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Original Research Article

In silico Evaluation of Promising Epigenetic Biomarkers for the Detection of Colon Adenocarcinoma

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Abstract

Introduction: Colon adenocarcinoma (COAD) is a common and fatal cancer in the world, with a high death rate in India as a result of late diagnosis. Conventional screening techniques, such as stool tests and colonoscopies, are expensive, invasive, and often insensitive in identifying early-stage illnesses. The development of reliable, non-invasive biomarkers is therefore crucial to improving prognosis and early diagnosis. Epigenetic alterations, especially DNA methylation changes, occur early in tumor development and can be detected in circulating cell-free DNA (cfDNA), making them promising candidates for liquid biopsy-based diagnostics.

Objective: The purpose of this study was to identify and validate epigenetic biomarkers for the non-invasive diagnosis and prognosis of COAD, with a particular emphasis on SEPT9 and SDC2. The objective was to utilize computational techniques to assess their expression patterns and methylation status, with a focus on developing methylation assays suitable for early diagnosis and disease monitoring.

Materials and Methods: The Human Protein Atlas, TCGA, UALCAN, GEPIA, and other publicly available multi-omics datasets were utilized to evaluate gene expression, promoter methylation, protein localization, and survival relationships for SDC2 and SEPT9. By comparing tumor and normal tissues, bioinformatics analyses revealed variations in methylation. The analysis of single-cell RNA sequencing data, with an emphasis on epithelial lineage, confirmed the expression of specific genes in distinct cell types.

Results and Discussion: Bioinformatics analysis revealed significant promoter hypermethylation of SEPT9 and SDC2 in COAD samples compared to normal colon tissue. SDC2 demonstrated subtype-specific downregulation, whereas SEPT9 showed significant overexpression, especially in non-mucinous malignancies. Immunohistochemistry confirmed variable SDC2 expression and elevated SEPT9 protein levels. RNA sequencing of single cells has shown that both genes are highly expressed in epithelial cells, indicating their specificity as epigenetic biomarkers. The increased expression of both genes correlated with reduced overall survival, as indicated by survival analysis, underscoring their potential as prognostic indicators.

Keywords: Colon Adenocarcinoma, cell-free DNA, Syndecan-2, Septin 9, Immunohistochemistry, circulating tumor DNA, DNA methyltransferases.

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Introduction

Colon Adenocarcinoma (COAD), the most prevalent histological type of colorectal cancer, is increasingly emerging as a major global health problem. In 2022, there were approximately 1.9 million new cases and over 0.9 million deaths due to the disease [1,2]. The disease process takes a multi-step adenoma-carcinoma course, involving a chain of genetic and epigenetic alterations that tend to be asymptomatic in their initial phases [3]. The survival rates after five years differ immensely, ranging above 90% in stage I disease but below 14% for those with stage IV disease, motivating the urgent necessity for early and accurate detection [2]. The screening methods introduced for colon cancer, such as the gold standard colonoscopy and computed tomography colonography, provide structural details but are invasive, expensive, and lack satisfactory patient compliance rates [4]. Non-invasive modalities, such as the faecal immunochemical test (FIT) and guaiac faecal occult blood test (gFOBT), are not extremely sensitive and particularly lack the ability to detect advanced adenomas or early cancers [5]. It is imperative to develop new biomarkers that are both more sensitive and specific, as protein-based blood indicators, such as carcinoembryonic antigen (CEA), lack sufficient specificity and

sensitivity for early diagnosis [6]. Liquid biopsy is now a revolutionary tool, enabling the detection of tumor-derived materials such as circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), and extracellular vesicles from body fluids like blood, urine, or saliva [7]. Of these, cfDNA and ctDNA harbor somatic mutations, copy number variations, and aberrant DNA methylation patterns - providing information about tumor biology even at the beginning of carcinogenesis [8]. DNA methylation, catalyzed by DNA methyltransferases (DNMTs), is the methylation of CpG dinucleotides and is a major determinant of gene expression. The tumorigenic process is associated with a global decrease in methylation levels, overmethylation of the promoters of tumor suppressor genes, and the resultant silencing of genes such as MLH1 and APC [9]. These methylation changes are detectable at the initial stages of COAD carcinogenesis, and DNA methylation is therefore an appealing target for non-invasive diagnostic screening.

