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Investigating DNA Methylation of Solute Carrier Genes in Colorectal Cancer: A Comprehensive Analysis Using Microarray and Bioinformatics Tools

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Abstract: Background: Colorectal cancer (CRC) is a major global health concern, with a significant impact on morbidity and mortality. The molecular mechanisms underlying CRC, especially DNA methylation patterns in solute carrier (SLC) genes, have gained attention due to their potential role in CRC initiation and progression. SLC genes encode transporters that play vital roles in maintaining cellular homeostasis, and alterations in their DNA methylation can influence nutrient and metabolite transport, impacting cancer cell behavior. This proposal aims to investigate the DNA methylation status of SLC genes in CRC, utilizing microarray technology and a suite of bioinformatics tools. **Methods:** Microarray methylation data from

CRC and adjacent normal tissues underwent preprocessing, differential methylation analysis, functional enrichment exploration, and correlation analysis for a detailed study of methylation patterns. **Results:** This study decoded the epigenetic landscape of SLC genes in CRC, promising innovative therapeutic strategies and improved patient outcomes to combat this prevalent malignancy.

Keywords: Colorectal cancer; DNA methylation; Solute carrier genes; Biomarker; Precision medicine; SDG 3 Good health and well-being

1. Introduction

Cancer contributes to about one out of every six fatalities, and it's predicted that the global burden of new cancer cases will rise as compared to previous years to approximately 20.3 million and global cancer-related deaths will rise to 13.2 million by the year 2030, surpassing previous figures [1-4]. Colorectal cancer (CRC) has been a significant global health concern, responsible for many cancer-related fatalities worldwide [5-7]. The disease's pathogenesis is multifaceted, with a plethora of genetic and epigenetic changes playing pivotal roles in its onset, progression, and metastasis [8-10]. Early detection of CRC presents challenges, and thus, a deeper understanding of these genetic and epigenetic factors is vital for enhancing early detection methods and prognosis [11-13]. Early diagnosis of CRC significantly increases the patient survival rates given that early-stage CRC exhibits survival rates of up to a 5-year survival rate of 90% [14, 15]. Among these factors, DNA methylation, an epigenetic modification that regulates gene expression, has emerged as a key player in CRC development and progression [16, 17].

Solute carrier (SLC) genes represent a large family of membrane transport proteins that facilitate the transport of a diverse range of substrates across biological membranes. These genes have been implicated in various physiological processes and pathological conditions, including cancer. The human genome contains at least 362 putatively functional protein-coding SLC genes, which are organized into 55 families [18]. These genes encode a diverse group of membrane transport proteins that play crucial roles in transporting various solutes across biological membranes [19]. In the context of CRC, SLC genes are involved in drug resistance, metabolic reprogramming, and other cancer-related processes [20, 21]. However, despite their potential importance in CRC, the methylation status of SLC genes in this disease remains largely unexplored. Understanding how DNA methylation changes in SLC genes contribute to CRC could provide valuable insights into the molecular mechanisms underlying this disease and potentially reveal novel therapeutic targets or biomarkers.

This study aims to investigate the DNA methylation patterns of SLC genes in CRC using microarray technology and bioinformatics tools. Microarray technology allows for high-throughput analysis of methylation patterns across the genome, providing a comprehensive view of epigenetic alterations in CRC. Coupled with bioinformatics tools, it

enables the identification of differentially methylated regions, functional enrichment analysis, and correlation studies.

By focusing on SLC genes, this study hopes to shed light on their potential role in CRC pathogenesis through DNA methylation changes. The findings could contribute to our understanding of the molecular mechanisms underlying CRC and may reveal novel therapeutic targets or biomarkers for this disease.

2. Materials and Methods

2.1. Human Methylation 450K data analysis

The in-house methylation microarray data from 54 CRC and 54 paired adjacent normal colon tissues were retrieved and subjected to bioinformatics analysis. The data is also available from the GEO under accession GSE193535. Quality control was performed using Genome Studio software version 2.0.4 (Illumina Inc.). The ChAMP R package [22] was used to further analyze the past IDAT files from 108 samples in a single analysis, and filters were applied to all probes, removing CpG sites with a detection p value > 0.01. This includes the removal probes located on the sex chromosomes as well. The peak-based correction method (PBC) [23] was used for data normalization prior to batch effect correction using ComBat [24]. The β-values were then extracted and subjected to statistical analysis. The Limma Bioconductor package [25] was employed to identify differentially methylated CpG sites, applying FDR correction with a p value < 0.01 for significance.

2.2. Pathway Enrichment Analysis

We conducted a functional enrichment analysis using the online software GeneCodis 4 [26] to uncover the enrichment of signaling pathways associated with these genes. Instead of utilizing probe IDs, we opted for the IDs of 336 SLC genes that exhibited differential methylation. This approach allowed us to focus specifically on the genes of interest and their potential roles in CRC. To aid in data interpretation, we generated visualizations in the form of bar charts.

2.3. Receiver operating characteristic (ROC) curve analysis

The diagnostic efficacy of the potential biomarker was assessed through ROC curve analysis, generated using GraphPad Prism 8.0.2. The area under the ROC curve (AUC), constructed with a 95% confidence interval (95% CI), served as an accuracy criterion for evaluating the potential biomarker. Methylation values from 2277 probes, identified as potential biomarkers in CRC cases, were contrasted with their respective controls. An ideal diagnostic marker possesses an AUC value of 1. An AUC value ranging from 0.7 to 0.8 is deemed acceptable, 0.8 to 0.9 is considered excellent, and anything above 0.9 is regarded as outstanding.

