

Significance of *Fusobacterium nucleatum* Combined with SFRP2 and SDC2 Gene Methylation Detection in Early Screening of Colorectal Cancer

FANGCHAO ZHONG^{1#}, XIAOXIA WEI^{1#}, MAOSEN HUANG^{1#}, HAOCHAO YAN¹, LIHUA FU¹,
XIAOLIANG LIU², HAIMING RU^{1,2}, XIANWEI MO^{1,2}, ZIJIE SU⁴ and LINHAI YAN^{1,3*}

¹Department of Gastrointestinal Surgery, Guangxi Medical University Cancer Hospital, Nanning, China

²Department of Surgery, Guangxi Medical University Cancer Hospital, Nanning, China

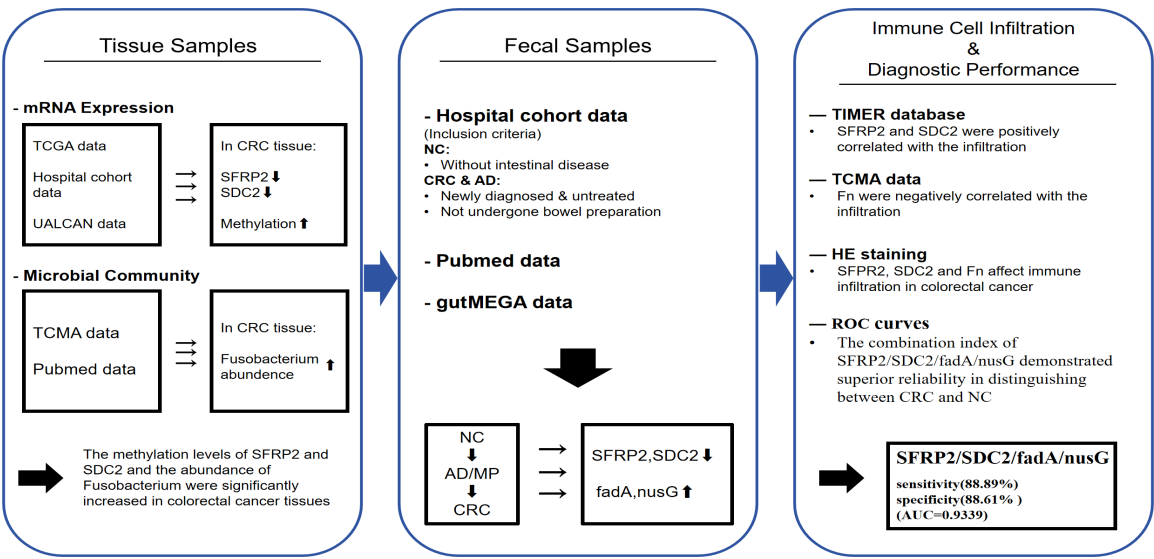
³Guangxi Clinical Research Center for Colorectal Cancer, Nanning, China

⁴Department of Research, Guangxi Medical University Cancer Hospital, Nanning, China

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Abstract

This study aimed to explore the combined detection of secreted frizzled-related protein-2 (SFRP2), Syndecan-2 (SDC2), and *Fusobacterium nucleatum* (Fn) in fecal samples for early colorectal cancer (CRC) screening. Public datasets were analyzed to evaluate the expression of SFRP2, SDC2, and Fn. The study included 30 pairs of tissue and 196 fecal samples. Gene expression of SFRP2, SDC2, *F. nucleatum* antigen adhesinA (fadA), and N-utilization substance G (nusG) was measured by qPCR. Correlations with Ki67, P53 expression, and immune infiltration were examined. The diagnostic performance of the combined markers was assessed using ROC curve analysis. SFRP2 and SDC2 were underexpressed ($p < 0.001$), while Fn was notably enriched in CRC ($p < 0.0001$). Expression of SFRP2 and SDC2 correlated with immune cell infiltration, whereas Fn abundance showed a negative correlation with immune infiltration. ROC analysis indicated that the combined detection of these markers outperformed single-gene tests, CEA, and FOBT in early CRC detection. This combined detection approach offers a promising, non-invasive, and cost-effective method for early CRC screening.



Key words: colorectal cancer, methylation, microbiome, fecal biomarker, early screening

[#] Fangchao Zhong, Xiaoxia Wei, and Maosen Huang contribute equally to this work and are co-first authors.

^{*} Corresponding author: L. Yan, Department of Gastrointestinal Surgery, Guangxi Medical University Cancer Hospital, Nanning, China; Guangxi Clinical Research Center for Colorectal Cancer, Nanning, China; e-mail: yanlinhai000@163.com

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Introduction

Colorectal cancer (CRC) is the second most prevalent cancer worldwide and the second leading cause of cancer-related mortality (Bray et al. 2018). The majority of CRC patients are diagnosed at an advanced stage or with metastasis, highlighting the need for early detection to improve outcomes (Bosch et al. 2011). Colonoscopy is the gold standard for early CRC diagnosis, but its invasiveness, high cost, and bowel preparation reduce compliance rates (Rabeneck et al. 2008; Niu et al. 2017). There is an urgent need for sensitive, non-invasive tests to improve early CRC detection and patient outcomes.

Recent screening tools, like fecal occult blood tests (FOBT) and blood tests for carcinoembryonic antigen (CEA), have effectively reduced CRC morbidity and mortality (Issa and Nouredine 2017). However, FOBT sensitivity and CEA accuracy are relatively low, limiting their application for early CRC screening (Tinmouth et al. 2015). The onset of CRC is associated with accumulating genetic mutations and epigenetic abnormalities (Nguyen et al. 2020). Aberrant DNA methylation is the most extensively studied epigenetic modification in cancer, including gastrointestinal cancer (Vedeld et al. 2018). DNA methylation has emerged as a promising tool for early CRC diagnosis, with many guidelines endorsing its use in screening (Pawa et al. 2011).