The SEPT9 gene contains a cytoskeletal protein GTPase, involved in cell division and cytokinesis. Large-scale validation has established that SEPT9 promoter hypermethylation is a valuable diagnostic biomarker for COAD [10]. The Epi proColon test, which measures methylated SEPT9 in plasma, was the first blood test to be FDA-approved for screening colorectal cancer (CRC) with a sensitivity of 68–88% and a specificity of approximately 80% [11]. Syndecan-2 (SDC2) is a heparan sulfate-bearing transmembrane proteoglycan involved in cellular proliferation, migration, cell matrix interaction, and angiogenesis. Methylation of SDC2 has been found to be abnormal in early COAD, with a diagnostic sensitivity of over 87.0% and a diagnostic specificity of 95.2% [12]. The diagnostic potential is further enhanced by multimarker methylation panels, such as ColoDefense, which include SDC2 and SEPT9 [13]. The application of in silico data mining has accelerated biomarker discovery based on the capability to systematically analyze gene expression, methylation signatures, and survival correlations in the framework of public data repositories like TCGA, UALCAN, and GEPIA [14,15]. Databases enable the validation of biomarkers across large patient groups and allow for stratification according to histological subtypes, including mucinous adenocarcinoma [16-18]. Bioinformatics methodologies enhance analytical accuracy and consistency by integrating beta value comparisons associated with methylation and survival outcomes for predictive purposes. Recent extensive studies have demonstrated the high sensitivity of methylation panels of ADHFE1, ADAMTS5, and MIR129-2 for identifying COAD in cfDNA, corroborating the effectiveness of computational methodologies [19]. Although there is increasing evidence on SEPT9 and SDC2 as methylation biomarkers, no study has been conducted in the Indian population. The current study seeks to bridge these gaps by conducting a comparative analysis of mRNA, methylation, and SEPT9 and SDC2 protein expression in COAD and normal tissues by utilizing the TCGA, UALCAN, and HPA databases and survival analysis with GEPIA to determine the prognostic significance. The TCGA, UALCAN, and HPA databases are utilized to conduct a comparative analysis of mRNA, methylation, and SEPT9 and SDC2 protein expression in COAD and normal tissues. In the present study, we conducted a survival analysis using GEPIA to ascertain the prognostic significance. By utilizing computer analysis, this research offers a cost-effective and data-driven approach to identifying key methylation markers in COAD, which can be beneficial in clinical practice. Hence, the present study was designed to evaluate promising epigenetic biomarkers for the detection of COAD in the Indian population, using an in-silico approach.

Materials and Methods

Study design: The objective of this computational work was to evaluate the diagnostic and prognostic value of two genes associated with COAD: SDC2 (Syndecan-2) and SEPT9 (Septin 9). The mRNA and protein expression levels, DNA methylation patterns, and correlations with overall survival of these genes in normal, adenocarcinoma, and mucinous adenocarcinoma colon tissues were investigated using publicly available datasets. Clinical and omics data from multiple sources, including TCGA, UALCAN, GEPIA, and the Human Protein Atlas, were incorporated into this all-encompassing strategy.

Gene selection and data sources: We chose the genes SEPT9 and SDC2 because they are known to be linked to the epigenetics of colon cancer and could be used as circulating methylation biomarkers (PMID: 29027401, 23291739). Identifying accurate biomarkers is a top priority, as early detection using non-invasive methods is crucial for enhancing patient outcomes. Public databases like TCGA, GEPIA, and the Human Protein Atlas offer essential genomic, transcriptomic, and proteomic data that can be utilized to identify and confirm putative biomarkers. This study utilizes these resources to investigate the expression, methylation, and protein localization of candidate genes, including SEPT9 and SDC2, aiming to evaluate their potential for early diagnosis and prognosis in COAD

Table 1:

Database	Data type	URL
TCGA-COAD (ULCAN)	mRNA expression, methylation beta values, clinical data	https://ualcan.path.uab.edu/analysis.html
GEPIA (Gene Expression Profiling Interactive Analysis)	Survival analysis, expression comparison	http://gepia.cancer-pku.cn/detail.php?gene=SEPT9 http://gepia.cancer-pku.cn/detail.php?gene=SDC2
Human Protein Atlas	Protein expression	https://www.proteinatlas.org/ENSG00000184640-SEPTIN9/single+cell/colon#tissue_cell_type https://www.proteinatlas.org/ENSG00000169439-SDC2/single+cell/colon#tissue_cell_type

Gene expression analysis and DNA methylation analysis: To further understand the functions of SEPT9 and SDC2 in colon adenocarcinoma, researchers examined gene expression and DNA methylation profiles. By utilizing UALCAN and normalized RNA-Seq data from TCGA, the mRNA expression profiles of these genes were collected and analyzed in normal colon tissue, COAD tissue, and mucinous COAD tissue. Student's t-tests were employed to assess the statistical significance of differences in gene expression across tissues. P-values were provided to compare normal tissue, colon adenocarcinoma (COAD), and mucinous COAD.

