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Integrated Methylation–Expression Profiling of Solute Carrier Genes Prioritizes Four Candidates for Further Evaluation in Early-Stage Colorectal Cancer

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Abstract: Colorectal cancer (CRC) is frequently diagnosed beyond the earliest stages, underscoring the need for molecular changes detectable during early disease. Previous studies identified widespread differential methylation of solute carrier (SLC) genes in CRC; however, whether these alterations are accompanied by concordant expression changes at Stage I, and which SLC genes warrant further prioritization, remain unclear. We integrated a previously generated CRC methylome dataset comprising 2,277 differentially methylated probes mapped to 336 SLC genes with stage-stratified TCGA colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) gene-expression and promoter-methylation data through UALCAN. Twenty-eight genes showed significant changes in both molecular layers, and 15 retained canonical inverse methylation–expression relationships in Stage I CRC. Four genes were prioritized based on concordance across COAD and READ and supporting CRC evidence. *SLC5A7* was downregulated and promoter-hypermethylated, with expression $p = 1.88 \times 10^{-5}$ (COAD) and 2.10×10^{-2} (READ), and methylation $p < 1 \times 10^{-12}$ and 3.12×10^{-3}, respectively. In contrast, *SLC7A5* was upregulated and hypomethylated (expression: 3.67×10^{-12} and 4.02×10^{-7}; methylation: 1.11×10^{-16} and 3.79×10^{-4}), as were *SLC39A10* (expression: 2.83×10^{-11} and 1.97×10^{-8}; methylation: 3.73×10^{-6} and 1.76×10^{-3}) and *SLC7A11* (expression: 2.92×10^{-8} and 3.06×10^{-2}; methylation: $<1 \times 10^{-12}$ and 3.00×10^{-4}), with values reported for COAD and READ, respectively. By integrating

promoter methylation with expression at Stage I, this study narrows previously reported SLC methylation alterations to four candidate genes showing concordant early-stage molecular dysregulation across colon and rectal cancer datasets. Together, these results prioritize four SLC genes for further functional and clinical validation in early-stage CRC. Their diagnostic utility remains to be established in independent cohorts.

Keywords: colorectal cancer; DNA methylation; solute carrier; cancer biomarker; early detection

1. Introduction

Colorectal cancer (CRC) remains a major cause of cancer morbidity and mortality. In Malaysia, CRC is among the most common malignancies and a substantial proportion of patients are diagnosed at advanced stages, when treatment options are more limited and survival is poorer. The Malaysian National Cancer Registry has reported a high national burden of CRC, and population-level survival analysis has shown a marked increase in mortality risk with advancing stage [1–3]. These observations reinforce the clinical value of biomarkers that can identify molecular changes associated with early disease.

DNA methylation is a central epigenetic mechanism regulating gene expression. Aberrant promoter hypermethylation can silence genes with tumour-suppressive functions, whereas hypomethylation may accompany transcriptional activation or broader genomic dysregulation [4,5]. Because methylation changes are stable and can occur early in carcinogenesis, they have attracted considerable interest as diagnostic and prognostic biomarkers [6–8].

The solute carrier (SLC) superfamily comprises membrane transporters that control the movement of nutrients, ions and metabolites across biological membranes. SLC transporters contribute to cellular homeostasis but can be repurposed by malignant cells to support altered metabolism, redox balance, drug transport and signalling [9,10]. Epigenetic regulation of SLC genes is therefore biologically relevant to cancer development. Previous colorectal methylome studies identified extensive differential methylation among SLC genes and suggested that these genes may have diagnostic and therapeutic value [11,12].

However, whether these methylation changes are accompanied by corresponding gene-expression changes in early-stage disease remains unresolved. Integrating promoter methylation with gene expression may therefore help distinguish epigenetic alterations that are more likely to have transcriptional relevance. In particular, promoter hypermethylation

accompanied by reduced expression, or promoter hypomethylation accompanied by increased expression, provides a biologically interpretable framework for candidate prioritization. The present study therefore integrated a previously generated CRC methylation dataset with stage-stratified TCGA COAD and READ expression and promoter-methylation data available through UALCAN [13,14]. The objective was to identify a shortlist of SLC genes showing canonical inverse promoter methylation–expression patterns in Stage I CRC across both colon and rectal adenocarcinoma and to prioritize candidates with supporting evidence for CRC-related biological or diagnostic relevance.

2. Materials and Methods

2.1. Study Design and Source Datasets

This study was designed as a secondary integrative analysis. The candidate SLC gene set was derived from previously published methylome profiling using the Infinium HumanMethylation450 BeadChip of 54 CRC tumours and 54 matched adjacent normal tissues (GEO accession GSE193535) (n = 108) [11,12]. In that work, genomic DNA was extracted from pathologist-reviewed tissue, subjected to bisulfite conversion and profiled using the Infinium HumanMethylation450 BeadChip. The array interrogated 485,577 CpG loci. The present study did not collect new human specimens; rather, it used the previously generated methylation results as a discovery set and public TCGA data for stage-stratified in silico evaluation. Accordingly, no additional institutional ethical approval was required for the present secondary analysis.

2.2. Methylation-Array Preprocessing and SLC Candidate Derivation

The previously reported methylation workflow used GenomeStudio for initial quality control and the ChAMP R package for downstream processing [15]. CpG sites with detection $p > 0.01$ were excluded, Peak-Based Correction [16] was used for normalization, and ComBat [17] was applied for batch-effect correction. Differentially methylated probes (DMPs) were identified using limma [18] with false-discovery-rate control at $p < 0.01$ [11,12]. This analysis yielded 157,846 significant DMPs. The number reflects the extent of genome-wide methylation differences detected between CRC and matched adjacent normal tissues under an FDR-controlled threshold, rather than the use of an unadjusted significance criterion. Restriction to SLC genes identified 2,277 DMPs mapping to 336 SLC genes, which formed the starting candidate set for the current analysis.

2.3. UALCAN-Based Expression and Promoter Methylation Analysis

UALCAN is an interactive portal that enables interrogation of TCGA molecular and clinical datasets [13,14]. The 336 differentially methylated SLC genes were queried separately in TCGA COAD and TCGA READ. For each gene, stage-stratified gene-expression and promoter-methylation outputs were evaluated. Genes with transcript abundance >1 TPM were retained as a pragmatic filter to exclude very low-abundance transcripts, for which UALCAN advises cautious interpretation. Pairwise comparisons between normal and tumour groups were performed within UALCAN using an unpaired two-sample t-test. A portal-reported $p < 0.05$ was used for candidate screening. These p-values were not subjected to additional multiple-testing correction in the present analysis and were therefore interpreted as part of a sequential candidate-prioritization framework rather than as definitive evidence of differential expression or methylation. The initial screening required significant differences between normal tissue and tumour for both gene expression and promoter methylation. After cross-referencing the expression and promoter-methylation outputs and excluding *SLC15A5* because relevant UALCAN data were unavailable, 28 genes were taken forward for Stage I evaluation.

