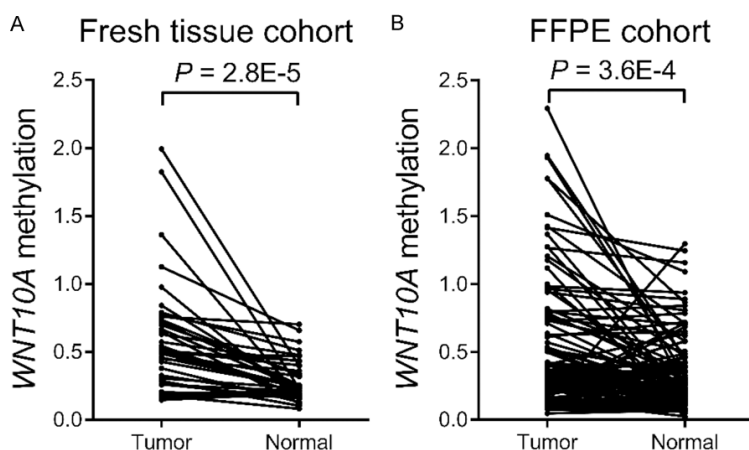


Figure 2. Comparison of *WNT10A* methylation between CRCs and controls in the cohorts of the present study. A: Comparison of *WNT10A* methylation between 40 paired CRCs and control samples in the fresh tissue cohort. B: Comparison of *WNT10A* methylation between 106 paired CRCs and control samples in the FFPE cohort.

established by the American Joint Committee on Cancer (AJCC).

DNA extraction

Genomic DNA of fresh samples and FFPE samples were separately extracted using the QIAamp DNA Mini Kit and DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Subsequently, the concentration and quality of the DNA was measured using the ultramicro nucleic acid ultraviolet tester (NANODROP 2000c, Thermo, USA).

Bisulfite treatment

Extracted genomic DNA was bisulphite-converted with ZYMO EZ DNA Methylation-Gold Kit according to the manufacturer's protocol (Zymo Research, Orange, CA, USA). The bisulphite-modified DNA was used for the following methylation analysis.

Quantitative methylation-specific polymerase chain reaction (qMSP)

Bisulphite modified DNA was amplified through fluorescence-based real-time quantitative methylation-specific PCR (qMSP) using SYBR Green Master Mix (Roche Diagnosis) with Roche 480II RT-PCR instrument (9-well plates, Roche Diagnostics). The qMSP primers for *WNT10A* were designed by MethPrimer ([Primer.cgi\) \[35\]. Briefly, we initially determined the gene description, such as position, CpG islands, histone modification status, and the position of each CpG sites, from UCSC Genome Browser \(<http://genome.ucsc.edu/>\). Subsequently, we identified and downloaded the sequences of target fragment of the *WNT10A* promoter region and input them into MethPrimer to design the qMSP primers \(Figure 1\). One pair of *WNT10A* primers that could amplify a specific product was used for the methylation analysis. Primers for *ACTB* gene were simultaneously amplified in each sample and used as internal control \[36\].](http://www.urogene.org/cgi-bin/methprimer2/Meth-</p></div><div data-bbox=)

The primers used for the *WNT10A* were as follows: sense, 5'-GCGGGTATAGGTAGGTAA-3'; antisense, 5'-CACACAACAAATTAACGAAA-3'. The primers used for *ACTB* were as follows: sense, 5'-TGGTGATGGAGGAGGTTTAGTAAGT-3'; antisense, 5'-AACCAATAAAACCTACTCCTCCCTTA-3'. The values obtained for the *WNT10A* gene were compared with these of the internal reference gene to calculate a ratio that was then multiplied by 1000 for simple tabulation ($WNT10A/ACTB \times 1000$) [36]. This calculation determined the relative levels of methylated *WNT10A* in each sample.

Validation dataset from the cancer genome atlas portal (TCGA) portal

To ascertain our results, we downloaded the Illumina HumanMethylation 450K methylation data and RNA-seq expression of *WNT10A*, combined with clinical characteristics from TCGA (<https://cancergenome.nih.gov/>). Collectively, a total of 443 patients' methylation frequencies and expression data of *WNT10A* along with the clinical characteristics were obtained, including 398 CRCs and 45 normal controls.