2.4. MethSurv Survival Analysis based on CpG Methylation Patterns

Survival analysis was performed using the online tool MethSurv (https://biit.cs.ut.ee/methsurv/) [27], which requires entry of a single CpG site per analysis. The Kaplan–Meier survival chart generated separately from the Cancer Genome Atlas Colon Adenocarcinoma (TCGA COAD) and Rectal Adenocarcinoma (TCGA READ) for the individual CpG sites. A total of 2277 CpG sites, along with CGI and genomic region data for the individual CpG sites, were used as input for the analysis. The correlation between the DNA methylation of each CpG site and the probability of survival was visualized in the Kaplan–Meier plot.

3. Results

3.1. Mapping the Genomic Locations of Differentially Methylated SLC Genes

In this study, we analyzed the differential methylation status of 54 CRC tissue samples and their corresponding adjacent normal samples, totaling 108 samples. We performed probe filtering to identify differentially methylated probes (DMPs) with an adjusted p-value less than 0.01, following the application of a false discovery rate (FDR) correction (Figure 1a). This process yielded a total of 157,846 DMPs. To focus our analysis on SLC genes, we implemented an additional filtering step, retaining only those probes specific to SLC genes. This resulted in a final selection of 2,277 probes for further analysis. These selected probes were then categorized as either hypermethylated or hypomethylated based on positive of negative β value difference (Δβ) of between the CRC and normal adjacent tissues. Our analysis identified a total of 1,891 probes showing hypomethylation and 386 probes exhibiting hypermethylation.

The differentially methylated probes (DMPs) were further classified based on their location relative to CpG islands (CGIs), resulting in four distinct regions: CpG island, shores, shelves, and open sea regions. As shown in Figure 1b, of the 1,891 hypomethylated probes, 934 probes (49%) were in the opensea region, 466 probes (25%) were in the shore, 248 probes (13%) were in the shelf, and the remaining 243 probes (13%) were in the island region. On the other hand, the majority of the hypermethylated probes were found in the island region (n = 234; 61%), followed by the shore (n = 85; 22%), opensea (n = 40, 12%), and shelf regions (n = 20, 5%). This distribution provides insights into the methylation patterns of the SLC genes in CRC.

The distribution of hypomethylated probes across genomic regions showed that the majority (n = 1174; 62%) were in the body region of SLC genes. This was followed by the TSS1500 region (n = 245; 13%), and the 3’ and 5’ UTR regions with 169 (9%) and 159 (9%) probes, respectively. The TSS200 region contained 80 probes (4%), and the 1st exon had 64 probes (3%). In contrast, the distribution of hypermethylated probes in SLC genes was more balanced. The body region contained 110 DMPs (29%), the TSS200 region had 93 DMPs (24%), the 5’UTR region had 76 DMPs (20%), the TSS1500 region had 63 DMPs (16%), the

1st exon had 28 DMPs (7%), and the 3'UTR region had 16 DMPs (4%). This distribution is illustrated in Figure 1c.