2.4. Stage I Prioritization and Canonical Methylation–Expression Filtering

The 28 genes were next evaluated specifically in Stage I CRC versus normal tissue in both TCGA COAD and READ. A canonical inverse methylation–expression pattern was defined a priori as either: (i) promoter hypermethylation accompanied by lower gene expression, or (ii) promoter hypomethylation accompanied by higher gene expression. This biological filter was used as a deliberately restrictive prioritization criterion to enrich for genes in which promoter methylation was directionally consistent with transcriptional regulation. Non-canonical methylation–expression relationships were not considered biologically irrelevant, as gene-body methylation, distal regulatory elements, chromatin state, transcription-factor activity and other regulatory mechanisms may produce more complex expression patterns [4,19].

2.5. Literature-Supported Prioritization

The 15 genes were qualitatively reviewed for published evidence linking each gene to CRC biology, early disease, diagnostic relevance or mechanistic regulation. PubMed was searched using combinations of the gene symbol with terms including “colorectal cancer”, “CRC”, “methylation”, “expression”, “biomarker”, “diagnosis”, “prognosis” and “functional regulation”. In addition, NCBI Gene and GeneCards were used primarily for gene annotation and cross-referencing. Studies were considered relevant when they provided gene-specific

evidence in CRC, including experimental, clinical or bioinformatic findings related to tumour biology, methylation–expression regulation or diagnostic potential. This step was used for qualitative candidate prioritization and was not conducted as a systematic review or meta-analysis.

2.6. Statistical Interpretation

The analysis used significance values displayed by UALCAN for group comparisons. A threshold of $p < 0.05$ was used for the UALCAN filtering steps. The prior microarray discovery analysis used FDR-adjusted $p < 0.01$ for DMP identification. Because the current study was a candidate-prioritization analysis based on public portal outputs, no new multivariable modelling, receiver-operating-characteristic analysis or independent validation was performed. Published AUC values cited for individual genes refer to external studies and were not generated in the present analysis. As the UALCAN-based screening relied on nominal portal-reported p-values without additional multiple-testing correction, these results were used for candidate prioritization and require validation in independent datasets.

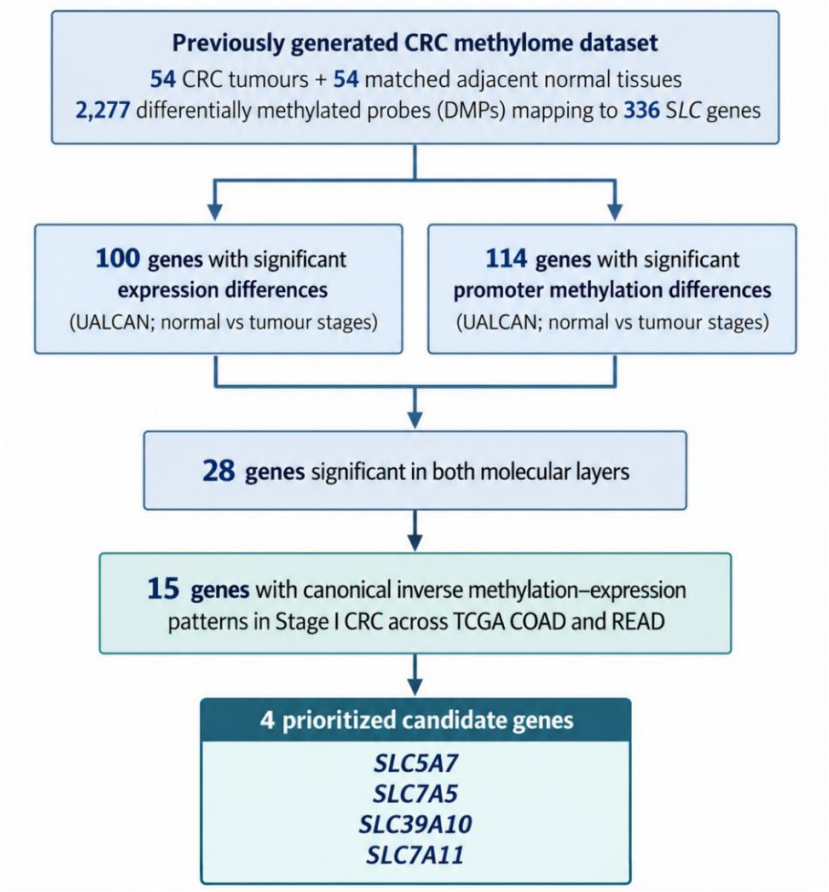


Figure 1. Study workflow for SLC-gene prioritization. The starting SLC list was derived from a previously generated colorectal methylome dataset, followed by stage-stratified expression and promoter methylation analysis using TCGA COAD and READ through UALCAN. DMP, differentially methylated probe; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas.

3. Results

3.1. Sequential Filtering Identifies 15 SLC Genes with Canonical Stage I Methylation–Expression Patterns

The starting set comprised 336 SLC genes containing 2,277 DMPs from the previously generated CRC methylome dataset. When these genes were evaluated through UALCAN, 100 genes showed significant expression differences and 114 showed significant promoter-methylation differences between normal tissue and all tumour stages. The intersection yielded 28 genes with significant changes in both molecular layers. Stage I versus normal comparison then identified 15 genes with canonical inverse methylation–expression patterns in both TCGA COAD and READ (Table 1). Eleven genes were upregulated with promoter hypomethylation, whereas four genes were downregulated with promoter hypermethylation.

Table 1. Fifteen SLC genes showing canonical inverse expression–promoter methylation patterns in Stage I CRC versus normal tissue in both TCGA COAD and READ.

Gene	Stage I expression	Stage I promoter methylation	Canonical interpretation	Prioritized
SLC11A1	Increased	Hypomethylated	Canonical	No
SLC11A2	Increased	Hypomethylated	Canonical	No
SLC18A2	Decreased	Hypermethylated	Canonical	No
SLC25A17	Increased	Hypomethylated	Canonical	No
SLC27A5	Increased	Hypomethylated	Canonical	No
SLC2A4	Decreased	Hypermethylated	Canonical	No
SLC2A8	Increased	Hypomethylated	Canonical	No
SLC35B1	Increased	Hypomethylated	Canonical	No
SLC35F1	Decreased	Hypermethylated	Canonical	No
SLC39A10	Increased	Hypomethylated	Canonical	Yes
SLC41A3	Increased	Hypomethylated	Canonical	No
SLC4A5	Increased	Hypomethylated	Canonical	No
SLC5A7	Decreased	Hypermethylated	Canonical	Yes
SLC7A11	Increased	Hypomethylated	Canonical	Yes
SLC7A5	Increased	Hypomethylated	Canonical	Yes

3.2. Four Genes Show Consistent Stage I Changes and Supporting CRC Evidence

Four genes were prioritized after integrating the Stage I molecular pattern with external CRC-related evidence: *SLC5A7*, *SLC7A5*, *SLC39A10* and *SLC7A11*. All four showed statistically significant Stage I-versus-normal differences for both expression and promoter methylation in TCGA COAD and READ (Table 2). The direction of change was concordant across the two anatomical datasets.