Regarding the methylation data of *WNT10A*, we abstracted the level 3 methylation of 49 CpGs sites. There were eight CpG sites (including cg19868691, cg00821731, cg12014818, cg16887890, cg00685723, cg05702737, cg19918230, and cg25607121) entered into further analysis, based on the following three cri-

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Table 1. Association of *WNT10A* methylation with clinical characteristics of CRC in the cohorts of the present study

Characteristics		N	Mean methylation	P
Gender	Male	86	55%	0.67
	Female	60	59%	
Age (year)	≤ 60	58	55%	0.73
	> 60	88	59%	
Stage	I	16	30%	0.24
	II	55	55%	
	III	63	61%	
	IV	12	64%	
Lymph metastasis	No	73	52%	0.41
	Yes	73	61%	
Distant metastasis	No	133	55%	0.35
	Yes	13	71%	
Tumor location	Colon	75	52%	0.35
	Rectum	71	61%	
Differentiation	Well	2	48%	0.21
	Moderate	100	44%	
	Poor	44	85%	
Tumor size	< 5 cm	83	56%	0.98
	≥ 5 cm	63	56%	
Histological classification	Adenocarcinoma	142	56%	0.56
	Mucinous adenocarcinoma	4	74%	

teria: 1) significantly correlated CpG sites according to Pearson's correlation; 2) located on the CpG island of the *WNT10A* promoter region annotated by Illumina HumanMethylation 450K annotation file downloaded from the Illumina official website (https://support.illumina.com/array/array_kits/infinium_humanmethylation-450_beadchip_kit/downloads.html); and 3) neighbored the targeted fragment in our study. The mean methylation levels of included eight CpG sites were computed to represent the methylation level of the promoter region of *WNT10A*.

Regarding the expression data of *WNT10A*, we also obtained the level 3 of RNASeq (Version 2). A total of 394 samples (including 373 CRC patients and 21 normal controls) simultaneously available of *WNT10A* methylation and expression data were used to investigate the expression of *WNT10A* and its correlation with *WNT10A* methylation in CRC.

Statistical analyses

All the statistical analyses were performed using the SPSS Version 18.0 (SPSS Inc.,

Chicago, IL, USA). Pearson's correlation was performed to identify the significant correlated CpG sites, and to analyze the correlation between *WNT10A* methylation and expression. Differences in continuous data between pairs of groups were detected by Student's *t* test. Namely, an independent *t* test was performed for unpaired subjects. Paired *t* test was utilized for paired subjects, and one-way analysis of variance (ANOVA) test was applied between multiple groups (i.e., more than two groups). The diagnostic value of *WNT10A* methylation was assessed using the receiver operating characteristic (ROC) curve and the area under the curve (AUC). In addition, the positive predict value (PPV) and negative

predict value (NPV) were also computed. A two-tailed *P* value of less than 0.05 was considered statistically significant. All the figures were drawn using the GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA) or R 3.1.2 software (<http://www.r-project.org/>).

Results

Hypermethylation of WNT10A in the study cohorts

The fragment of the CpG island in the promoter region was amplified using qMSP to verify the methylation level of *WNT10A* (**Figure 1**). In the fresh-tissue cohort, bisulphite modified DNA from 40 CRC and corresponding normal samples were analyzed. The results showed higher methylation level of *WNT10A* in CRCs was higher than that observed in controls ($P = 2.8E-5$, **Figure 2A**). In an independent validation set, the methylation frequencies of *WNT10A* in 106 bisulphite-converted DNA from samples from FFPE CRC and cutting-edge tissues were studied. These results corroborated the aforementioned findings, indicating a higher methylation