Fecal samples from CRC patients often contain shed tumor cells, and detecting methylated DNA from specific genes is an effective method for early screening (Vedeld et al. 2018). Fecal testing offers several advantages over other screening methods, including no need for bowel preparation, non-invasiveness, safety, ease of administration, cost-effectiveness, and effectiveness in detecting precancerous lesions (Osborn and Ahlquist 2005). Numerous highly sensitive DNA markers, including secreted frizzled-related protein-2 (SFRP2), Syndecan-2 (SDC2), and Septin9, have been identified as potential biomarkers for CRC diagnosis (Zhao et al. 2020; 2021). Emerging evidence underscores the intimate link between the gut microbiota and the initiation and progression of CRC (Mima et al. 2016). Certain microbial virulence factors, exemplified by *Fusobacterium nucleatum* (Fn), play a role in inducing CRC development through the expression of the virulence factor fadA (Irrazábal et al. 2014). FadA, a surface adhesive molecule, promotes microbial attachment to colonic epithelial cells, disrupting cell junctions and increasing paracellular permeability, which

allows other bacteria to penetrate the epithelium (Ikegami et al. 2009). The microbial community further stimulates the host immune system, ultimately fostering the occurrence and development of CRC (Fardini et al. 2011). A study sequencing the nusA and nusG genes, along with variable regions V2, V3, and V6 of the bacterial 16S rRNA gene in tumor tissues, aimed to detect *Fusobacterium* DNA and explore its link to clinical features, CRC-specific mortality, and somatic mutations (Borozan et al. 2022). Experimental studies have shown that Fn is enriched in human colorectal adenomas and cancer tissues compared to adjacent normal tissues, highlighting its potential diagnostic value in CRC screening (Mima et al. 2016).

However, single-gene methylation testing and individual microbial detection often exhibit lower sensitivity. Numerous studies have indicated that multi-gene methylation testing can enhance the sensitivity and specificity of CRC detection. The methylation levels of SFRP2 and SDC2, along with the abundance of Fn, are markedly elevated in CRC patients compared to normal individuals. Currently, limited research exists on the amalgamation of fecal DNA methylation and microbial detection in CRC. Therefore, the combined detection of two DNA methylation biomarkers, SFRP2 and SDC2, along with Fn in feces, may yield promising diagnostic performance for CRC. This study endeavors to establish a composite index, SFRP2/SDC2/fadA/nusG, and compare it with single gene markers, serum tumor markers, and traditional FOBT to assess its diagnostic efficacy in early CRC screening.

Experimental

Materials and Methods

Study population. We procured 30 tissue samples and 228 fecal samples for experimental analysis to enhance the efficiency and reliability of diagnosing and screening CRC. After excluding the unqualified stool samples, a total of 196 samples were included in the study. The fecal samples comprised 132 cases from CRC patients, 22 from adenoma (AD) patients, and 42 from healthy volunteers. The study population of this study was from 132 patients with colorectal cancer and 22 patients with adenoma who were hospitalized in our cancer hospital from March 31, 2021, to April 27, 2023, and 42 healthy volunteers were recruited during this period. The CRC group consisted of 54 females and 78 males, aged 23 to 89 years, with a mean age of 57.33.

The AD group included 12 females and 10 males, aged 34 to 79 years, with a mean age of 55.82. The control group comprised 17 females and 25 males, aged 24 to 63 years, with a mean age of 43.90. The chi-square test and one-way analysis of variance were used to compare the gender and age among the three groups, and the differences among the three groups were not statistically significant and were comparable ($p > 0.05$). The inclusion and exclusion criteria for the study population are provided in Table SI. All participants provided prior consent for the study, which the Ethics Committee of Guangxi Medical University Cancer Hospital approved.

Data collection. Gene expression profiles of CRC patients were retrieved from the TCGA database, encompassing 698 tissues (647 tumor tissues and 51 normal tissues). Fecal metagenomic sequencing data published by Yachida et al. (2019) were also included. Clinical information for 132 CRC patients was sourced from the Affiliated Hospital of Guangxi Medical University. The protein marker results of immunohistochemistry (Ki67 and P53) were obtained from the hospital's pathology department.

Public database analysis. The UALCAN database (<https://ualcan.path.uab.edu>) (Chandrashekar et al. 2017; 2022) was utilized to examine the methylation levels of SFRP2 and SDC2 in CRC and normal tissues. The Traditional Chinese Medicine Association (TCMA) database (<https://tcma.pratt.duke.edu>) (Dohlman et al. 2021) was employed to assess the abundance of major microbial species in CRC and adjacent tissues. The gutMEGA database (<http://gutmega.omicsbio.info>) (Zhang et al. 2021) was used to analyze the relative abundance of *Fusobacterium* in fecal samples from both CRC patients and healthy controls. The Tumor Immune Estimation Resource (TIMER) (<https://cistrome.shinyapps.io/timer>) (Li et al. 2016; Li et al. 2017) was applied to investigate the correlation between the expression levels of SFRP2 and SDC2 and tumor-infiltrating immune cells.

Detection of methylated DNA and Fn-specific markers. DNA was extracted from tissue and fecal samples using the Fast Pure Tissue DNA Isolation Mini Kit (Vazyme, China) and the HiPure Stool DNA Mini Kit (Magen, China), respectively, following the manufacturer's protocols. DNA from both sources was chemically modified using sodium bisulfite with the EpiArt DNA Methylation Bisulfite Kit (Vazyme, China). Microbial detection did not require bisulfite conversion. RT-qPCR amplification with Taqman probes

was performed to analyze the methylation status of SFRP2 and SDC2 genes and to detect bacterial gene markers. The primers and probes are provided in Table SII. Actin beta (ACTB) and quantitative actin beta (Q-ACTB) were used as reference genes.

Hematoxylin-eosin (HE) staining. HE staining was conducted on CRC tissue samples using the HE staining kit (Sangon Biotech, China), following the manufacturer's instructions.

Data processing and statistical analysis. Independent sample *t*-tests and Wilcoxon rank sum tests were applied for non-paired samples. The Kruskal-Wallis test was used to assess differential expression in fecal samples from different populations. Chi-square tests evaluated the correlation between positive rates of SFRP2, SDC2, fadA, nusG, and CEA with clinicopathological features. One-way analysis of variance (ANOVA) compared the relative expression of two Fn biomarkers, and unpaired *t*-tests assessed correlations between Ki67 positivity (threshold 60%) and the expression of SFRP2, fadA, and P53 positivity (threshold 50%) with the expression of four genes. Mann-Whitney tests were used for Ki67 correlation with SDC2 and nusG expression.

The area under the receiver operating characteristic (ROC) curve (AUC) and its 95% confidence interval (CI) were calculated. The optimal cutoff value was determined by maximizing the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$). Numerical data are presented as mean $\pm$ standard error of the mean (SEM) or median (interquartile range, IQR). Statistical analysis was conducted using GraphPad Prism v9.4.0 (GraphPad Software, USA, www.graphpad.com), IBM SPSS Statistics for Windows v26.0 (IBM Corp., USA), and R v4.2.1 software (R Core Team 2022). A *p*-value < 0.05 was considered statistically significant.