Table 2. UALCAN-reported p-values for Stage I CRC versus normal tissue for the four prioritized SLC genes.

Gene	Stage I molecular pattern	Expression p TCGA COAD	Expression p TCGA READ	Promoter methylation p TCGA COAD	Promoter methylation p TCGA READ
SLC5A7	Downregulated / hypermethylated	1.88×10^{-5}	2.10×10^{-2}	$<1 \times 10^{-12}$	3.12×10^{-3}
SLC7A5	Upregulated / hypomethylated	3.67×10^{-12}	4.02×10^{-7}	1.11×10^{-16}	3.79×10^{-4}
SLC39A10	Upregulated / hypomethylated	2.83×10^{-11}	1.97×10^{-8}	3.73×10^{-6}	1.76×10^{-3}
SLC7A11	Upregulated / hypomethylated	2.92×10^{-8}	3.06×10^{-2}	$<1 \times 10^{-12}$	3.00×10^{-4}

p-values are UALCAN-reported values for Stage I CRC versus normal tissue comparisons; no additional multiple-testing correction was applied in the present study.

3.2.1. SLC5A7

SLC5A7 showed lower expression together with promoter hypermethylation in Stage I CRC in both TCGA datasets. In COAD, expression and promoter methylation differed at $p = 1.88 \times 10^{-5}$ and $p < 1 \times 10^{-12}$, respectively; corresponding READ p-values were 2.10×10^{-2} and 3.12×10^{-3}. These findings indicate that reduced *SLC5A7* expression with promoter hypermethylation is already evident at Stage I in both COAD and READ. Independent experimental evidence has described *SLC5A7* as a tumour-suppressive choline transporter in CRC: increased *SLC5A7* activity impaired tumour growth through p53 stabilization, and targeted promoter demethylation restored *SLC5A7* expression and suppressed tumour progression [14]. This direct link between promoter methylation, expression and tumour-suppressive function makes *SLC5A7* a particularly strong mechanistically supported candidate in the present prioritization. The stage-stratified expression and promoter-methylation patterns are shown in Figure 2.

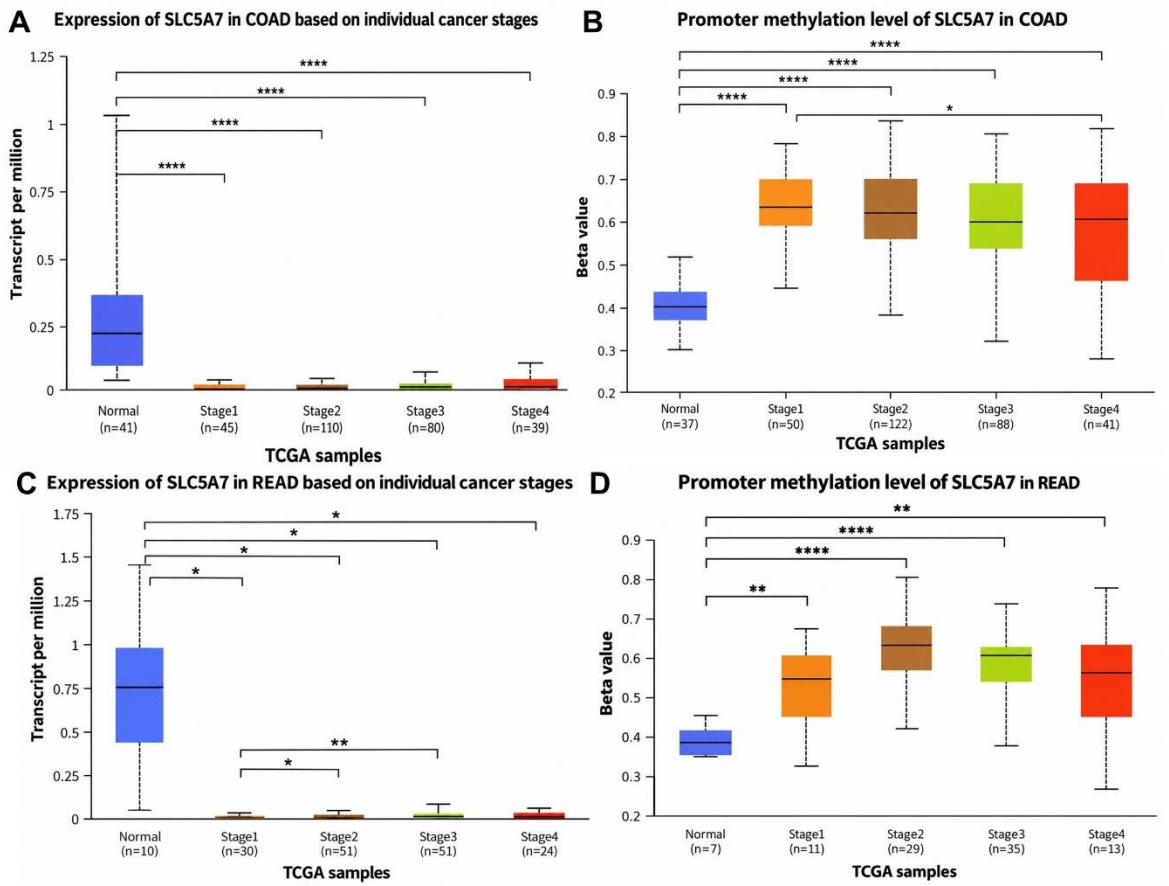


Figure 2. Stage-stratified *SLC5A7* expression and promoter methylation in TCGA colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). (A) *SLC5A7* expression in COAD; (B) promoter methylation in COAD; (C) *SLC5A7* expression in READ; and (D) promoter methylation in READ. Asterisks indicate UALCAN-reported pairwise statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Only statistically significant pairwise comparisons are annotated; non-significant stage-to-stage comparisons are omitted for clarity. Sample sizes for each group are indicated below the corresponding boxplots.