Hypermethylated WNT10A in CRC

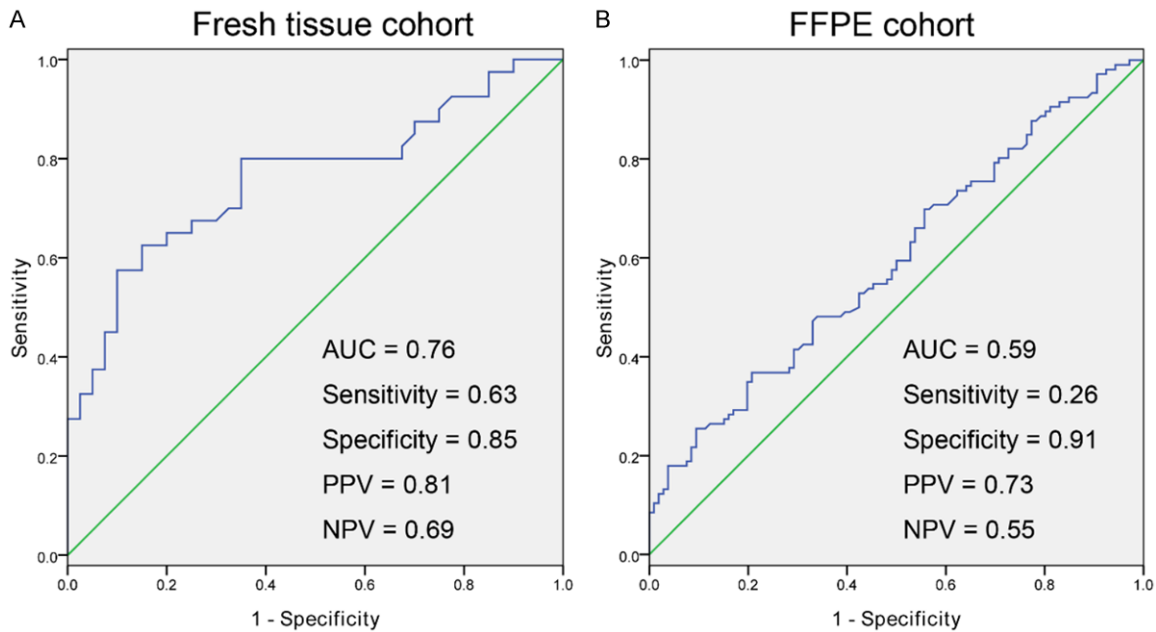


Figure 3. Diagnostic power of methylated *WNT10A* in distinguishing of CRC from controls in the cohorts of the present study. A: Diagnostic power of detection of methylated *WNT10A* in distinguishing of CRC from controls in the fresh tissue cohort. B: Diagnostic power of detection of methylated *WNT10A* in distinguishing of CRC from controls in the FFPE cohort.

level of *WNT10A* in patients with CRCs than in normal controls ($P = 3.6E-4$, **Figure 2B**).

Subsequently, a methylation difference analysis based on clinical characteristics was performed. Although, the observed differences in the study cohorts of the present study was not statistically significant (**Table 1**), an upward trend for the mean methylation frequency of *WNT10A* related to the advanced stage of CRC was still notable. Furthermore, the mean methylation level was increased in patients with lymph metastasis or distant metastasis versus those without metastasis. These results indicate the potential important role of *WNT10A* methylation in the progression of CRC.

Diagnostic value of methylated *WNT10A* in CRC

Subsequently, an ROC analysis was conducted to determine the diagnostic power of methylation *WNT10A* in identification of CRC from normal control in both the fresh-tissue cohort and FFPE cohorts. In the fresh-tissue cohort, the under area of ROC curve (AUC) was 0.76 with 85% specificity and 63% sensitivity. And the PPV and NPV were 0.81 and 0.69, respectively

(**Figure 3A**). In the FFPE cohort, the value of AUC was 0.59 with 91% specificity and 26% sensitivity; the PPV is 0.73 and NPV were 0.55, respectively (**Figure 3B**).

Validation datasets from TCGA

To ascertain our results, methylation data of *WNT10A* from 398 CRC samples and 45 normal controls were download from TCGA portal. The results demonstrated elevated methylation frequency in CRC samples than in controls ($P = 1.9E-83$, **Figure 4A**).

Then, 394 CRC samples with available data for clinical characteristics were used for subgroup comparison analyses based on gender, age, different stage of disease, lymph invasive status, as well as distant metastasis status. In TCGA cohorts, a higher methylation level of *WNT10A* was observed with statistical significance in elderly patients (aged > 60 years) ($P = 0.037$), patients with distant metastasis ($P = 0.033$), and those diagnosed with rectal cancer ($P = 0.03$) and mucinous adenocarcinoma ($P = 0.02$) (**Table 2**). However, there were no associations between *WNT10A* methylation and the clinical stage of disease, lymph metastasis status or gender.