The methylation profiles of SEPT9 and SDC2 for DNA methylation analysis were acquired from TCGA using UALCAN. The methylation levels were assessed utilizing beta values, which span from 0 (unmethylated) to 1 (completely methylated). The methylation status was categorized according to beta value ranges: hypomethylation (0.25- 0.30) and hypermethylation (0.50 - 0.70). The methylation levels were analyzed across normal, colon adenocarcinoma, and mucinous COAD tissues. Statistical significance was established at a p-value of less than 0.05, with highly significant results (e.g., p-values below 1.0×10^{-12}) denoting pronounced differential methylation between tissue types.

Protein expression analysis: Immunohistochemistry (IHC) images of colon sections were used to generate protein expression levels from the Human Protein Atlas (HPA).

We classified the SEPT9 and SDC2 expression levels as High, Medium, Low, or Not Detected based on the staining intensity and the proportion of stained cells. Normal colon tissues and COAD tissues were examined to identify any potentially divergent expression levels. Moreover, by utilising STRING and GeneMANIA, we successfully identified the protein interaction partners of SEPT9 and SDC2. This essay clarifies their molecular mechanisms and physiological functions**.**

Survival Analysis: The predictive potential of SEPT9 and SDC2 for future outcomes was examined using GEPIA's survival analysis approach. We generated Kaplan-Meier survival graphs after classifying patients into high- and low-expression groups using median cutoff values. The TCGA clinical information provided the survival data. We noted down the log-rank p-values and the hazard ratios (HR). It was considered that p-values less than 0.05 were statistically significant. The risk tables and confidence intervals made the images easier to interpret.

Statistical method and parameters: We obtained all statistical indicators, including p-values, beta values, and hazard ratios, directly from the analytical platforms (UALCAN, GEPIA, HPA). When the databases had to make multiple comparisons, they used the Benjamini-Hochberg false discovery rate (FDR) adjustment. No adjustments were made by hand, and the data was not reprocessed. The default options for each web platform were utilized to produce data visualizations like survival curves, scatter plots, and box plots, unless otherwise specified.

Software and computational environment: All studies were conducted using cloud-based platforms and publicly accessible websites, eliminating the need for local software or programming. We used Microsoft Excel 365 to collect and organize the data, and then we used Microsoft Word and GraphPad Prism 9 to present and visualize the results.

Results and Discussions

1. Tissue-wide transcriptomic and proteomic expression profiles of the SEPT9 and SDC2 gene in human tissues

1a. Expression Analysis of SEPT9: Gene and protein expressions of SEPT9 were analyzed in normal human tissues. The data is presented in Figure 1a. The RNA-seq data (Figure 1a, left panel) from GTEx and Illumina Body Map demonstrated the abundant presence of SEPT9 mRNA in various human tissues. High expression of SEPT9 mRNA levels was observed in tissues such as bone marrow and white blood cells, highlighting their importance in hematopoietic and immune processes. Testis and

placenta also show high levels, indicating their developmental significance. A moderate to high expression of SEPT9 mRNA (10-100 FPKM) was observed in colon tissue. This finding suggests that the SEPT9 gene is actively expressed in colonic epithelial cells even under normal physiological conditions, indicating a functional role.

Protein expression of SEPT9 was analyzed using the Human Protein Atlas for immunohistochemistry data for normal human tissue samples (Figure 1a, right panel). The data showed high levels of SEPT9 protein in each tissue, as indicated by log10 values. The high levels of protein in the testis, pancreas, and certain immune-rich tissues were consistent with the known biological roles of SEPT9, particularly with respect to cell division and cytoskeletal stability. In the case of colon tissue, moderate to high expression of the SEPT9 protein suggests functional activity that aligns with mRNA expression.

In cancer, particularly COAD, increased production of SEPT9 protein may lead to disruption of cytokinesis, chromosomal instability, and tumor progression. These findings suggest that SEPT9 may play a role as a biomarker in cases of colon adenocarcinoma.