Results

mRNA expression levels of SFRP2 and SDC2 in different tissue samples. Analysis of the TCGA database revealed a significant reduction in the mRNA expression of SFRP2 and SDC2 in CRC tissues compared to normal tissues (Fig. 1a and 1b). Paired comparisons of 50 cancer and adjacent non-cancerous tissue samples confirmed that both genes were notably downregulated in CRC tissues (Fig. 1c and 1d). Additionally, investigation using the UALCAN database showed significantly increased methylation levels of SFRP2 and SDC2 in CRC tissues compared to normal

tissues (Fig. 1e and 1f). Validation through qPCR on postoperative tissue specimens from 30 CRC patients at Guangxi Medical University Cancer Hospital confirmed higher methylation levels of SFRP2 and SDC2 in tumor tissues compared to adjacent non-cancerous tissues (Fig. 1g and 1h). Pathway enrichment analysis was performed on genes associated with these markers to explore the biological significance further (Fig. S1).

Microbial community differences between samples in CRC and control groups. To assess the differences in the classification of bacterial communities between normal individuals and patients with CRC, we initially analyzed data sourced from the public TCMA database. Fig. 2a illustrates the dominant microbial communities found in CRC tissues ($n = 170$) and adjacent tissues ($n = 25$). However, only *Fusobacterium*, *Parabacteroides*, and *Oscillibacter* showed significant differences in abundance between tumor tissues and adjacent tissues (Fig. 2c). Subsequently, a heatmap was generated to visualize the enrichment abundance of the top 15 microbial genera in cancer tissues and adjacent tissues (Fig. 2e). Furthermore, we analyzed published metagenomic sequencing data from the PubMed database. The results were consistent with the analysis of the TCMA database (Fig. 2b, 2d, and 2f). Through the above analysis, we established significant differences in the abundance of *Fusobacterium* between healthy controls and tumor samples.

Evaluation of DNA methylation markers and Fn as indicators for the detection of CRC and AD in fecal samples. In our study, we analyzed 196 fecal samples to assess the methylation status of specific genes and the abundance of bacterial markers using RT-qPCR. Compared to the healthy control group, the CRC group exhibited significantly elevated levels of SFRP2 and SDC2 methylation in stool samples ($p < 0.0001$) (Fig. 3a and 3b). The expression levels of Fn in fecal samples from CRC patients were markedly higher than in normal fecal samples ($p < 0.0001$) (data from Yachida et al. 2019) (Fig. 3c). Additionally, we found a significant increase in the abundance of Fn in fecal samples from CRC patients in the gut MEGA database ($p < 0.01$) (Fig. 3f). Moreover, the relative expression levels of fadA and nusG were significantly elevated in CRC fecal samples compared to the normal control (NC) group ($p < 0.0001$). (Fig. 3d and 3e).

We further analyzed the positivity rates of four genes in 196 fecal samples (Fig. 3g). In summary, the positivity rates of these four genes were significantly higher in CRC and AD samples compared to the NC group (NC vs. AD, $p = 0.0006$; NC vs. CRC, $p < 0.0001$).

Additionally, Tables SIII and SIV summarize the methylation results of SFRP2 and SDC2 in CRC samples, the positive rates of fadA and nusG in CRC samples, and their relationship with different clinicopathological features. The results indicated a significant correlation between the methylation of SFRP2 and SDC2 genes and lymph node metastasis ($p = 0.0443$; $p = 0.0002$) (Fig. 3h). Moreover, the correlation of SFRP2, SDC2, fadA, and NusG with Ki67 and P53 expression was analyzed, which revealed a positive correlation between the methylation levels of SFRP2 and SDC2 and the relative expression levels of fadA and nusG with the expressions of Ki67 and P53 (Fig. S2).

Correlation between DNA methylation markers, Fn, and levels of immune cell infiltration. In CRC, SFRP2 and SDC2 were positively correlated with the infiltration of most immune cells, while negatively correlated with tumor purity (Fig. 4a and 4b). Moreover, based on the data from the TCMA database, a negative correlation was demonstrated between the abundance of Fn and lymphocytes and neutrophils (Fig. 4c, 4d, and 4e). Histological examination along with HE staining revealed that the higher the methylation level of SFRP2 and SDC2 in the stool samples of patients, and the higher the expression of fadA and nusG genes, the more obvious the infiltration of immune cells in the cancer tissues. In the same way, patients with high expressions of the fadA and nusG genes in stool samples, the infiltration of lymphocytes in their cancer tissues was only mild to moderate (Fig. 5). These findings suggest that a high abundance of Fn in tissue may lead to a decrease in lymphocyte count, inhibiting anti-tumor immune responses, and reducing immune function, thereby promoting the onset and progression of CRC.

Diagnostic performance of combined detection of SFRP2/SDC2/fadA/nusG surpassed that of single-gene testing, serum tumor marker CEA, and FOBT. We used colonoscopy and pathological examination as the diagnostic gold standard, with qPCR results as the evaluation index. ROC curve analysis was adopted to assess the diagnostic efficacy of SFRP2, SDC2, Fn (fadA + nusG), CEA, FOBT, and SFRP2/SDC2/fadA/nusG in fecal samples (Fig. 6). The prevalence of CEA positivity in CRC patients was assessed, and its association with various clinical parameters was investigated (Table SV). The results indicated no correlation between the positive rate of CEA and various clinical parameters. Table SVI outlines the performance of fecal-based biomarkers in CRC screening. The SFRP2/SDC2/fadA/nusG combination index demonstrated superior reliability in distinguishing between CRC and