Hypermethylated WNT10A in CRC

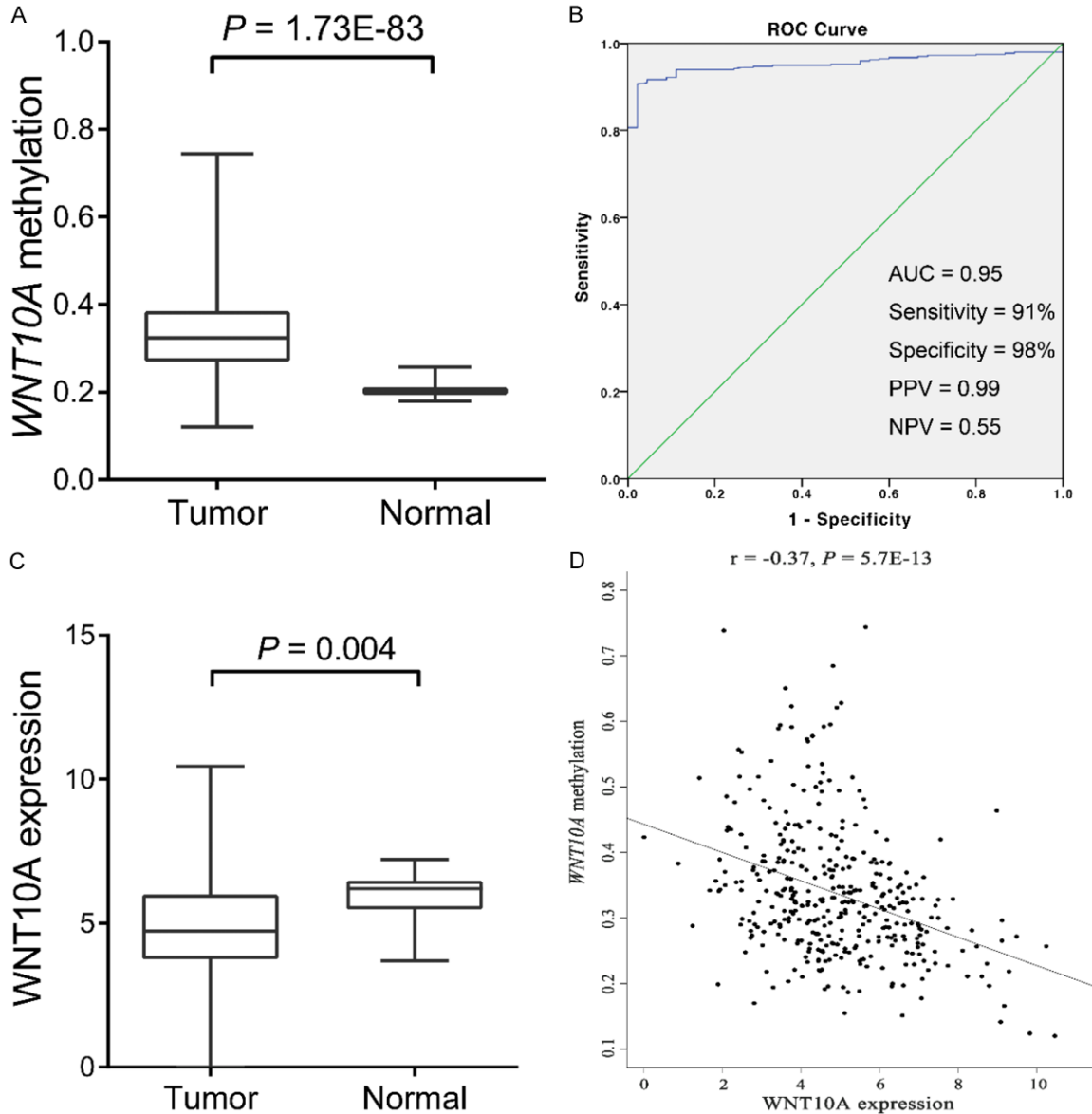


Figure 4. Validation of *WNT10A* methylation from TCGA dataset. A: Comparison of *WNT10A* methylation between patients with CRC and controls in TCGA cohorts. B: Diagnostic power of methylated *WNT10A* in distinguishing of patients with CRC from controls in TCGA cohorts. C: Comparison of *WNT10A* expression between patients with CRC and controls from TCGA portal. D: Negative correlation of *WNT10A* methylation and expression from TCGA portal.