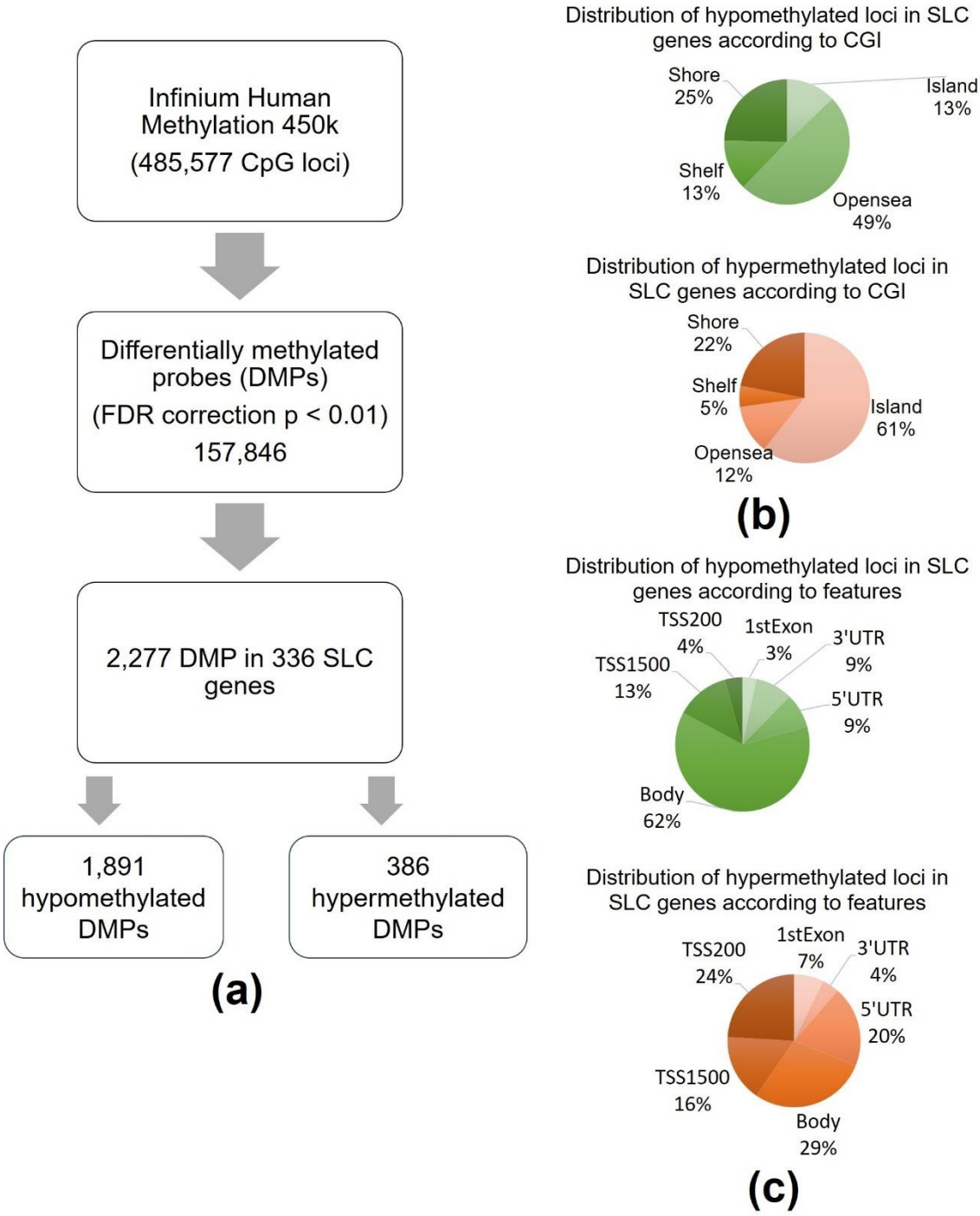

Figure 1. Identification and Distribution of Differentially Methylated Probes in Solute Carrier Genes. (a) The process of identifying differentially methylated probes (DMPs) in Solute Carrier (SLC) genes. (b) Distribution of DMPs in relation to CGIs. It shows that of the 1,891 hypomethylated probes, the majority were in the open sea and shore regions. In contrast, most hypermethylated probes were found in the island region. (c) Distribution of these probes across genomic regions. For hypomethylated probes, the majority were in the body region, while for hypermethylated probes, the distribution was more balanced across the body, TSS200, and 5'UTR regions.

3.2. Analysis of Differentially Methylated SLC Genes

The in-house methylation microarray data from 54 CRC and 54 paired adjacent normal colon tissues were retrieved and subjected to bioinformatics analysis. Quality control and data normalization were performed using Genome Studio software and the ChAMP R package. The Limma Bioconductor package was employed to identify differentially methylated CpG sites. Probes with a $\Delta\beta$ value of tumor versus normal ≥ 0.2 were considered as hypermethylated and $\Delta\beta \leq -0.2$ as hypomethylated.

The results of this analysis are presented in the Figure 2 below, which shows the top 10 SLC genes with the highest number of differentially methylated loci.

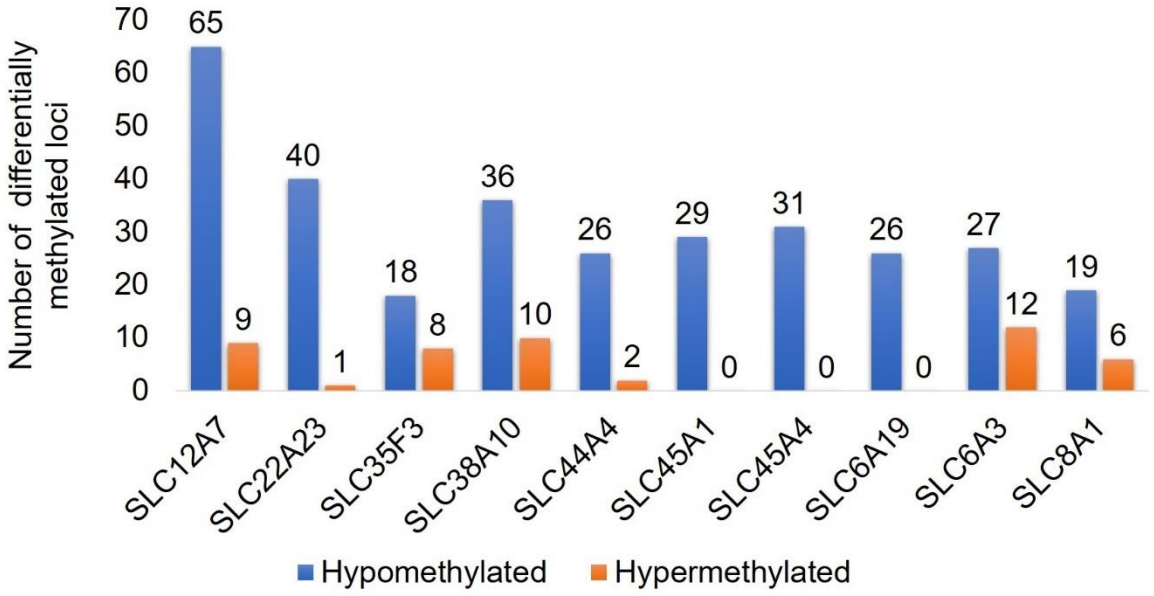

Figure 2. Top 10 Solute Carrier (SLC) Genes with the Highest Number of Differentially Methylated Loci.

For example, the *SLC12A7* gene has 65 hypomethylated loci and 9 hypermethylated loci, making a total of 74 differentially methylated loci. This suggests that *SLC12A7* underwent significant methylation changes in CRC, which could potentially influence disease progression and outcome.