3.2.2. SLC7A5

SLC7A5 expression was upregulated and promoter hypomethylated in Stage I CRC in both COAD and READ. The Stage I differences were highly significant for expression (COAD $p = 3.67 \times 10^{-12}$; READ $p = 4.02 \times 10^{-7}$) and promoter methylation (COAD $p = 1.11 \times 10^{-16}$; READ $p = 3.79 \times 10^{-4}$). This pattern indicates that *SLC7A5* upregulation with promoter hypomethylation is detectable from Stage I in both datasets. *SLC7A5*, also known as LAT1, supports amino acid transport and has been linked to mTORC1-driven metabolic reprogramming, proliferation and migration in CRC [15–17]. Published work reported strong diagnostic performance for *SLC7A5*-associated serum and tissue measurements, including AUC values of 0.9846 and 1.000, respectively [16]. These external AUC values support

diagnostic relevance but were not calculated in the current study. The stage-stratified expression and promoter-methylation patterns are shown in Figure 3.

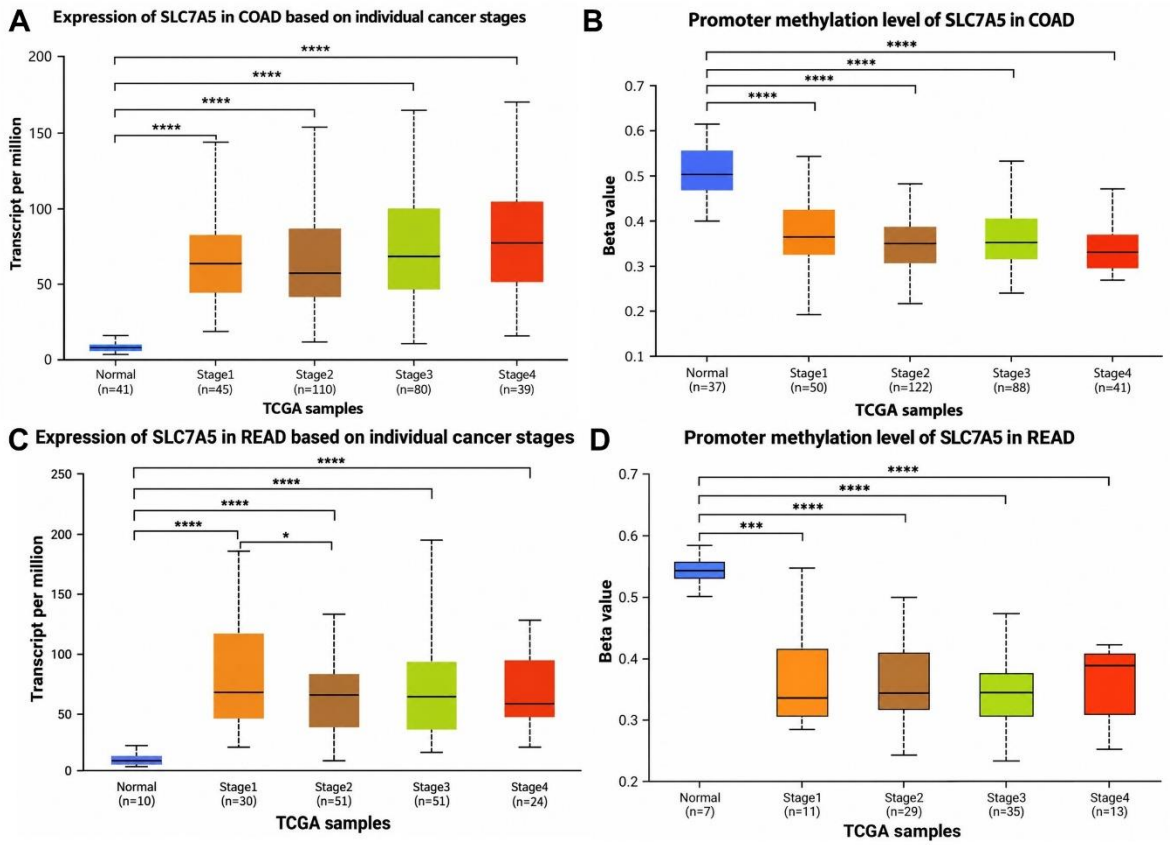


Figure 3. Stage-stratified *SLC7A5* expression and promoter methylation in TCGA colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). (A) *SLC7A5* expression in COAD; (B) promoter methylation in COAD; (C) *SLC7A5* expression in READ; and (D) promoter methylation in READ. Asterisks indicate UALCAN-reported pairwise statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Only statistically significant pairwise comparisons are annotated; non-significant stage-to-stage comparisons are omitted for clarity. Sample sizes for each group are indicated below the corresponding boxplots.

3.2.3. SLC39A10

SLC39A10 showed increased expression with promoter hypomethylation in Stage I CRC. Expression *p*-values were 2.83×10^{-11} in COAD and 1.97×10^{-8} in READ, while promoter methylation *p*-values were 3.73×10^{-6} and 1.76×10^{-3}, respectively. The concordant Stage I increase in expression and promoter hypomethylation across COAD and READ supports further investigation of *SLC39A10* as an early molecular alteration. *SLC39A10* encodes the zinc transporter ZIP10 and has been implicated in exosome-mediated intercellular communication in CRC. A published bioinformatic analysis reported an AUC

of 0.987 for distinguishing CRC from normal tissue and linked *SLC39A10* expression to tumour stage and microsatellite-instability-related biology [18]. Although direct functional evidence for promoter methylation remains limited, the concordant TCGA pattern supports further investigation. The stage-stratified expression and promoter-methylation patterns are shown in Figure 4.