Moreover, the diagnostic power of methylated *WNT10A* in TCGA cohort was studied. In this cohort, the AUC was 0.95 with 98% specificity and 91% sensitivity, and the PPV and NPV were 0.99 and 0.55, respectively (Figure 4B). These results represented the that *WNT10A* have potential to be served as useful biomarker for identification of CRC from normal control, which is still needed further prospective study to ascertain our results.

Expression data from TCGA

Numerous studies revealed a negative correlation between methylation and expression of *WNT10A*. The expression level of *WNT10A* was also studied in present study. Due to the absence of an adequate number of samples, we applied the expression data available at TCGA database. Firstly, the expression of *WNT10A* in CRC and controls was compared.

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Table 2. Association of *WNT10A* methylation with clinical characteristics of CRC in TCGA cohorts

Characteristics		N	Mean methylation	P
Gender	Male	212	33%	0.11
	Female	182	35%	
Age (year)	≤ 60	153	32%	0.037*
	> 60	241	35%	
Stage	I	58	31%	0.12
	II	153	34%	
	III	127	34%	
	IV	56	35%	
Lymph metastasis	No	217	33%	0.31
	Yes	177	34%	
Distant metastasis	No	341	31%	0.033*
	Yes	53	34%	
Tumor location	Colon	296	32%	0.03*
	Rectum	98	35%	
Histological classification	Adenocarcinoma	344	33%	0.02*
	Mucinous adenocarcinoma	50	37%	

* $P < 0.05$.

This comparison revealed a reduced expression of *WNT10A* in patients with CRC was observed than in controls ($P = 4.0E-3$, **Figure 4C**). Secondly, the Pearson's analysis showed an inverse correlation between *WNT10A* expression and methylation ($r = -0.37$, $P = 5.7E-13$, **Figure 4D**).

Discussion

Although the overall incidence of CRC is gradually decreased, the rejuvenation of CRC is observed and the number of individual who is diagnosed as CRC is still large in China [3]. Despite the introduction and improvement of endoscopy and biopsies technology, the number of diagnosed CRC is just the tip of the iceberg for untypical clinical symptom of most of early stage of this disease. Approximately 35% of patients are diagnosed as localized stage CRC, for which the 5-year survival is 90% [2]. However, the survival rate declines to 71% and 14% for patients diagnosed with regional and advanced stage disease, respectively [2, 37]. Therefore, early detection is of crucial importance to improve the survival rate of patients with CRC, in whom curative surgical resection or endoscopic treatment is possible. The application of molecular markers specific to CRC offers new possibilities for early detection [38].

Although proteins or other small molecular markers derived from cancers proved useful in certain cases, markers based on changes in the genetic material offer the powerful advantage of allowing signal amplification using PCR, hence increasing sensitivity [39]. Thus far, studies strongly suggest that the analysis of DNA methylation patterns may become a powerful tool for the accurate and early diagnosis of CRC, with unparalleled specificity and sensitivity [40-42].

The Wnt- β -catenin-TCF signaling pathway consists of WNTs, Frizzled

genes (*FZD*), intracellular signaling molecules, and TCF transcription factors, which are implicated in carcinogenesis and embryogenesis [43]. Wnts are secreted-type glycoproteins with conserved cysteine residues [44, 45]. They bind to seven-transmembrane-type WNT receptors containing a cysteine-rich domain, encoded by the *FZD* genes.[44, 46] Genetic alterations in the members of the canonical Wnt/ β -catenin pathway moleculars, such as *APC*, *AXIN1*, β -*catenin* and *TCF* are involved in carcinogenesis through transcriptional activation of the Wnt target genes [47, 48]. The human WNT gene family consists of at least 19 members. A previous study revealed that *WNT10A* is clustered with *WNT6* on chromosome 2q35 in a head-to-tail manner [49]. The *WNT10A* gene encodes a 417-amino-acid polypeptide with a Wnt core domain, which is 64% identical to *WNT10B* [49, 50]. Developmentally, *WNT10A* is extensively studied in the context of ectodermal lineages, primarily in the deregulation of ectodermal tissues resulting in a variety of disorders [51, 52]. Furthermore, *WNT10A* was previously implicated in a variety of cancers and has been shown to promote proliferation, migration, and chemo-resistance in renal cell carcinoma cell lines via regulation of β -*catenin* [32, 53-55]. Besides, Kurasa and his colleagues reported that *WNT10A* was associated