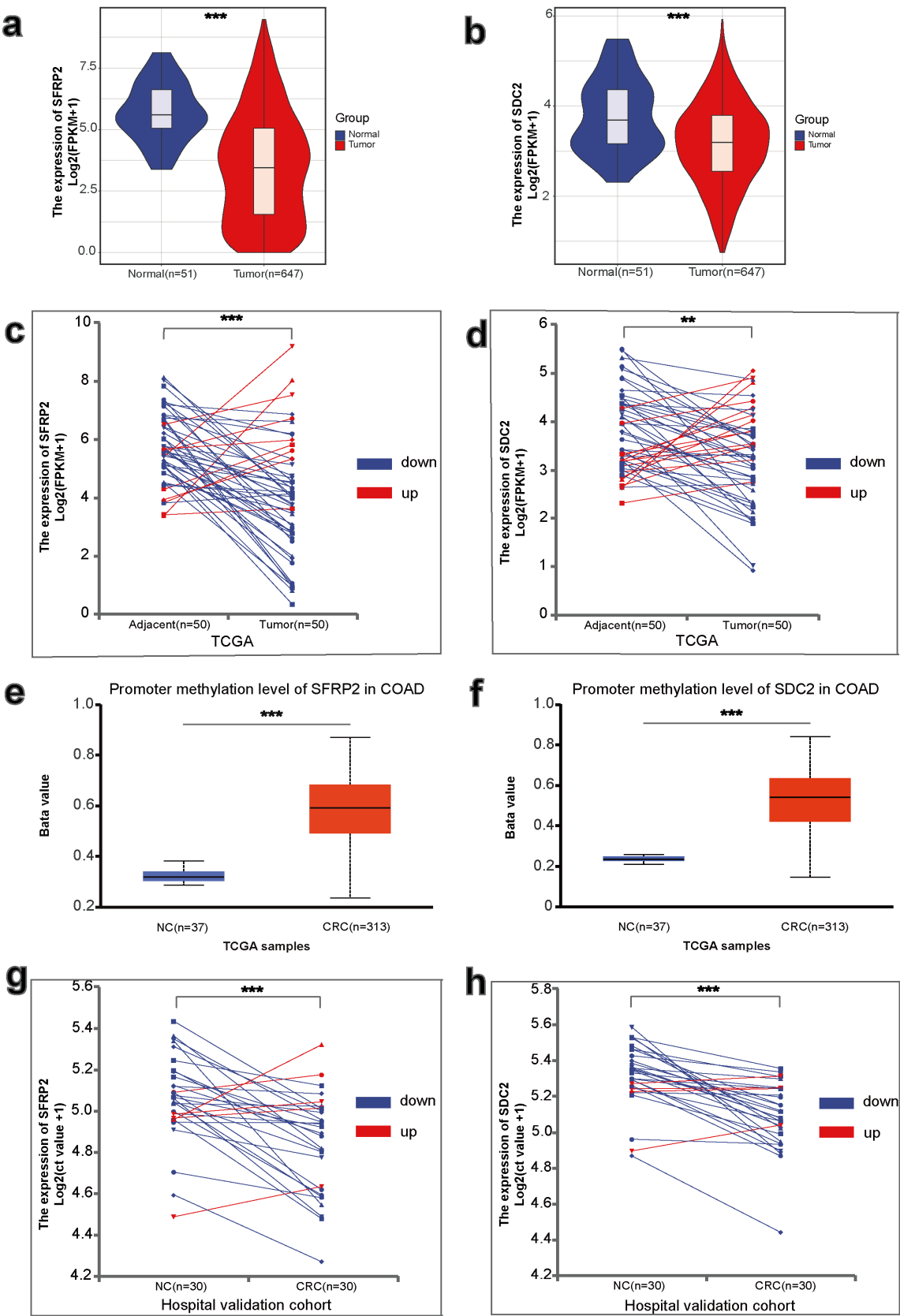


Fig. 1. Expression of SFRP2 and SDC2 in different tissues. Non-paired (a, b) and paired (c, d) expression of SFRP2 and SDC2 in colorectal cancer tumors and normal tissues from the TCGA database. SFRP2 (e) and SDC2 (f) methylation levels in primary tumors and normal tissues from the UALCAN database. Expression of SFRP2 (g) and SDC2 (h) in our recruited cohort. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

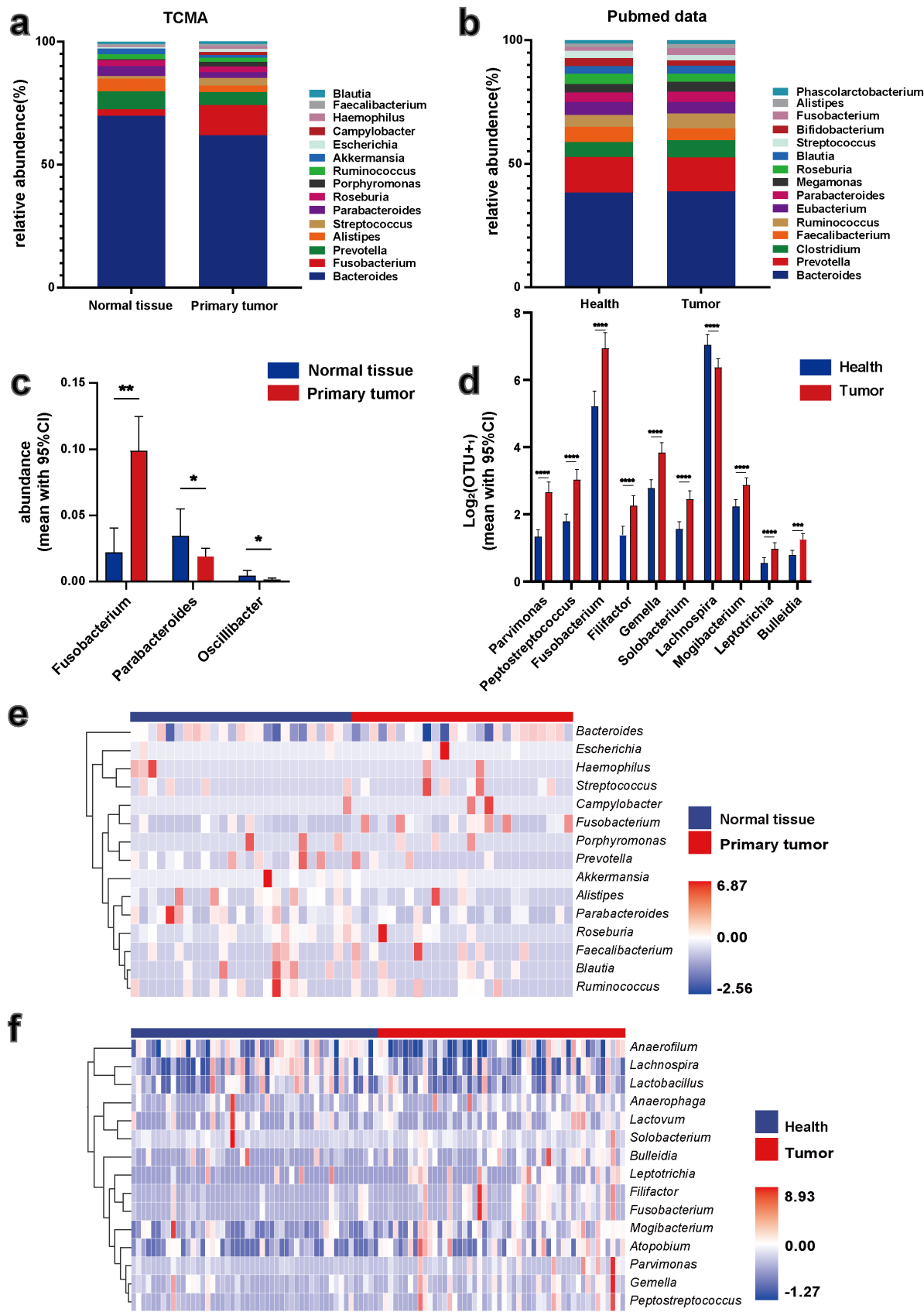


Fig. 2. Microbial composition at the genus level between colorectal cancer and control samples. Stacked plots (a, b), OTU abundance bars (c, d), and heat maps of the top 15 OTU rankings (e, f) for tumors and normal tissues from different data sources. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