In terms of patterns, the number of hypomethylated loci is generally higher than the number of hypermethylated loci for most genes. However, there are exceptions such as *SLC6A3* and *SLC8A1* where the number of hypermethylated loci is higher. These findings highlight the complex nature of methylation changes in CRC and underscore the need for further research to fully understand their implications. While these genes could serve as potential biomarkers for CRC, further validation and exploration of these findings could provide valuable insights into the molecular mechanisms underlying CRC.

3.3. Functional Analysis of Differentially Methylated SLC Genes - Uncovering Biological Significance through Enrichment Analysis

In this study, we utilized GeneCodis for pathway enrichment analysis. This analysis was based on the BioPlanet curated database [28]. Our analysis revealed significant enrichment in 66 pathways which predominantly involve transmembrane transport processes, including the transport of small molecules, inorganic cations/anions, amino acids/oligopeptides, glucose and other sugars, bile salts and organic acids, metal ions, and amine compounds (https://genecodis.genyo.es/gc4/analysisResults?job=sVOQdPB8qP7thA). This suggests that alterations in these transport processes could be a key factor in CRC pathogenesis.

Our analysis pinpointed the SLC-mediated transmembrane transport pathway as the most significantly enriched in our dataset (Figure 3), exhibiting an adjusted p-value of 1.07E-314 and a relative enrichment score of 32.57. This pathway, orchestrated by SLC proteins, governs the translocation of various substances across the cell membrane. Given our focus on SLC genes in this analysis, the prominence of this pathway aligns with our expectations. SLC proteins, instrumental in ferrying a wide array of solutes across biological membranes, appear to be modulated by the differential methylation of SLC genes, potentially influencing the progression of CRC.

Additionally, we identified the transmembrane transport of small molecules as the second most significantly enriched pathway, marked by an adjusted p-value of 6.67E-246 and a relative enrichment score of 19.02. This pathway, integral to numerous cellular functions such as nutrient uptake, waste removal, and cell signaling, oversees the movement of various small molecules across the cell membrane. The significant enrichment of this pathway implies its potential role in CRC pathogenesis.

Interestingly, our results also highlighted pathways related to neurotransmitter transport and release, including sodium/chloride-dependent neurotransmitter transporters and the neurotransmitter release cycle. This suggests a potential link between CRC and neurological processes. Several metabolic processes were also significantly enriched, including the metabolism of vitamins and cofactors, bile acid and bile salt metabolism, carbohydrate metabolism, and glucose metabolism. This indicates that metabolic alterations could play a crucial role in CRC. Furthermore, we found several pathways related to the transport and metabolism of vitamins and related molecules, suggesting a potential role of nutritional processes in CRC. The enrichment of pathways associated with metal ions, such as zinc transporters and zinc efflux, suggests a potential association between SLC genes and metal homeostasis in CRC. Moreover, pathways related to cell surface interactions at the vascular wall were also significantly enriched, indicating a potential role of cell-cell interactions, and signaling in CRC. Further research into these pathways could provide valuable insights into the molecular mechanisms underlying CRC and may reveal novel therapeutic targets or biomarkers for this disease.