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with epithelial-mesenchymal transition (EMT) and progression of cancer through mesenchymal-specific DNA hypermethylation [34]. Other reports indicate that *WNT10A* is upregulated in esophageal cancer, gastric, and colon cancer cells and tumors [32]. However, hypermethylation of the *WNT10A* promoter region resulting in lower expression has been identified in head and neck squamous cell carcinoma and oligodendroglioma cell lines [33, 34]. Numerous studies have addressed the causal relationship between promoter hypermethylation and abated transcriptional.

The current data regarding the methylation and expression of *WNT10A* in human cancers are inconsistent. Thus, in the present study, we analyzed its methylation and expression in CRC. Herein, the results demonstrated that *WNT10A* is frequently methylated in CRC primary tumors, but less frequently in normal colon tissues. Kurasawa and his colleagues have demonstrated the hypermethylation level of *WNT10A* in head and neck carcinomas and the associations of methylation and clinicopathology; specifically, the elevated level of *WNT10A* methylation in the advanced stages of the disease [34]. Although significant associations between *WNT10A* methylation and clinical features were not found in this study, an upward tendency of methylation in advanced CRC was observed in the present cohort. Public data were also accessed/analyzed to verify our results, showing increased methylation level in patients with lymph metastasis and distant metastasis. This finding is in accordance with previous study results. Furthermore, in TCGA dataset, a higher level of *WNT10A* methylation was observed in patients diagnosed with rectal cancer than in those with colon cancer. Andrew and his colleagues have conducted an assessment of loci methylation level throughout the genome of ascending colon and rectal normal colon epithelial cells, revealing multiple differentially methylated genes [56]. An explanation for this observation may be that different sublocations arise from different embryological sources and different methylation levels are associated with particular types tissues and cell lineages [57, 58]. Previous studies also reported multiple differentially methylated genes among four different sublocations of CRC [59, 60]. These findings suggest that

assessing the diagnostic value of gene methylation may be useful in distinguishing different sublocations of CRC. Hypermethylation of the gene promoter region is accompanied with dysregulation of expression [61, 62]. However, upregulation of *WNT10A* was reported in numerous human cancers, such as gastric cancer, esophageal cancer or other cancers [32]. Nevertheless, in the current study, we analyzed the expression level of *WNT10A* in CRC using public data. The results showed lower expression of *WNT10A* in CRC tissues and a negative correlation between gene expression and methylation in CRC.

An indication that DNA methylation may be used as biomarkers provided by the use of hypermethylation events at the genes O6-methylguanine-DNA methyltransferase (*MGMT*) in glioma [63] and the glutathione S-transferase pi 1 (*GSTP1*) in prostate cancer [64]. These hypermethylation events were shown to be effective in determining an appropriate treatment choice for glioma and in the diagnosis of prostate cancer, respectively. Therefore, the detection of epigenetic alterations is a promising tool for the diagnosis and prognosis of disease. In the current study, the diagnostic power of methylated *WNT10A* was also analyzed in each of cohort. In our study cohorts A and cohort B, the AUC were 0.76 and 0.59 with high specificity and middle/low sensitivity, respectively. The PPV for CRC was more than 70%, whereas the NPV was not quite satisfactory. The disparity in AUC due to the different types of samples included in these two cohorts. The FFPE samples of cohort B were embedded in formalin, which induced the fragmentation of DNA. Furthermore, the public data from TCGA were used to verify the results. This analysis provided a consolidated conclusion, indicating that the detection of *WNT10A* methylation is potentially useful in distinguishing CRC from normal controls.

In summary, the present findings revealed the hypermethylation of *WNT10A* in CRC, which was negatively correlated with the expression of *WNT10A*. An upward tendency of *WNT10A* hypermethylation was observed in CRC. Further studies with large sample-size are warranted to confirm these results. The methylation status of *WNT10A* is a potentially useful biomarker for the diagnosis of CRC.

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Disclosure of conflict of interest

None.

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