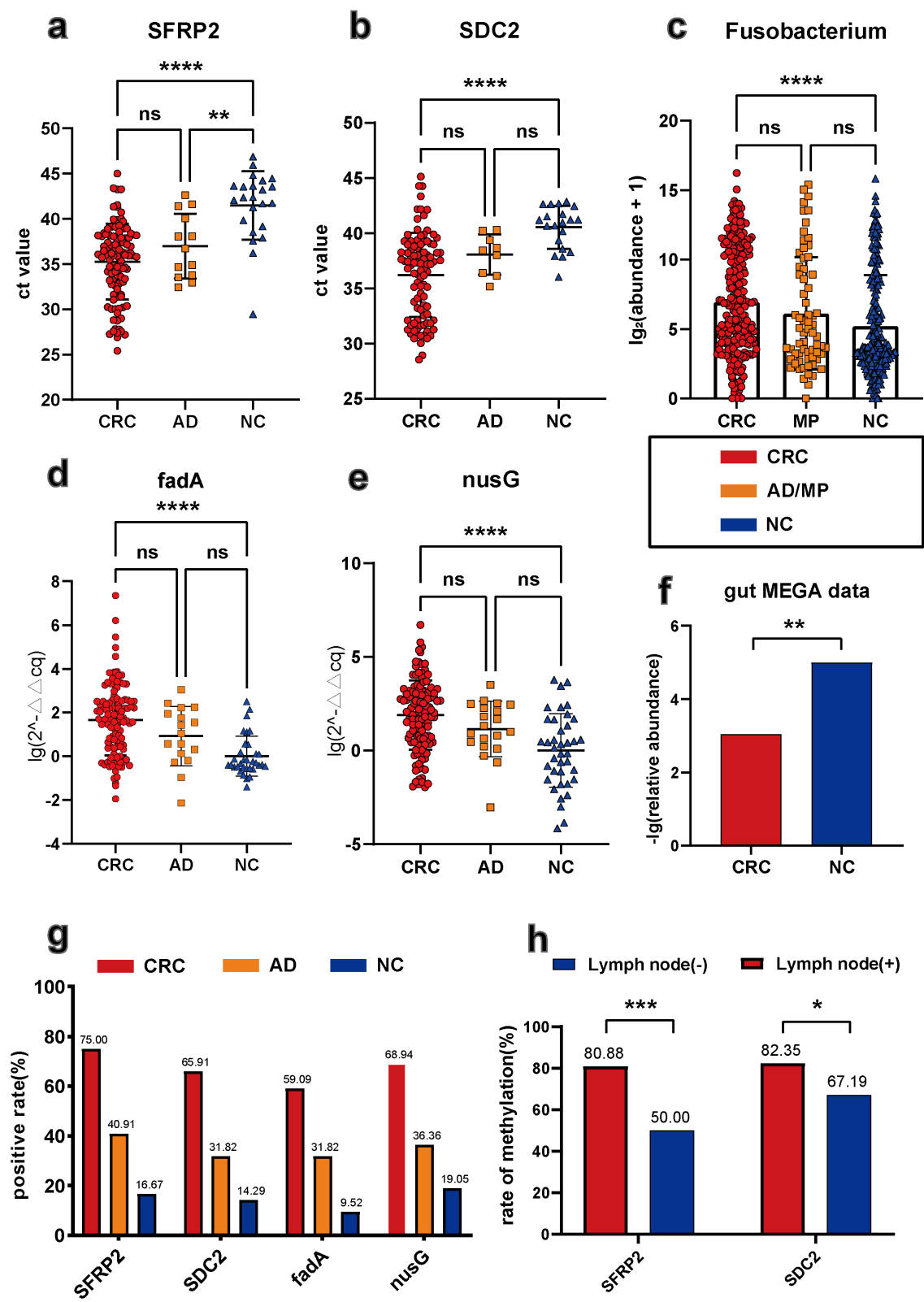


Fig. 3. Detection of DNA methylation markers and *Fusobacterium nucleatum* (Fn) markers in stool samples. Methylation levels of SFRP2 (a) and SDC2 (b). Relative abundance of *Fusobacterium* in stool samples based on the data from Yachida et al. (2019) (c). Relative expression levels of the *fadA* (d) and *nusG* (e) genes in fecal samples from NC, AD, and CRC. Fn relative abundance in stool samples was analyzed using the gutMEGA database (f). Proportion of positive occurrences of SFRP2, SDC2, *fadA*, and *nusG* in fecal samples (g). Association between lymph node metastasis and methylation of SFRP2 and SDC2 (h). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