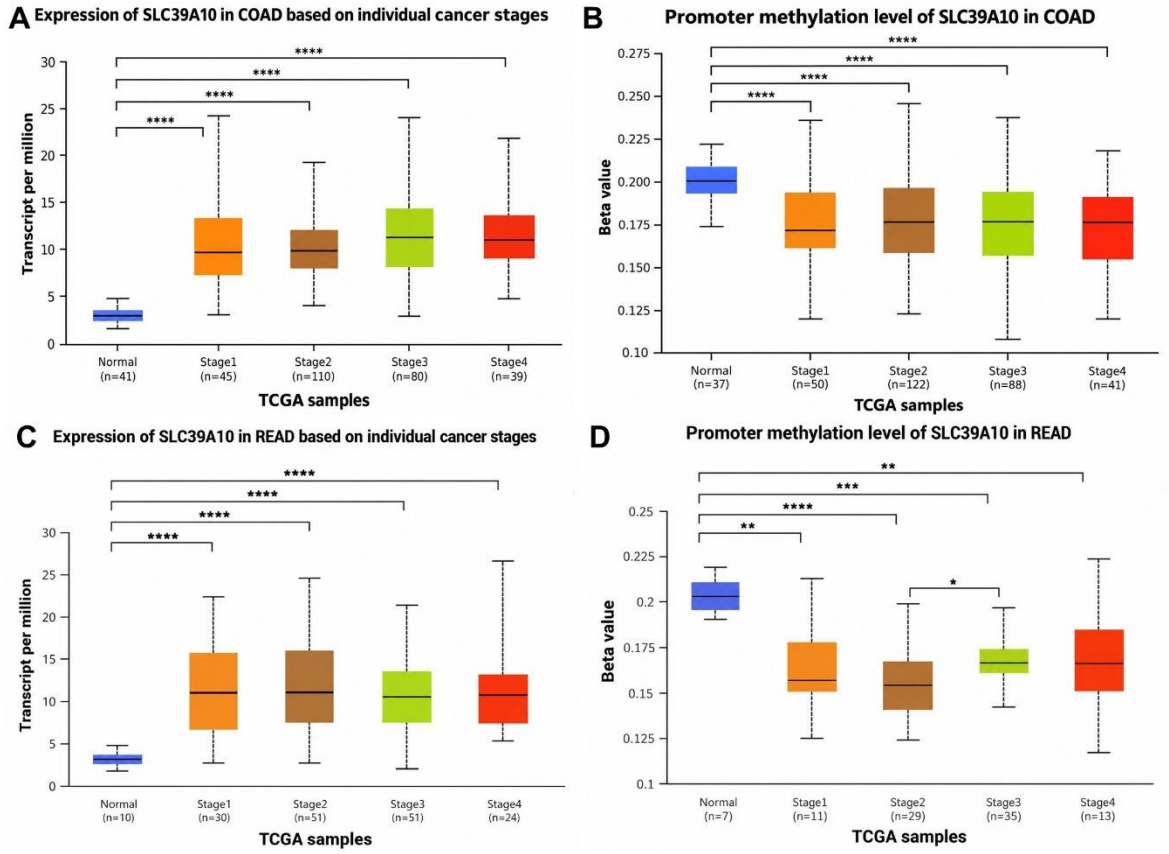


Figure 4. Stage-stratified *SLC39A10* expression and promoter methylation in TCGA colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). (A) SLC39A10 expression in COAD; (B) promoter methylation in COAD; (C) *SLC39A10* expression in READ; and (D) promoter methylation in READ. Asterisks indicate UALCAN-reported pairwise statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Only statistically significant pairwise comparisons are annotated; non-significant stage-to-stage comparisons are omitted for clarity. Sample sizes for each group are indicated below the corresponding boxplots.

3.2.4. SLC7A11

SLC7A11 was also upregulated with promoter hypomethylation in Stage I CRC. The expression differences were significant in COAD ($p = 2.92 \times 10^{-8}$) and READ ($p = 3.06 \times 10^{-2}$), as were promoter methylation differences (COAD $p < 1 \times 10^{-12}$; READ $p = 3.00 \times 10^{-4}$). Similarly, increased *SLC7A11* expression with promoter hypomethylation was

detectable at Stage I in both COAD and READ. *SLC7A11* encodes xCT, a core component of system Xc^- that supports cystine uptake, glutathione synthesis and resistance to oxidative stress. The gene has been included in a published blood-based multigene CRC signature and is closely linked to ferroptosis and therapy resistance [19,20]. A separate study showed that curdione can suppress *SLC7A11* through m6A-dependent post-transcriptional regulation, further supporting its biological relevance, although this mechanism is distinct from promoter DNA methylation [21]. The stage-stratified expression and promoter-methylation patterns are shown in Figure 5.

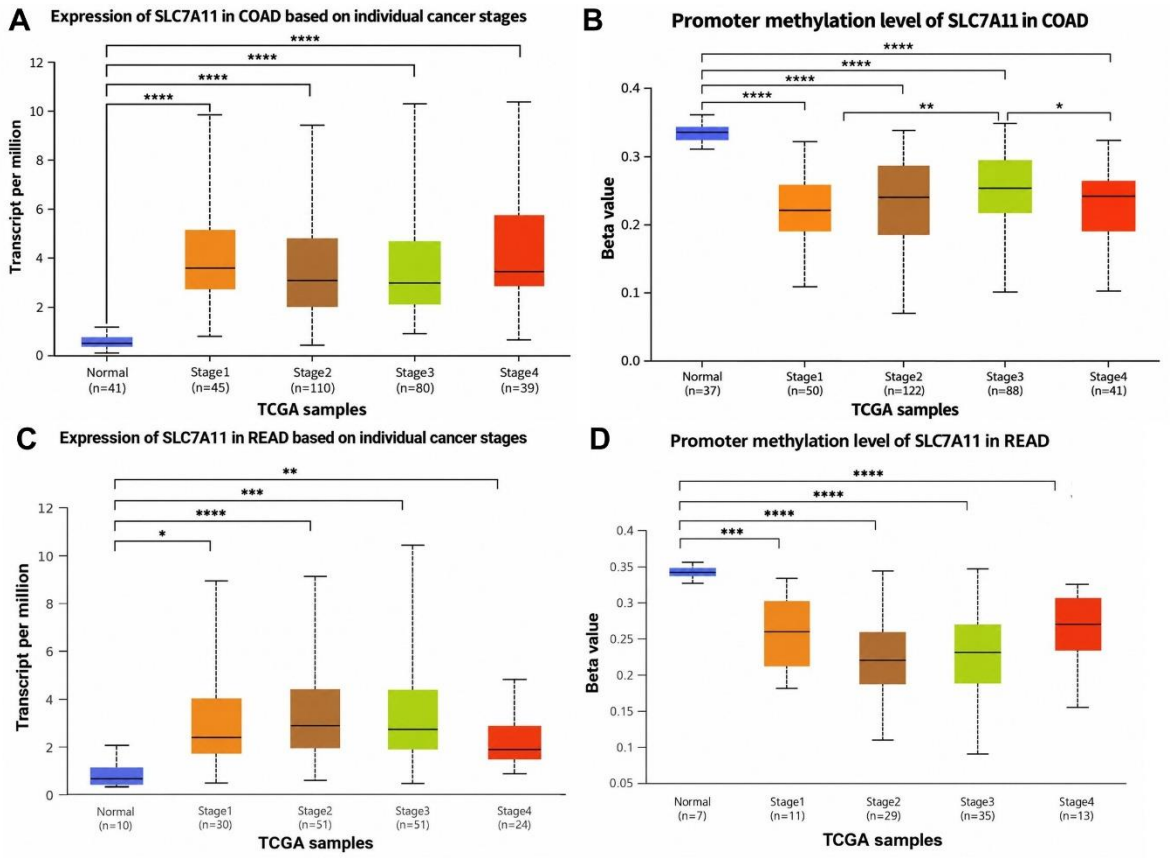


Figure 5. Stage-stratified *SLC7A11* expression and promoter methylation in TCGA colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). (A) *SLC7A11* expression in COAD; (B) promoter methylation in COAD; (C) *SLC7A11* expression in READ; and (D) promoter methylation in READ. Asterisks indicate UALCAN-reported pairwise statistical significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Only statistically significant pairwise comparisons are annotated; non-significant stage-to-stage comparisons are omitted for clarity. Sample sizes for each group are indicated below the corresponding boxplots.