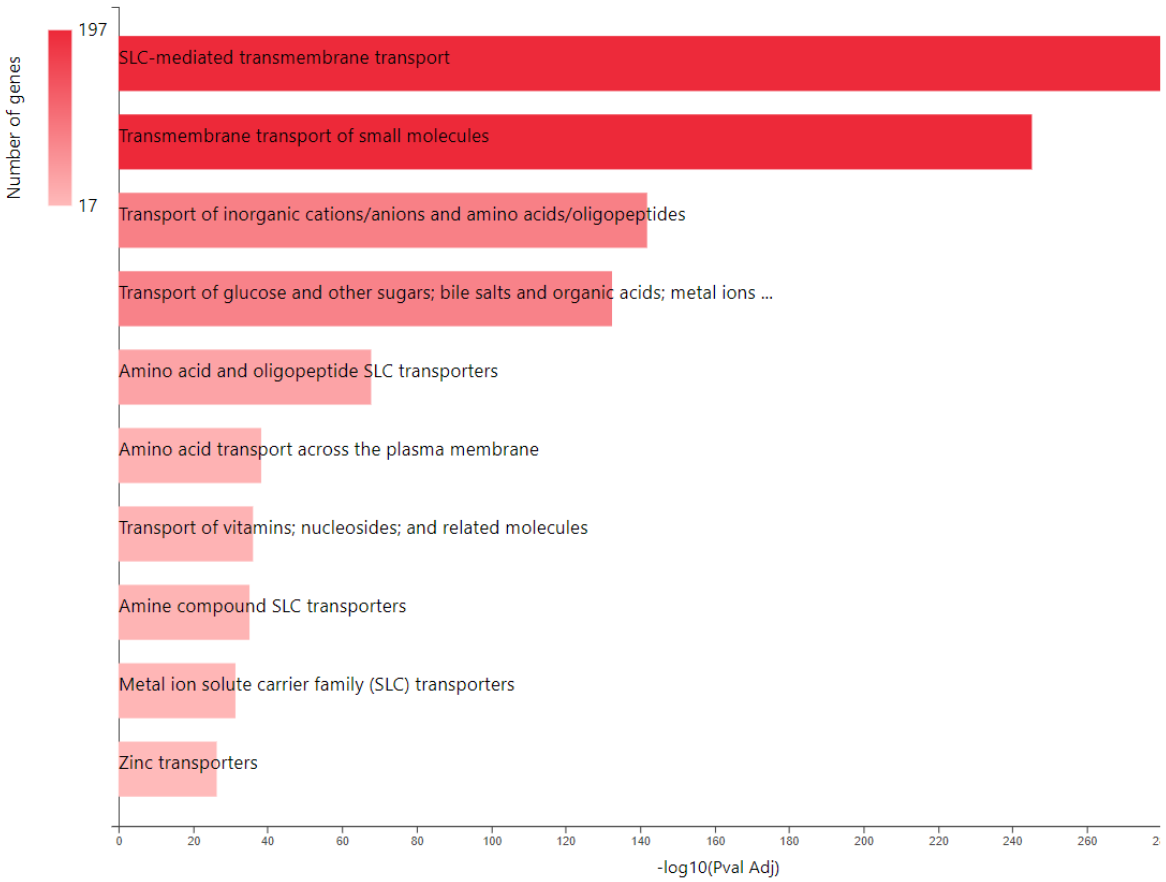

Figure 3. The Ten Most Significantly Enriched Pathways Derived from Differentially Methylated SLC Genes

3.4. Impact of Differential Methylation in SLC Genes on Drug Response

Additionally, enrichment was performed against PharmGKB database [29] to understand the potential impact of the differentially methylated SLC genes on drug response in CRC. PharmGKB is an NIH-funded resource developed by Stanford University and is the largest pharmacogenetic database. Four drugs were found to be significantly enriched by the differentially methylated SLC genes (Table 1), which are metformin, zidovudine, tipiracil hydrochloride, and trifluridine.

Our analysis revealed a significant association between the antidiabetic drug metformin and differentially methylated SLC genes in CRC. The adjusted p-value (pval adj) for this association was 3.61e-04, indicating a high level of statistical significance. Furthermore, the relative enrichment score was 6.32, suggesting a strong overrepresentation of differentially methylated SLC genes among all genes associated with metformin. Specifically, we identified 10 SLC genes that were significantly differentially methylated in association with metformin. These genes are *SLC30A8*, *SLC22A3*, *SLC29A4*, *SLC47A1*, *SLC22A1*, *SLC22A4*, *SLC2A2*, *SLC47A2*, *SLC22A2*, and *SLC19A3*.

Table 1. *In silico* Analysis of Association of Differentially Methylated SLC Genes with Various Drugs in CRC.

Drug (PharmGKB)	Genes	Adjusted p value	Relative enrichment score	Use
Metformin	*SLC30A8*, *SLC22A3*, *SLC29A4*, *SLC47A1*, *SLC22A1*, *SLC22A4*, *SLC2A2*, *SLC47A2*, *SLC22A2*, *SLC19A3*	3.61e-04	6.32	Used to treat type 2 diabetes [30]
Zidovudine	*SLC22A1*, *SLCO3A1*, *SLC22A11*, *SLC22A8*, *SLC22A7*, *SLC28A3*, *SLC22A6*, *SLC28A1*	3.61e-04	7.89	Management and treatment of HIV-1 [31]
Trifluridine	*SLC47A1*, *SLC29A1*, *SLC22A2*	3.75e-03	24.65	A component of a chemotherapy combination that includes trifluridine and tipiracil hydrochloride [32]
Tipiracil Hydrochloride	*SLC47A1*, *SLC29A1*, *SLC22A2*	3.75e-03	24.65	A part of a chemotherapy combination that contains trifluridine and tipiracil hydrochloride [21]

Both trifluridine and tipiracil hydrochloride, components of a chemotherapy combination, showed a significant association with the same set of three SLC genes. The adjusted p-value of 3.75e-03 and a relative enrichment score of 24.65 for both drugs indicate a strong correlation. These findings suggest that these SLC genes may play a crucial role in the drug's mechanism of action in chemotherapy and influence their effectiveness in cancer treatment. This underscores the potential importance of SLC genes in the therapeutic response to this chemotherapy combination in CRC.

Zidovudine, an antiretroviral medication used for the management and treatment of HIV-1, was also significantly associated with eight SLC genes even though this drug is not typically associated with cancer.

3.5. Exploring the Correlation between DNA Methylation Patterns of SLC Transporter Genes and Survival Rates in CRC Patients

Survival analysis was conducted using the MethSurv online tool, which necessitates the input of a single CpG site for each analysis. The Kaplan–Meier survival chart was independently generated for each individual CpG site from the TCGA COAD and TCGA READ datasets. Out of 2277 differentially methylated probes (DMPs), 114 exhibited a significant association with survival in either the TCGA COAD or TCGA READ datasets. Interestingly, six DMPs demonstrated a significant survival association across both datasets, as detailed in Table 2. Table 3 presents the top 10 differentially methylated SLC genes that

are significantly correlated with survival rates in CRC patients. For instance, the *SLC12A7* gene has 23 DMPs associated with survival, 13 of which are found in the COAD dataset and 12 in the READ dataset. Notably, more than 30% of DMPs in *SLC12A7* are significantly associated with CRC survival, indicating that these DMPs could potentially serve as valuable prognostic markers.

Table 2. Significantly Associated DMPs for Survival Across Both TCGA COAD and TCGA READ Datasets.