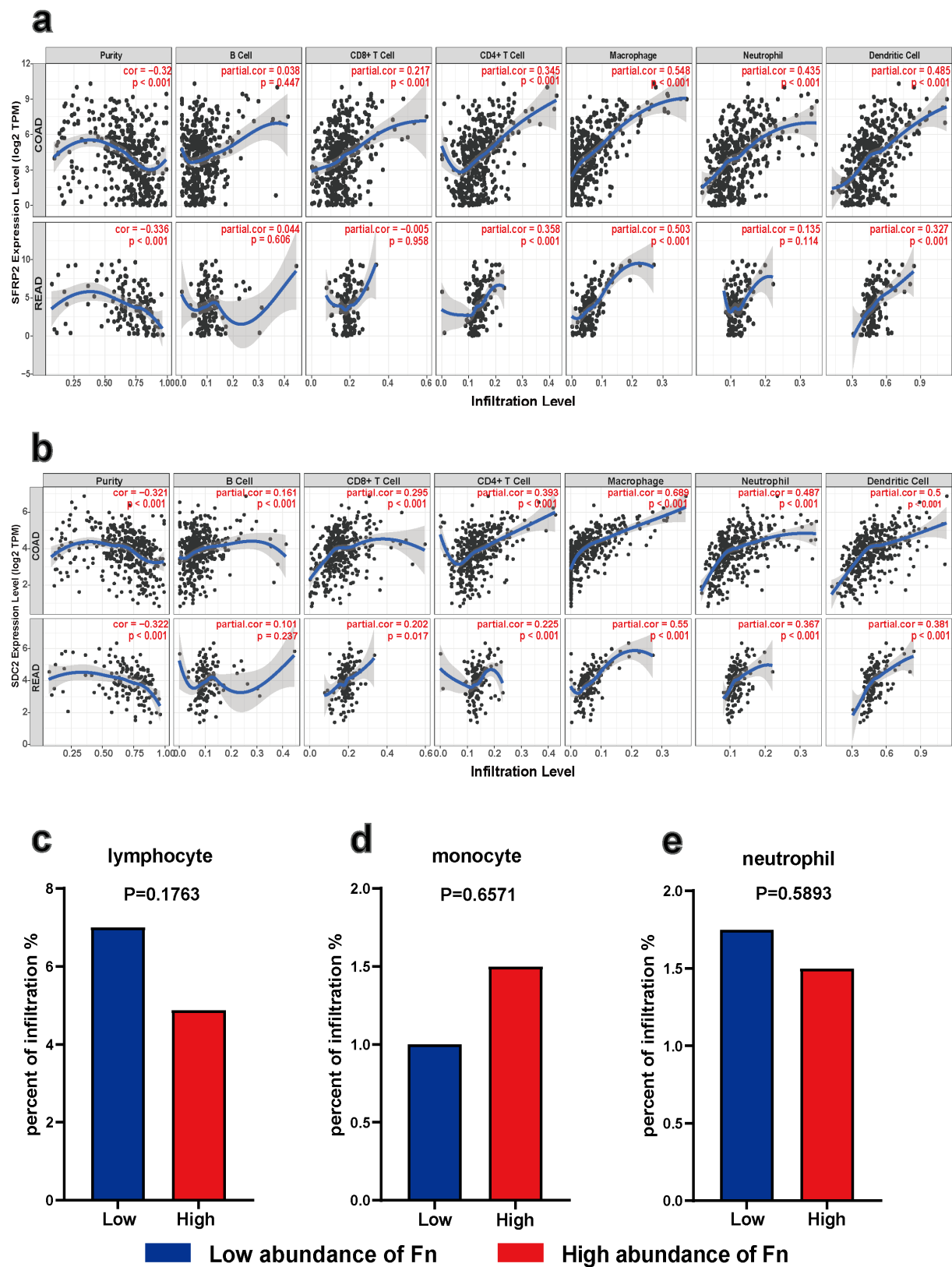


Fig. 4. Correlation between the expression of SFRP2 and SDC2, Fn, and immune-infiltrating cells. Correlations between SFRP2 (a) and SDC2 (b) expression and immune cells in COAD and READ by TIMER. Relationship between Fn and lymphocyte (c), monocyte (d), and neutrophil (e) levels in the TCMA database.

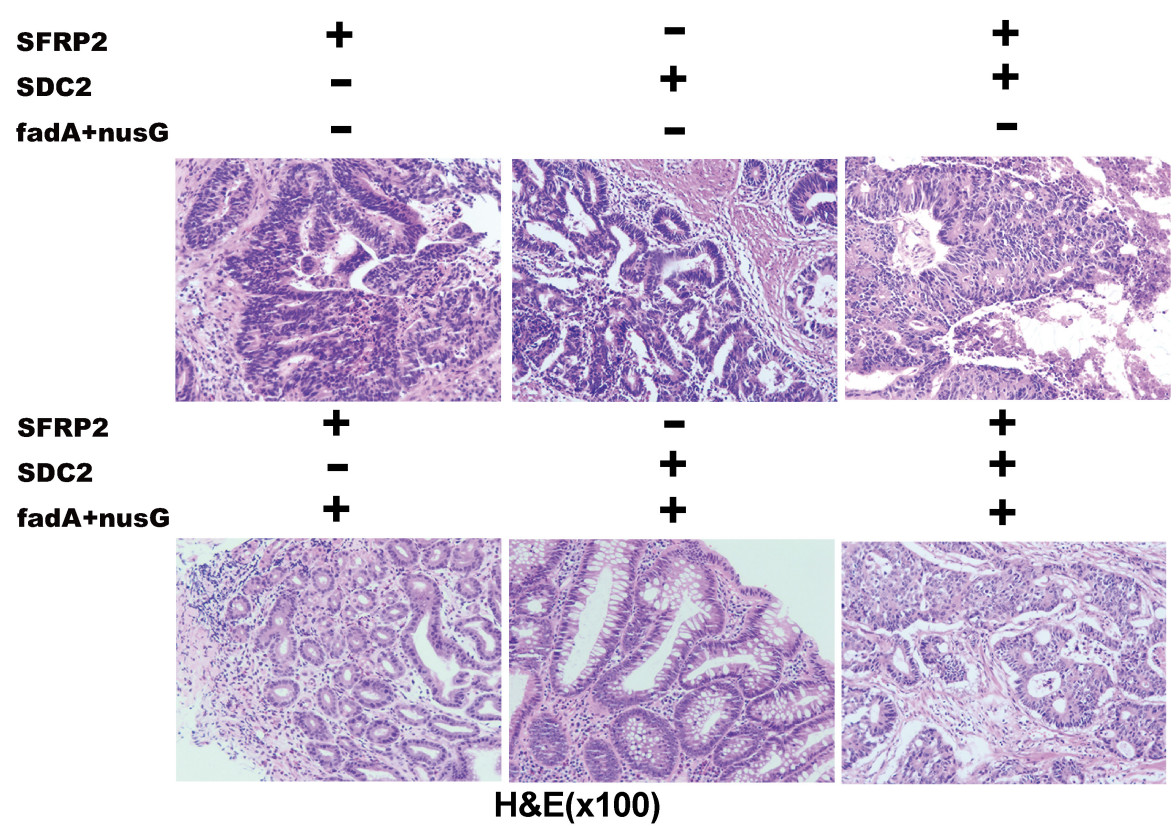


Fig. 5. H&E staining of colorectal cancer tissue.
+ – Hypermethylation (SFRP2, SDC2) or hyperexpression (fadA, nusG); - – hypomethylation or unmethylation (SFRP2, SDC2), and undetected or hypoexpression (fadA, nusG).