Collectively, the four prioritized genes showed distinct but biologically plausible methylation–expression patterns, with varying levels of supporting mechanistic and diagnostic evidence. A comparative summary of their molecular profiles and rationale for prioritization is presented in Table 3.

Table 3. Integrated molecular and biological interpretation of the four prioritized SLC candidate biomarkers.

Gene	Stage I molecular pattern	Supporting evidence	Priority interpretation
SLC5A7	Downregulated; promoter hypermethylated	CRC tumour-suppressive role; p53 stabilization; targeted demethylation restored expression	Strong mechanistic candidate
SLC7A5	Upregulated; promoter hypomethylated	CRC tissue/serum overexpression; reported high diagnostic AUC; mTORC1-linked metabolism	Strong diagnostic/biological candidate
SLC39A10	Upregulated; promoter hypomethylated	Reported AUC 0.987; zinc transport; exosome-related CRC network and metastasis biology	Promising, but methylation mechanism requires validation
SLC7A11	Upregulated; promoter hypomethylated	Blood-based CRC signature; ferroptosis and oxidative-stress regulation; therapy resistance	Promising non-invasive/functional candidate

4. Discussion

This study used a sequential, biology-informed filtering strategy to reduce a broad set of differentially methylated SLC genes to a focused panel of early-stage CRC candidates. From 336 SLC genes identified in a prior colorectal methylome analysis, 28 were taken forward after cross-referencing UALCAN expression and promoter-methylation outputs. Of these, 15 retained a canonical inverse relationship in Stage I disease. Four genes, which are *SLC5A7*, *SLC7A5*, *SLC39A10* and *SLC7A11*, were prioritized after integrating cross-dataset consistency with published CRC evidence. SLC transporters are biologically relevant because they can influence metabolic exchange, adaptive responses and tumour-microenvironment interactions [20]. Importantly, the present study extends our previous CRC methylome analyses rather than simply reproducing differential methylation findings. Baharudin *et al*. established the genome-wide CRC methylation landscape, while Zabidi *et al*. subsequently characterized differential methylation within the SLC gene family [11,12]. The current analysis adds a distinct layer by integrating these methylation findings with stage-stratified TCGA expression and promoter methylation data and by applying a restrictive inverse methylation–expression filter in Stage I disease. This sequential strategy narrowed 336 differentially methylated SLC genes to four biologically supported candidates for further early-stage validation.

The canonical inverse relationship is especially relevant because promoter methylation can directly influence transcriptional accessibility. Nevertheless, methylation–expression relationships in cancer are not universally inverse. Gene-body methylation, distal regulatory regions, chromatin state, transcription-factor activity and copy-number alterations can modify the direction or magnitude of transcriptional effects [4,12]. Accordingly, the 13 genes removed at the canonical-filtering step should not be interpreted as unimportant to CRC. Rather, they were outside the deliberately restrictive prioritization framework used here.

An important feature of the four prioritized genes is that their coordinated methylation–expression alterations were already detectable in Stage I CRC in both TCGA COAD and READ. This is particularly relevant for biomarker development because molecular alterations that are present at the earliest clinically defined stage may have greater potential for early detection than changes that emerge only in advanced disease. Previous studies and systematic reviews have shown that aberrant DNA methylation can be detected in early-stage CRC and that methylation-based markers in tissue, stool and circulating DNA are promising candidates for non-invasive screening and early detection, although sensitivity in early-stage disease remains a key challenge [21–24]. In the present analysis, *SLC5A7* showed reduced expression with promoter hypermethylation, whereas *SLC7A5*, *SLC39A10*, and *SLC7A11* showed increased expression with promoter hypomethylation at Stage I in both anatomical datasets. The consistency of these patterns across colon and rectal adenocarcinoma strengthens their biological plausibility and suggests that they may reflect early CRC-associated epigenetic dysregulation rather than changes restricted to a single tumour location. Although the current study does not establish diagnostic performance or Stage I specificity, the presence of these alterations at Stage I supports their prioritization for validation in early-stage tissue cohorts and, ultimately, in minimally invasive biospecimens such as circulating DNA or blood-based assays.

Among the four candidates, *SLC5A7* has the clearest direct mechanistic connection between promoter methylation and tumour-suppressive function. The observed Stage I hypermethylation and reduced expression in both COAD and READ are consistent with experimental work showing that restoration of *SLC5A7* can inhibit CRC growth through p53 stabilization [25]. This concordance across epigenetic, transcriptional and functional levels strongly justifies targeted validation of *SLC5A7* methylation in independent early-stage tissues and, ultimately, circulating DNA. A clinically useful assay would need to establish whether the methylation difference is sufficiently large, technically reproducible and specific to CRC or colorectal neoplasia.

SLC7A5, *SLC39A10* and *SLC7A11* showed the opposite pattern: promoter hypomethylation with increased expression. This direction is biologically compatible with the metabolic demands of malignant cells. *SLC7A5* supports amino-acid uptake and mTORC1-linked growth, while *SLC7A11* supports cystine uptake and redox defence [26–30]. Their upregulation may therefore provide a selective advantage even at early stages. In colorectal and gastric cancers, *SLC39A10* functions as a key zinc importer and exosomal network hub that drives cell migration, proliferation, and tumor microenvironment remodeling (including M2 macrophage polarization) through activation of downstream signaling pathways such as MAPK/ERK and PI3K/AKT [31,32]. The consistency of these trends in both colon and rectal TCGA datasets strengthens their candidacy, although it does not establish that promoter methylation is the sole driver of altered expression. In contrast to *SLC5A7*, direct experimental evidence demonstrating that promoter methylation regulates *SLC7A5*, *SLC39A10* or *SLC7A11* expression in CRC remains limited. Their inverse methylation–expression patterns in the present analysis should therefore be interpreted as associations rather than evidence of direct epigenetic causality. Other regulatory mechanisms, including methylation outside promoter regions, transcription-factor activity, chromatin state, copy-number alterations and post-transcriptional regulation, may also contribute. For example, *SLC7A5* expression can be influenced through LINC00857/YTHDC1-mediated transcript stabilization, while *SLC7A11* is also subject to m6A-dependent post-transcriptional regulation [28–30].