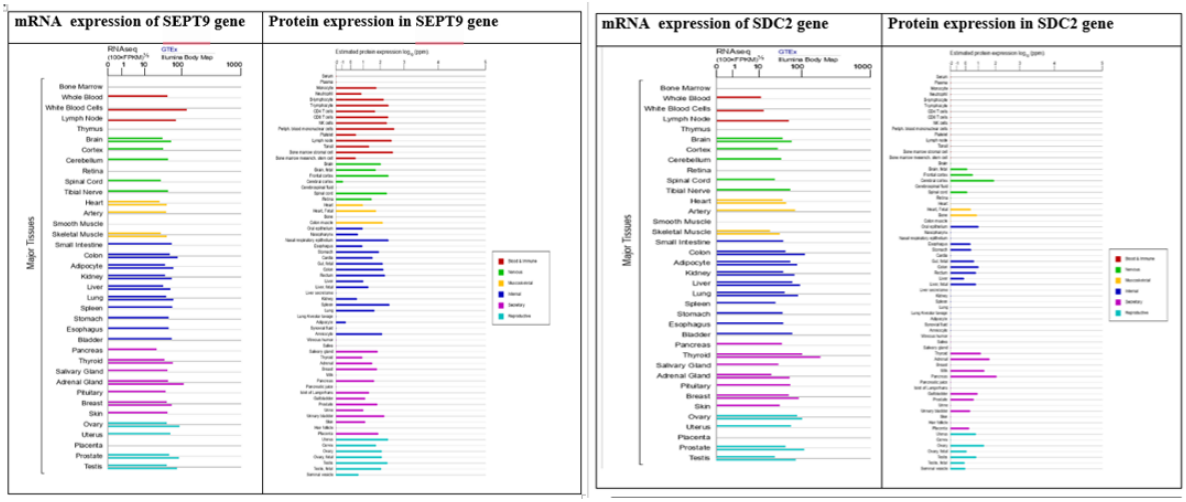

Figure 1a: Transcriptomic and proteomic profiles of SEPT9 gene expression in human tissues. & Figure 1b: mRNA and protein expression levels for the SDC2 gene in some major human tissues

(Left) mRNA (levels) expression of SEPT9 in several relevant human tissue types, including the colon, using RNA-seq data from GTEx and Illumina Body Map. SEPT9 transcriptional expression levels are moderate to high in the colon in normal conditions, likely conferring a functional role. (Right) Protein expression levels of SEPT9 (log10's estimated values) were programmed into the Atlas in several human tissues, using data from an immunohistochemistry assay. Colon tissues showed high cytoplasmic protein expression levels of SEPT9, consistent with the mRNA expression levels. The findings provided evidence of biological activity for SEPT9 in normal colonic action, while suggesting possible downstream dysregulation in the abdomen leading to colon cancer.

Figure 1b: mRNA and protein expression levels for the SDC2 gene in some major human tissues: (Left) Transcriptomic analysis shows the mRNA expression levels of SDC2 derived from GTEx and Illumina Body Map RNA-seq datasets. Moderate to high expression levels in colon tissues suggest that SDC2 may play a significant role in the signalling of intestinal epithelial cells. (Right) Protein expression data (log10-scaled) derived from immunohistochemistry, as reported in the Human Protein Atlas, suggest that SDC2 is translated at a consistent amount in the colon. The agreement between mRNA and protein expression in normal tissues and the established promoter hypermethylation of SDC2 in colon cancer suggests that SDC2 may be a novel biomarker for clinical utility in the early detection and progression monitoring of tumor biology in colon adenocarcinoma.

1b. Expression Analysis of SDC2: The mRNA and protein expressions of SDC2 were also analyzed in normal human tissues, and the results are presented in Figure 1b. The data for SDC2 mRNA (Figure 1b, left panel), based on RNA-seq data using GTEx and Illumina Body Map, showed moderate to high levels in the colon. This finding indicated that SDC-2 is normally expressed in healthy colonic epithelium and has affinity related to cell adhesion, migration, and Wnt signaling. Overexpression of SDC2 was also observed in the lung, esophagus, placenta, and small intestine, indicating a general role of SDC2 in interactions between epithelium and mesenchyme.

In colon cancer, particularly in the early stage of COAD, the SDC2 promoter is frequently hypermethylated, which can affect proper expression or alternative signaling. In later stages, SDC2 may be overexpressed due to a loss of balance between methylation and expression, which is consistent with this database.

The data for SDC2 protein expression (Figure 1b, right panel) in different tissues are obtained on a log10 scale from the Human Protein Atlas. This data showed moderate levels of SDC2 protein in colon tissue corresponding to mRNA patterns, indicating continued translation and functional roles in colon maintenance. SDC2 protein levels were relatively high in tissues such as the esophagus, placenta, and thyroid, reflecting its functional role in preserving epithelial integrity and facilitating continued hypotactic growth and angiogenesis.