NC, achieving a sensitivity of 88.89% and specificity of 88.61% (AUC = 0.9339). In contrast, the sensitivity and specificity of CEA were 100% and 23.48%, respectively (AUC = 0.6197); meanwhile, for FOBT, the sensitivity and specificity were 69.05% and 65.91%, respectively (AUC = 0.6748). (Table SVI; Fig. 6a, 6d, and 6g). When comparing CRC to AD, SDC2 showed the highest sensitivity at 100%, while maintaining a specificity of 36.73% (AUC = 0.6565). The combination of SFRP2/SDC2/fadA/nusG achieved a sensitivity of 77.22% and specificity of 71.43% (AUC = 0.7649). In contrast, the sensitivity and specificity of CEA were 100% and 19.70%, respectively (AUC = 0.5114); for FOBT, the sensitivity and specificity were 45.45% and 65.91%, respectively (AUC = 0.5568) (Table SVI; Fig. 6b, 6e, and 6h). In the comparison between AD and NC, the SFRP2/SDC2/fadA/nusG combination achieved a sensitivity of 100% and specificity of 57.14% (AUC = 0.8254); the sensitivity and specificity of CEA and FOBT were 68.18% and 59.52%, and 54.55% and 69.05%, respectively (AUC = 0.6407 and 0.6180) (Table SVI; Fig. 6c, 6f, and 6i). Thus, the combined detection

of SFRP2/SDC2/fadA/nusG surpassed single-gene, CEA, and FOBT detection in effectively distinguishing CRC from NC. Moreover, among 132 CRC patients diagnosed with CRC, the positive detection rates of conventional serum tumor biomarkers CA125, CA153, and CA199 were relatively low across stages I–IV. In stages I–III, the positive detection rate of the combined indicator was substantially higher compared to that of FOBT, CEA, CA125, CA153, CA199, as well as higher than that of these combined indicators comprising these conventional serum tumor biomarkers (Fig. 6j). These results underscored that the combined testing of fecal SFRP2/SDC2/fadA/nusG significantly improved the effectiveness of CRC screening compared to both FOBT and conventional serum tumor biomarkers.

Discussion

About 20% of CRC patients present with metastasis at diagnosis, and over 50% develop metastasis during disease progression (Leporrier et al. 2006). Early

screening, diagnosis, and treatment are crucial. Studies have identified SFRP2 and SDC2 genes as reliable CRC biomarkers, and research suggests gut microbiota-related gene markers could aid early diagnosis (Wu et al. 2021; Zhao et al. 2021). We validated the expression of SFRP2 and SDC2 in cancer and normal tissues using the UALCAN database and our own data. The abundance of Fn in fecal samples from CRC and control groups was confirmed using the gutMEGA database. As expected, SFRP2 and SDC2 were aberrantly expressed in cancer tissues, and Fn abundance was higher in cancer samples, consistent with previous findings (Hua et al. 2020). Datorre et al. (2024) demonstrated in a previous study that detecting Fn in the leftovers of fecal immunochemical tests (FIT) could enhance colorectal cancer screening. The diagnostic efficacy of Fn detection in FIT-positive samples for predicting colorectal cancer was favorable, consistent with our findings (Datorre et al. 2024). The principle underlying FIT is akin to that of the fecal occult blood test (FOBT). Compared to SFRP2 and SDC2, the sensitivity of FIT or FOBT was high, but their specificity was less than optimal. We identified four CRC-related biomarkers – SFRP2, SDC2, fadA, and nusG – and validated their potential for non-invasive CRC diagnosis using qPCR. A combined indicator set (SFRP2 + SDC2 + fadA + nusG) improved early CRC screening compared to single markers like FOBT and serum CEA. Additionally, we explored the correlation between SFRP2, SDC2, and their co-expressed counterparts in CRC (Oberwalder et al. 2008). Pearson correlation and PPI analyses of SFRP2 and SDC2-related proteins suggested their co-regulatory role in cell growth. KEGG pathway analysis linked SFRP2, SDC2, and their co-expressed genes to the Calcium signaling pathway, which mediates cell proliferation and migration. This may explain the increased cancer susceptibility when SFRP2 and SDC2 are downregulated, reducing their inhibitory effect on the Calcium pathway. Recent research has highlighted the role of intestinal microbiota dysbiosis in CRC development, with microbial products interacting with host DNA to regulate cell growth (Irrazábal et al. 2014). Furthermore, a confirmed significant increase in the copy number of the fadA gene in individuals with CRC has been reported (Rubinstein et al. 2013). Huang et al. (2016) demonstrated that Loop-mediated isothermal amplification (LAMP) detection of fadA and nusG can sensitively and specifically detect Fn. Given the high phenotypic heterogeneity of colorectal

tumors and disparities in molecular patterns and genotypes, the diagnostic potential of a single molecular marker is limited (Li et al. 2023). Based on the above findings, the American Cancer Society has officially sanctioned multi-target fecal DNA testing as a novel screening strategy for CRC within its clinical guidelines (Ahlquist et al. 2012). To our knowledge, this study represents a pioneering effort in utilizing qPCR to detect four CRC-related gene markers and establish a composite index, SFRP2/SDC2/fadA/nusG, for the early screening of CRC in feces.

We investigated the correlation between SFRP2, SDC2, fadA, nusG, and the expressions of Ki67 and P53. Our results showed a positive correlation between SFRP2 methylation and Ki67, consistent with previous studies (Ruf et al. 2016). Notably, the inhibition of SDC2 complementary DNA (cDNA) could induce G(0)/G(1) cell cycle arrest and elevate p53 expression, thereby promoting tumor proliferation (Park et al. 2002). Early detection of SFRP2 and SDC2 methylation, along with fadA and nusG expression in stool samples, could help predict tumor differentiation, assess prognosis, and guide therapeutic strategies for CRC patients.

We also explored the correlation between these four genes and immune cell infiltration. Our findings indicated that SFRP2 and SDC2 may recruit immune cells like CD4+ T cells, macrophages, and dendritic cells into the tumor microenvironment, triggering an anti-tumor immune response. These results support the role of SFRP2 and SDC2 in CRC initiation and progression, consistent with previous studies (Hua et al. 2020). We found that lymphocyte and neutrophil infiltration levels were negatively correlated with *F. nucleatum* abundance. While the comparison was not statistically significant due to sample size limitations, it offers an important lead for further analysis. The increased fusobacteria abundance in CRC patient fecal samples was associated with reduced lymphocyte and neutrophil infiltration, suggesting that fusobacteria may inhibit immune response, promoting CRC onset and progression. These findings align with previous research by Gao et al. (2021).