SLC genes	Probes ID	Delta-beta value	LR test P-value	
			COAD	READ
SLC12A7	cg17851021	-0.078979475	0.023	0.047
SLC12A7	cg00697639	-0.099582235	0.017	0.045
SLC35F3	cg10878114	0.090591202	0.047	0.0092
SLC44A4	cg08506113	-0.086719725	0.025	0.0098
SLC44A4	cg07643404	-0.080037400	0.015	0.0012
SLC6A19	cg10035234	-0.141271346	0.0051	0.039

Table 3. Top 10 Differentially Methylated SLC Genes Significantly Correlated with Survival Rates in CRC Patients.

SLC genes	Total number of DMP associated with survival	COAD	READ
SLC12A7	23	13	12
SLC22A23	11	6	5
SLC35F3	7	3	5
SLC38A10	19	10	9
SLC44A4	10	4	8
SLC45A1	8	6	2
SLC45A4	7	3	4
SLC6A19	10	4	7
SLC6A3	9	6	3
SLC8A1	10	1	9

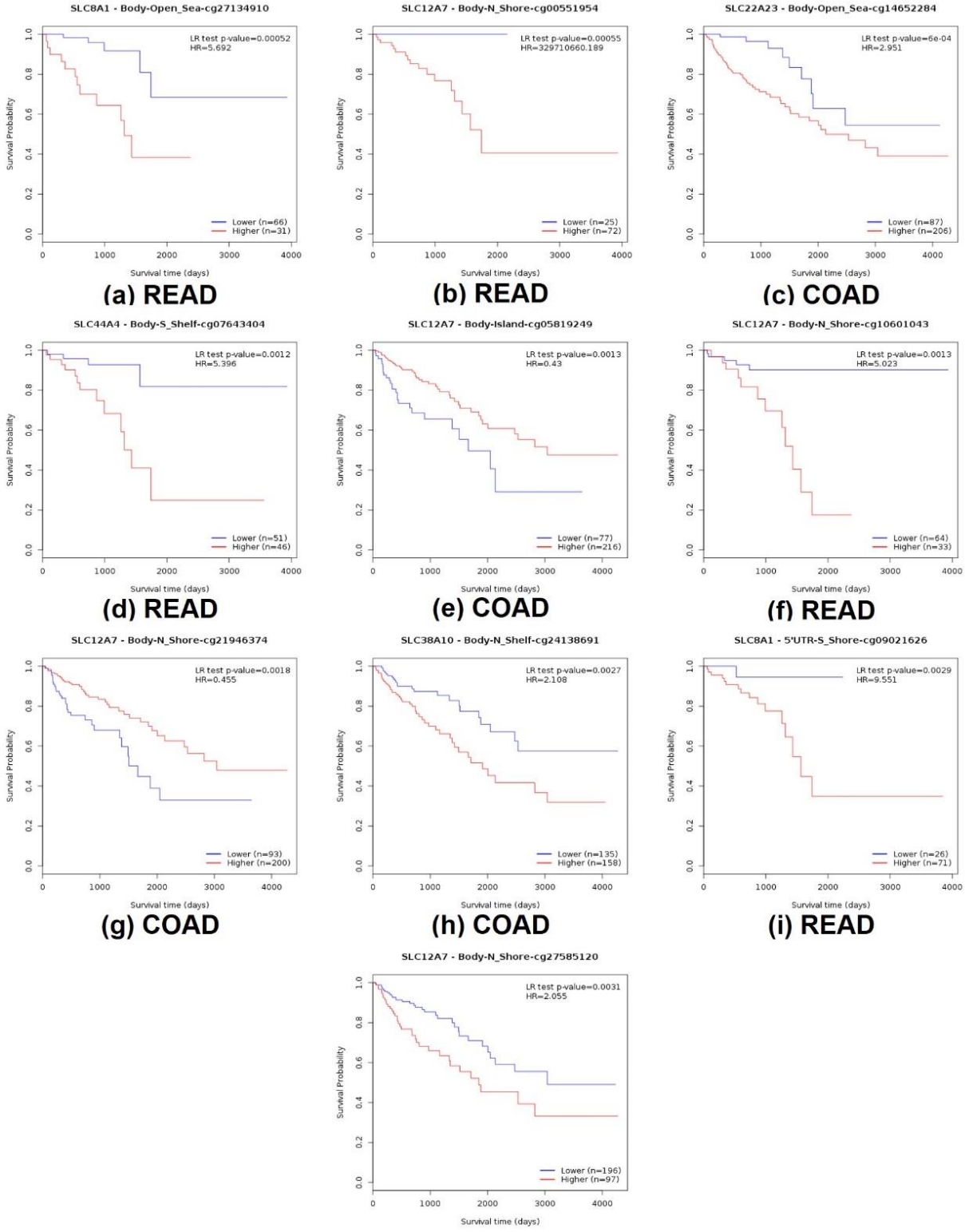

Figure 4. The Ten Most Significant Differentially Methylated Probes (DMPs) within SLC Genes and Their Association with Survival Outcomes. COAD: Colorectal Adenocarcinoma, READ: Rectal Adenocarcinoma.

Figure 4 presents the ten most significant DMPs associated with survival outcomes. For instance, the cg27134910 in the *SLC8A1* gene which has a delta-beta value of -0.254277375, is located in the 'Opensea' region of the gene body, was identified in the READ dataset, and has a LR-test p-value of 0.00052, indicating a statistically significant correlation with survival outcomes. These findings highlight the potential of these SLC genes and their DMPs as prognostic markers for CRC.