The present results also illustrate why literature-supported prioritization requires cautious interpretation. Published diagnostic AUC values for *SLC7A5* and *SLC39A10* were generated in external studies using different molecular analytes, study populations and analytical designs [27,31]. For *SLC7A5*, the reported diagnostic performance was based on serum and tissue measurements rather than promoter methylation, while the *SLC39A10* AUC was derived from a bioinformatic tumour-versus-normal expression analysis. These findings therefore support biological and diagnostic interest in the respective genes but do not validate their methylation status or the combined four-gene prioritization reported here. Similarly, inclusion of *SLC7A11* in a blood-based multigene signature suggests potential for non-invasive detection but does not demonstrate that its promoter methylation is detectable or discriminatory in plasma [29]. The appropriate next step is therefore prospective molecular validation rather than immediate diagnostic translation.

The remaining canonical genes may still have biological value. *SLC11A2* has been linked to iron uptake, Notch-related cell fate and JAK–STAT3 signalling in CRC [33,34]. *SLC35B1* has been reported within prognostic transporter analyses [35]. *SLC2A4* has also been

associated with CRC clinical characteristics and hypoxia-related prognostic models [36]. However, the study identified weaker or less direct evidence for promoter methylation, Stage I specificity or diagnostic application for these genes. The four-gene prioritization therefore reflects the convergence of early-stage molecular changes and supporting evidence, not a claim that the other 11 genes are biologically irrelevant.

Several limitations should be considered. First, the analysis focused on promoter methylation and did not evaluate gene-body, enhancer or other non-promoter CpG regions. Second, the UALCAN step relied on portal-level group comparisons rather than reanalysis of individual-level TCGA data. Consequently, we were unable to adjust for potential covariates such as age, sex, molecular subtype or other clinical characteristics, and study-specific effect sizes, multivariable models, ROC analyses and combined classifier performance could not be calculated. Third, tumour purity and cellular composition were not adjusted in the portal-based analysis. Because bulk tumour specimens contain variable proportions of malignant epithelial, stromal, immune and other cell populations, some of the observed expression or methylation differences may partly reflect cellular admixture rather than tumour-cell-intrinsic regulation [37]. Fourth, the discovery methylome and TCGA analyses were derived from different cohorts. Although cross-cohort concordance strengthens candidate prioritization, differences in population background, tissue composition, sample processing and analytical workflow may introduce heterogeneity and limit direct comparability. The Malaysian discovery cohort may also differ from the population composition represented in TCGA, supporting the need for validation in Malaysian and broader Southeast Asian populations. Fifth, the candidate-ranking literature review was qualitative rather than systematic and may therefore be affected by publication and selection bias, particularly because well-studied genes are more likely to have supporting evidence than less-characterized candidates. Finally, no independent tissue cohort, functional experiment or liquid-biopsy assay was performed. These limitations mean that the findings should be interpreted as a hypothesis-generating prioritization framework rather than evidence of established diagnostic performance.

Future work should validate the four candidates in an independent cohort enriched for Stage I CRC, together with non-neoplastic controls and representation of later disease stages to determine whether the observed alterations are specific to early disease or remain present across progression. Targeted promoter methylation should first be confirmed using an orthogonal quantitative method such as bisulfite pyrosequencing or methylation-specific quantitative PCR, alongside RT-qPCR assessment of gene expression. Subsequent studies should extend the analysis to gene-body and enhancer methylation and integrate

transcriptomic, proteomic and metabolomic data. Functional experiments in appropriate *in vitro* and *in vivo* models will be required to determine whether the observed methylation–expression relationships contribute directly to CRC biology. Validation in Malaysian and broader Southeast Asian cohorts would address population generalizability, while subsequent testing in plasma cell-free DNA or other minimally invasive biospecimens would directly assess early-detection potential. Because reliable effect-size estimates are not available from the portal-level analysis, effect sizes should first be established in an independent pilot cohort and then used for formal power and sample-size calculations in subsequent diagnostic validation studies.

5. Conclusions

This study extends previous SLC methylation analyses by integrating promoter methylation with stage-stratified gene expression, thereby narrowing a broad set of differentially methylated SLC genes to four prioritized candidates, *SLC5A7*, *SLC7A5*, *SLC39A10*, and *SLC7A11*, with coordinated molecular alterations detectable as early as Stage I CRC in both TCGA COAD and READ. The principal contribution of this work is the identification of a focused, biologically supported shortlist for further investigation rather than the establishment of a diagnostic biomarker panel. These findings are hypothesis-generating and require validation in independent early-stage cohorts, together with functional studies and assessment in minimally invasive biospecimens, before their potential clinical utility can be determined.

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References

1. Malaysian_National_Cancer_Registry_Report_2007-2011.pdf.
2. Muhamad NA, Ma'amor NH, Rosli IA, *et al.* Colorectal cancer survival among Malaysia population: data from the Malaysian National Cancer Registry. Frontiers in Oncology 2023; 13. doi: 10.3389/fonc.2023.1132417.
3. Yusof DSN binti, Raman DS a/l, Hamzah DN binti, *et al.* Summary of The Malaysia National Cancer Registry Report 2017-2021. 2024; : 1–8.