Nevertheless, our study had several limitations. Firstly, the relatively small number of adenoma samples is attributable to the fact that most colorectal cancer patients are diagnosed at advanced stages. The limited sample size may impact the statistical analysis of the adenoma group, and thus, the applicability of these

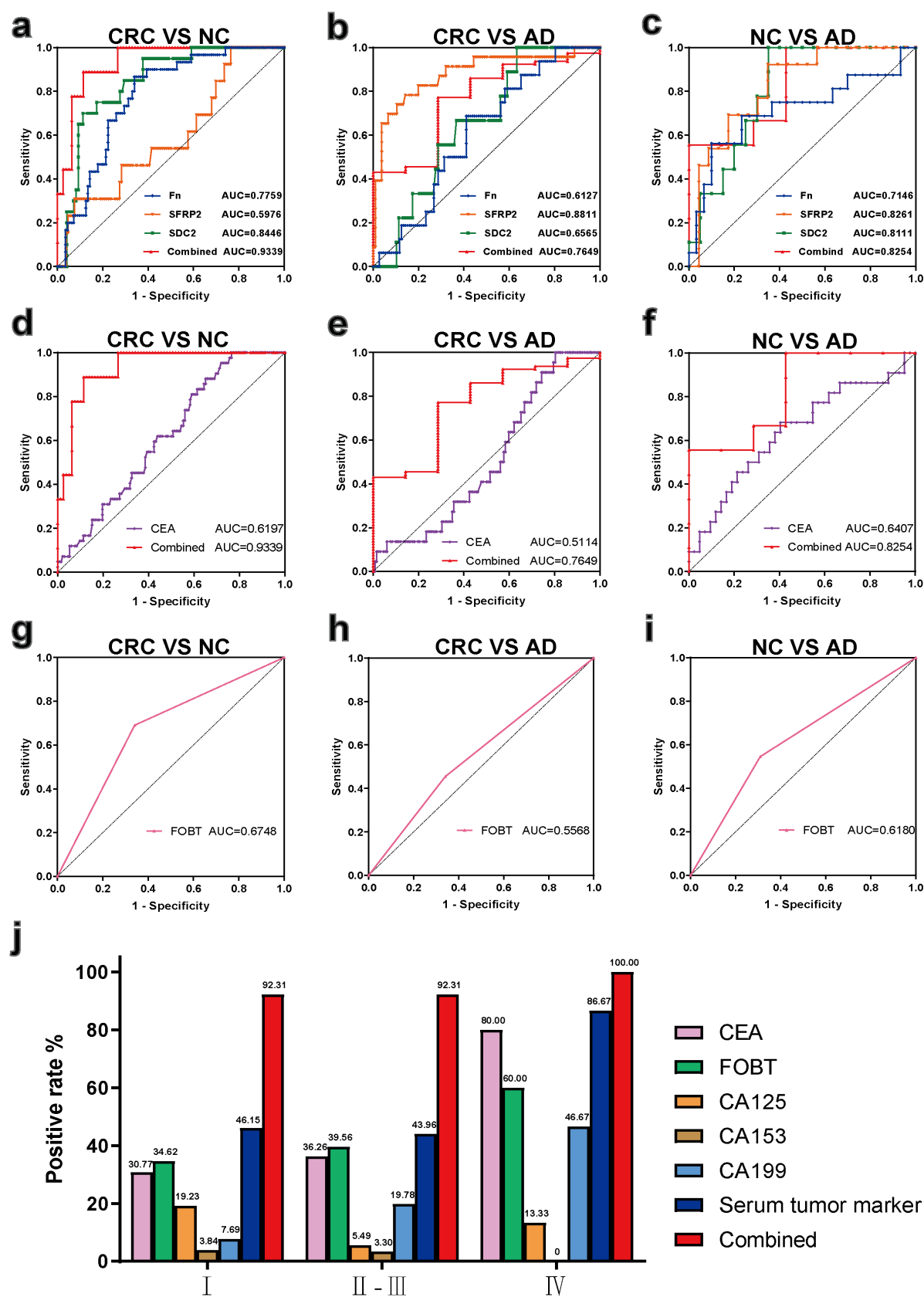


Fig. 6. Diagnostic performance of combined detection, single-gene detection, CEA, and FOBT. ROC curves illustrating the sensitivity and specificity of SFRP2, SDC2, Fn (fa_A + nu_G), CEA, FOBT and combined indicators in CRC vs. NC (a, d, g), CRC vs. AD (b, e, h), and NC vs. AD (c, f, i) in fecal samples, along with their corresponding AUC values. Positive rates of four CRC biomarker combination indicators, FOBT, and serum tumor markers in CRC stages I, II, III, and IV (j).

results for early adenoma screening remains to be further assessed. Secondly, this study utilized data from multiple databases, with gene expression and fecal microbiota composition data sourced from different study cohorts, which may have influenced the study's findings. Moreover, the primary focus of this study was on early screening and prevention of colorectal cancer, and tumor progression was not a focus. As Aitchison et al. (2022) noted, Fn in fecal samples may serve as a biomarker for increased disease risk. However, it cannot predict the molecular progression of colorectal cancer (Aitchison et al. 2022). Future research should aim further to explore the relationship between gut microbiota and cancer progression. Lastly, since the participants in this study were exclusively from Guangxi Medical University Cancer Hospital, collaborating with additional hospitals in a multicenter cohort study will be necessary to enhance the robustness and generalizability of the findings.

Conclusions

In conclusion, our findings underscored the high efficacy of combined SFRP2/SDC2/fadA/nusG detection in fecal DNA for early CRC screening. This approach demonstrated superior performance to traditional methods, such as FOBT, CEA assessment, and other serum tumor biomarkers. The combined index also showed better diagnostic accuracy than single-gene or individual Fn detection (fadA + nusG). With robust validation, this study suggests the combined index as a non-invasive, convenient, and effective tool for early CRC screening in clinical settings.

Abbreviations

AUC – Area Under the Curve
 CA125 – Carbohydrate Antigen 125
 CA153 – Carbohydrate Antigen 153
 CA199 – Carbohydrate Antigen 199
 CEA – Carcinoembryonic Antigen
 CRC – Colorectal cancer
 AD – Adenoma
 Ct (Cq) – Threshold cycle value.
 fadA – *Fusobacterium nucleatum* antigen adhesinA
 Fn – *Fusobacterium nucleatum*
 FOBT – Fecal Occult Blood Test
 MP – Multiple polypoid adenomas with low-grade dysplasia
 NC – Normal Controls
 nusG – N-utilization substance G
 QPCR – Real-time Quantitative Polymerase Chain Reaction
 SDC2 – Syndecan-2
 SFRP2 – Secreted Frizzled Related Protein-2

Ethical statement

This project obtained ethical approval from the Clinical Research Ethics Committee of Guangxi Medical University Cancer Hospital (No: KY2021279, LW2024112). All patients and healthy controls participating in the study have obtained informed consent. The research was conducted in accordance with the principles of the Helsinki Declaration.

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Author contributions

Fangchao Zhong is responsible for conducting the experiment and writing the manuscript. Xiaoxia Wei is responsible for collecting the specimens and the clinical data. Maosen Huang, Haochao Yan, Xiaoliang Liu and Lihua Fu are responsible for the clinical data analyses and interpretation. Haiming Ru and Xianwei Mo participate in the review and editing of manuscript. Zijie Su is responsible for the design of the experimental part of the article. Linhai Yan participate in the design and supervision of the full text and provided the main source of funding for the article.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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