3.6. Evaluating the Diagnostic Potential of Differentially Methylated SLC Genes Using ROC Analysis

The Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the candidate biomarkers. The methylation values of 2277 probes in SLC genes were plotted against their corresponding controls. The area under the ROC curve (AUC) was used as an accuracy criterion for the examination of the candidate biomarker.

Table 3. The Ten Most Significant Differentially Methylated SLC Probes, Ranked by the Highest Area Under the Receiver Operating Characteristic (ROC) Curve.

SLC genes	Probes	Area under curve	Std. Error	95% confidence interval	P value
SLC38A11	cg01770810	0.939	0.02028	0.8992 to 0.9787	<0.0001
SLC6A3	cg24178621	0.9304	0.02487	0.8816 to 0.9791	<0.0001
SLC20A2	cg10308027	0.9287	0.02322	0.8832 to 0.9742	<0.0001
SLC6A16	cg19920353	0.9266	0.02585	0.8759 to 0.9773	<0.0001
SLC9A11	cg24720132	0.9246	0.02581	0.8740 to 0.9751	<0.0001
SLC25A24	cg16307793	0.9242	0.02446	0.8763 to 0.9722	<0.0001
SLC16A3	cg23141183	0.9228	0.02669	0.8705 to 0.9752	<0.0001
SLC38A10	cg05919238	0.9208	0.02566	0.8705 to 0.9711	<0.0001
SLC39A10	cg08310476	0.9208	0.02614	0.8696 to 0.9720	<0.0001
SLC25A4	cg16667710	0.9204	0.02575	0.8700 to 0.9709	<0.0001

The results of the ROC curve analysis for the ten most significant differentially methylated SLC probes are presented in Table 3. The probe cg01770810 in the gene *SLC38A11* had the highest AUC value of 0.939, indicating an outstanding diagnostic potential. The 95% confidence interval for this probe was 0.8992 to 0.9787, and the p-value was less than 0.0001, indicating a statistically significant result. The other probes also showed high AUC values, ranging from 0.9204 to 0.939, and all had p-values less than 0.0001. These results suggest that these differentially methylated SLC probes could serve as potential biomarkers for CRC.

4. Discussion

The Solute Carrier (SLC) family of genes plays a crucial role in cancer, with links to DNA methylation, metabolic adaptation, drug response, and immune reactivity [33]. DNA methylation is a key regulatory mechanism of gene expression, and alterations in methylation patterns can influence cancer cell fate. Interestingly, the expression of relevant SLCs was correlated with hypo- and hyper-methylation of promoter and body region, showing an established DNA methylation pattern. For instance, the positive association of SLC7A11 cg06690548 methylation with cancer outcome suggests the independent predictive role of DNA methylation at a single nucleotide resolution [33].

The field of research focusing on the methylation of SLC genes in CRC has indeed made substantial strides. This has led to a deeper understanding of the role these genes play in the development and progression of CRC. However, despite these advancements, there are still numerous areas that require further exploration and clarification. The SLC gene family is vast and varied, with each member potentially having a unique role in the progression of colorectal cancer. Comprehensive studies are needed to elucidate the specific functions and mechanisms of each SLC gene in this context.

In this research, we discovered that the *SLC12A7* gene exhibits the highest number of differentially methylated loci. This differential methylation appears to significantly impact survival rates in CRC. The *SLC12A7* gene, also known as KCC4, is a protein-coding gene involved in various processes, including cell volume homeostasis and inorganic ion transmembrane transport [34]. This trans-membrane protein, which is 1083 amino acids in length, plays a crucial role in regulating cell volume through the transport of potassium and chloride [35, 36]. Interestingly, research has shown that an increase in the expression of *SLC12A7* can enhance the malignant behavior of various types of cancer. Specifically, *SLC12A7* is found to be overexpressed in gynecologic [37, 38] and breast cancers [39]. This overexpression, along with that of other members of the SLC12 gene family, has been associated with local tumor invasion, lymph node metastases, and unfavorable clinical

outcomes [40]. Our findings suggest that the hypomethylation status of *SLC12A7* could serve as a potential prognostic marker for CRC. This means that the methylation status of this gene could help predict the course or outcome of the disease, providing valuable information for treatment planning. Although there are currently no published studies specifically investigating the methylation of *SLC12A7* in CRC, existing research does suggest a potential role for *SLC12A7* in cancer progression [34]. However, these studies are not specific to CRC and do not conclusively establish a role for *SLC12A7* in this type of cancer. Therefore, while these findings provide some insight, more targeted research is needed to fully understand the role of *SLC12A7* in CRC. Further studies are essential to validate these preliminary findings and to explore any potential therapeutic implications.

Our investigation into the pathways significantly enriched due to the differential methylation of SLC genes in CRC has yielded crucial insights into the molecular mechanisms implicated in the oncogenesis and progression of this disease. The research has unveiled several enriched pathways that encompass a diverse array of cellular processes, thereby highlighting the complex interplay of SLC gene methylation within the CRC landscape. One pathway of particular interest is the SLC-mediated transmembrane transport pathway. This pathway facilitates the transport of a variety of molecules across the cell membrane, a process in which SLC genes play an instrumental role [41]. The differential methylation of these genes could potentially modify the efficiency or specificity of this transport mechanism. Such modifications could, in turn, contribute to the progression of CRC by altering cellular homeostasis and promoting malignant transformation. Given the emphasis of our analysis on SLC genes, the prominence of this pathway is consistent with our expectations. This observation also underscores the significance of SLC genes in CRC pathogenesis and their potential as therapeutic targets. The differential methylation of SLC genes could serve as a novel avenue for therapeutic intervention, offering the possibility of personalized treatment strategies based on the patient's unique methylation profile.