4. Moore LD, Le T, Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacology 2013; 38(1): 23–38. doi: 10.1038/npp.2012.112.
5. Das PM, Singal R. DNA Methylation and Cancer. Journal of Clinical Oncology 2004; 22(22): 4632–4642. doi: 10.1200/JCO.2004.07.151.
6. Ishak M, Baharudin R, Mohamed Rose I, *et al.* Genome-Wide Open Chromatin Methylome Profiles in Colorectal Cancer. Biomolecules 2020; 10(5): 719. doi: 10.3390/biom10050719.
7. Ishak M, Baharudin R, Tan LT-H, *et al.* Landscape Of HOXA Genes Methylation in Colorectal Cancer. Progress In Microbes & Molecular Biology 2020; 3(1). doi: 10.36877/pmmb.a0000085.
8. Abu N, Rus Bakarurraini NAA, Nasir SN, *et al.* Methylation Profile of Cancer Testis Antigens in Colorectal Cancer. Iranian Journal of Immunology 2023; 20(1). doi: 10.22034/iji.2023.92600.2171.
9. Pizzagalli MD, Bensimon A, Superti-Furga G. A guide to plasma membrane solute carrier proteins. The FEBS Journal 2021; 288(9): 2784–2835. doi: 10.1111/febs.15531.
10. Nyquist MD, Prasad B, Mostaghel EA. Harnessing Solute Carrier Transporters for Precision Oncology. Molecules 2017; 22(4): 539. doi: 10.3390/molecules22040539.
11. Baharudin R, Ishak M, Muhamad Yusof A, *et al.* Epigenome-Wide DNA Methylation Profiling in Colorectal Cancer and Normal Adjacent Colon Using Infinium Human Methylation 450K. Diagnostics 2022; 12(1): 198. doi: 10.3390/diagnostics12010198.
12. Zabidi NAN, Baharudin R, Rus Bakarurraini NQ, *et al.* Investigating DNA Methylation of Solute Carrier Genes in Colorectal Cancer: A Comprehensive Analysis Using Microarray and Bioinformatics Tools. Progress In Microbes & Molecular Biology 2023; 6(1). doi: 10.36877/pmmb.a0000393.
13. Chandrashekar DS, Bashel B, Balasubramanya SAH, *et al.* UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017; 19(8): 649–658. doi: 10.1016/j.neo.2017.05.002.
14. Chandrashekar DS, Karthikeyan SK, Korla PK, *et al.* UALCAN: An update to the integrated cancer data analysis platform. Neoplasia 2022; 25: 18–27. doi: 10.1016/j.neo.2022.01.001.
15. Tian Y, Morris TJ, Webster AP, *et al.* ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Bioinformatics 2017; 33(24): 3982–3984. doi: 10.1093/bioinformatics/btx513.
16. Dedeurwaerder S, Defrance M, Calonne E, *et al.* Evaluation of the Infinium Methylation 450K technology. Epigenomics 2011; 3(6): 771–784. doi: 10.2217/epi.11.105.
17. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 2007; 8(1): 118–127. doi: 10.1093/biostatistics/kxj037.
18. Ritchie ME, Phipson B, Wu D, *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research 2015; 43(7): e47. doi: 10.1093/nar/gkv007.
19. Moarii M, Boeva V, Vert J-P, *et al.* Changes in correlation between promoter methylation and gene expression in cancer. BMC genomics 2015; 16: 873. doi: 10.1186/s12864-015-1994-2.
20. Hossen MdS, Islam MSU, Yasin M, *et al.* A Review on the Role of Human Solute Carriers Transporters in Cancer. Health Science Reports 2025; 8(1): e70343. doi: 10.1002/hsr2.70343.
21. Petit J, Carroll G, Gould T, *et al.* Cell-Free DNA as a Diagnostic Blood-Based Biomarker for Colorectal Cancer: A Systematic Review. Journal of Surgical Research 2019; 236: 184–197. doi: 10.1016/j.jss.2018.11.029.

22. Anghel SA, Ioniță-Mîndrican C-B, Luca I, *et al.* Promising Epigenetic Biomarkers for the Early Detection of Colorectal Cancer: A Systematic Review. Cancers 2021; 13(19): 4965. doi: 10.3390/cancers13194965.
23. Rasmussen SL, Krarup HB, Sunesen KG, *et al.* Hypermethylated DNA as a biomarker for colorectal cancer: a systematic review. Colorectal Disease 2016; 18(6): 549–561. doi: 10.1111/codi.13336.
24. Khabbazpour M, Tat M, Karbasi A, *et al.* Advances in blood DNA methylation-based assay for colorectal cancer early detection: a systematic updated review. Gastroenterology and Hepatology from Bed to Bench 2024; 17(3). doi: 10.22037/ghfbb.v17i3.2978.
25. Yin Y, Jiang Z, Fu J, *et al.* Choline-induced SLC5A7 impairs colorectal cancer growth by stabilizing p53 protein. Cancer Letters 2022; 525: 55–66. doi: 10.1016/j.canlet.2021.09.027.
26. Huang H, Dai Y, Duan Y, *et al.* Effective prediction of potential ferroptosis critical genes in clinical colorectal cancer. Frontiers in Oncology 2022; 12. doi: 10.3389/fonc.2022.1033044.
27. Yoon ML, Chun H, Lee H, *et al.* Identification and Validation of Serum Biomarkers to Improve Colorectal Cancer Diagnosis. Cancer Medicine 2024; 13(23): e70460. doi: 10.1002/cam4.70460.
28. Tang S, Liu Q, Xu M. LINC00857 promotes cell proliferation and migration in colorectal cancer by interacting with YTHDC1 and stabilizing SLC7A5. Oncology Letters 2021; 22(2): 1–10. doi: 10.3892/ol.2021.12839.
29. Kaya IH, Al-Harazi O, Colak D. Transcriptomic data analysis coupled with copy number aberrations reveals a blood-based 17-gene signature for diagnosis and prognosis of patients with colorectal cancer. Frontiers in Genetics 2023; 13. doi: 10.3389/fgene.2022.1031086.
30. Wang Y, Zhang Z, Sun W, *et al.* Ferroptosis in colorectal cancer: Potential mechanisms and effective therapeutic targets. Biomedicine & Pharmacotherapy 2022; 153: 113524. doi: 10.1016/j.biopha.2022.113524.
31. Ma J, Wang P, Huang L, *et al.* Bioinformatic analysis reveals an exosomal miRNA-mRNA network in colorectal cancer. BMC Medical Genomics 2021; 14(1): 60. doi: 10.1186/s12920-021-00905-2.
32. Chen H, Chen C, Chen J, *et al.* Unveiling the prognostic and therapeutic landscape of the zinc transporter protein SLC39A family in colorectal cancer through multi-omics and machine learning approaches. Clinical and Experimental Medicine 2026; 26(1): 202. doi: 10.1007/s10238-025-01996-2.
33. Xue X, Ramakrishnan SK, Weisz K, *et al.* Iron Uptake via DMT1 Integrates Cell Cycle with JAK-STAT3 Signaling to Promote Colorectal Tumorigenesis. Cell Metabolism 2016; 24(3): 447–461. doi: 10.1016/j.cmet.2016.07.015.
34. Hounjet J, Groot AJ, Piepers JP, *et al.* Iron-responsive element of Divalent metal transporter 1 (Dmt1) controls Notch-mediated cell fates. The FEBS Journal 2023; 290(24): 5811–5834. doi: 10.1111/febs.16946.
35. Chen D, Zhang Y, Nie H, *et al.* Comprehensive analyses of solute carrier family members identify SLC12A2 as a novel therapy target for colorectal cancer. Scientific Reports 2024; 14(1): 4459. doi: 10.1038/s41598-024-55048-y.
36. Hu J, Liu X, Chi J, *et al.* Expressions of IGF-1, ERK, GLUT4, IRS-1 in metabolic syndrome complicated with colorectal cancer and their associations with the clinical characteristics of CRC. Cancer Biomarkers 2018; 21(4): 883–891. doi: 10.3233/CBM-170942.

37. Zheng X, Zhang N, Wu H-J, *et al.* Estimating and accounting for tumor purity in the analysis of DNA methylation data from cancer studies. Genome Biology 2017; 18(1): 17. doi: 10.1186/s13059-016-1143-5.

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