Another important pathway identified is the transmembrane transport of small molecules pathway. Transmembrane transport is a fundamental cellular process that involves the movement of small molecules across the cell membrane [42]. This process is crucial for maintaining cellular homeostasis and facilitating various cellular functions [42]. In the context of cancer, the role of transmembrane transport becomes even more significant due to its potential involvement in the disease’s progression and response to treatment. The differential methylation of SLC genes, as observed in our study, could potentially alter the efficiency or specificity of transmembrane transport. Such alterations might contribute to the progression of CRC by disrupting cellular homeostasis and promoting malignant transformation.

Moreover, changes in the transmembrane transport of small molecules have been implicated in the development of drug resistance in CRC [43]. Such alterations in the transmembrane transport of small molecules, potentially due to differential methylation of SLC genes, could lead to reduced drug uptake or increased drug efflux, thereby contributing to drug resistance.

We also explored the association between differentially methylated SLC genes in CRC and various drugs. Metformin, a drug commonly used to treat type 2 diabetes [44], showed a significant association with ten SLC genes in our analysis. Metformin has been shown to have anti-proliferative, chemo-preventive, apoptosis-inducing effects, and it can also act as an adjuvant and radio-chemosensitizer in CRC [45, 46]. However, some studies suggest that metformin does not function as an anti-proliferative agent and is not a beneficial adjunct therapy for certain types of cancer [47, 48]. Zidovudine, an antiretroviral medication used for the management and treatment of HIV-1, was associated with eight SLC genes. Our data suggests a significant relationship between Zidovudine and these SLC genes, potentially influencing the drug's effectiveness in HIV-1 treatment. Zidovudine is not typically used in the treatment of cancer and is not listed among the FDA-approved drugs for CRC. However, one study found that zidovudine significantly reduced the proliferation of murine and human cancer cell lines [49].

As of now, numerous clinical trials are being conducted to investigate the effectiveness of Trifluridine/Tipiracil Hydrochloride, also known as TAS-102, in the treatment of metastatic CRC [50-56]. TAS-102 operates through a unique mechanism. Trifluridine, a nucleoside analog, can penetrate cells and integrate into their DNA, thereby preventing its replication and halting cell division. On the other hand, tipiracil acts as an inhibitor of thymidine phosphorylase, an enzyme that breaks down trifluridine in the body. This action of tipiracil not only allows trifluridine to remain in the body longer to destroy more cancer cells but also aids in maintaining the blood concentration of trifluridine by inhibiting its metabolism [57-59].

Despite these ongoing studies, there is currently no published evidence that establishes a direct link between the use of TAS-102 and the DNA methylation *SLC47A1*, *SLC29A1*, and *SLC22A2*. Alterations in the methylation status of these genes could potentially influence their expression and the functionality of the proteins they encode. Given the roles of these genes and the mechanism of action of TAS-102, it is plausible to hypothesize that changes in the methylation status of these genes could impact the uptake or efficacy of TAS-102 in cancer cells. However, this hypothesis would require validation through rigorous experimental studies. It is essential to continue research in this area to

uncover the potential interplay between these genetic factors and the effectiveness of TAS-102 in treating metastatic CRC. This could pave the way for more personalized and effective treatment strategies in the future.

Recent advancements in nanomedicine have opened up new avenues for improving the pharmacological response and clinical outcomes in patients undergoing chemotherapy [60]. This is particularly relevant when studying the DNA methylation of SLC genes in CRC, as nanoparticle-based drug delivery systems could potentially enhance the therapeutic properties of drugs targeting these epigenetic modifications. In parallel, the field of cancer classification and therapeutics have also seen significant progress with the advent of deep learning algorithms and *in silico* analysis [61-63]. These algorithms have demonstrated promising results in analyzing multi-omics data, including DNA methylation [64-68]. These advanced computational algorithms could potentially enhance the analysis and interpretation of complex multi-omics data.

5. Conclusions

The comprehensive analysis of the microarray methylation data from CRC tissue samples and their corresponding adjacent normal tissues has led to significant findings. A total of 2,277 differentially methylated probes of SLC genes were identified, indicating a potential role of these genes in CRC pathogenesis. Furthermore, several SLC genes were found to be significantly associated with the survival of CRC patients, suggesting their potential as prognostic markers. Additionally, certain SLC transporter genes emerged as promising candidates for specific and accurate diagnostic biomarkers for CRC. These findings underscore the potential of SLC genes in enhancing our understanding of CRC and in the development of novel diagnostic and therapeutic strategies.

While the study provides valuable insights into the role of SLC genes in CRC, it does have certain limitations. The study is based on a limited sample size of 54 CRC tissue samples, and while this provides a good starting point, larger studies could offer more robust results. Additionally, this study identifies differentially methylated SLC genes associated with CRC but does not provide functional or clinical validation of these genes. Experimental and clinical studies are needed to confirm the biological and diagnostic significance of these findings. Lastly, the study does not account for inter-individual variability in DNA methylation patterns, which can be influenced by factors such as age, lifestyle, and genetic background.

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