In colon cancer, the dysregulation in glycosaminoglycan chain synthesis and overproduction of SDC2 increase tumor cell migration, matrix remodeling, and metastatic tendencies. The agreement between mRNA and protein expression in normal tissues and the established promoter hypermethylation of SDC2 in colon cancer suggests that SDC2 may be a novel biomarker for clinical utility in the early detection and progression monitoring of tumor biology in colon adenocarcinoma**.**

2a. Heatmap analysis and insights on SEPT9 Expression: The y-axis (Figure 2a) represents different cell types in the colon, such as enterocytes, mitotic cells, immune cells, endothelial cells, and fibroblast cells. The x-axis displays marker genes associated with each cell type, such as EPCAM for epithelial cells, ACTG2 for smooth muscle cells, CD68 for macrophages, and CD3E for T cells. The color scale ranges from black (highest expression) to yellow/white (very low or no expression). Mitotic cells in the colon exhibit high co-expression of SEPT9 with genes such as TOP2A, RRM2, and HMMR, indicating the active involvement of SEPT9 in cell division and tumor progression. Colon epithelial cells display moderate SEPT9 expression, linked to lineage markers such as EPCAM, consistent with its normal role; however, dysregulation may indicate adenocarcinoma.

Immune cells (T-cells, macrophages, and plasma cells) exhibit minimal or no SEPT9 expression, suggesting that SEPT9 is specifically expressed in tumour epithelial cells. Endothelial and fibroblast cells show low to moderate SEPT9 levels, implying a secondary role in angiogenesis and extracellular matrix remodelling. The strong association of SEPT9 with proliferative and tumor cells, combined with its limited expression in immune and stromal compartments, makes it a promising biomarker. This is particularly relevant for non-invasive detection methods, such as cfDNA methylation assays, and for targeted diagnostics, including immunohistochemistry staining of tumor biopsies.

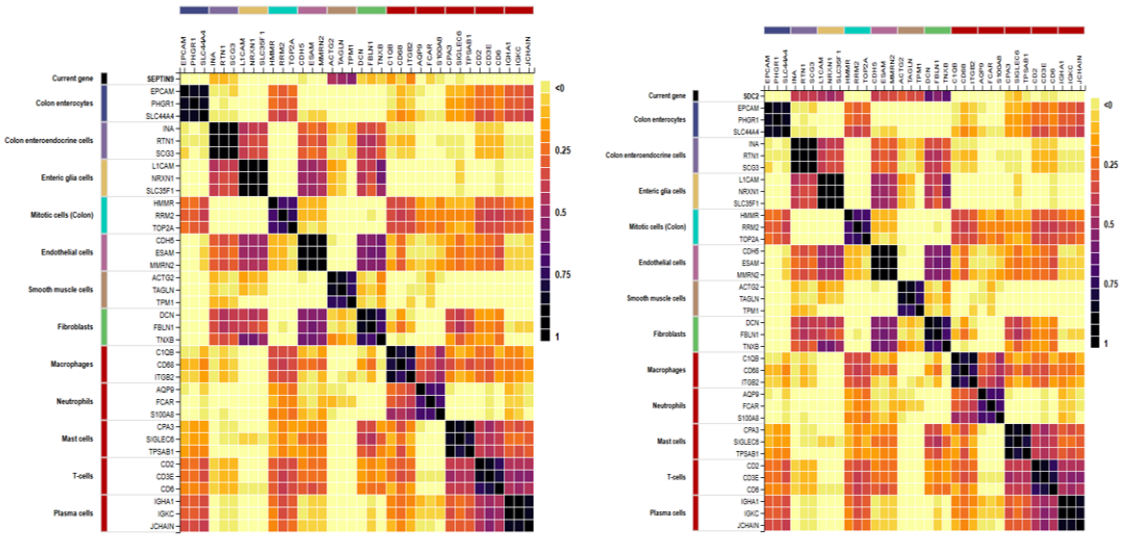

Figure 2a: Heatmap highlighting the cell-type-specific expression of SEPT9 as well as other canonical marker genes across colon and immune cell types. & Figure 2b: The cell-type-specific expression heatmap for SDC2 and canonical marker genes across colonic and immune cell populations.

Heatmap showing the relative level of expression (black = high, yellow = low) of SEPT9 and 40 marker genes that represent distinct colon tissue cell types. Expression of SEPT9 is highly enriched in mitotic epithelial cells, where it is co-expressed with genes involved in cell division (TOP2A, RRM2, HMMR) and is moderately expressed in colon enterocytes. There were levels of minimal

expression in immune cells (T-cells, macrophages, and plasma cells), consistent with the tumor-epithelial-specific nature of SEPT9. Cellular-level resolution confirmed SEPT9 as a mitosis-linked biomarker that can be diagnostically used as a prognostic marker in colon adenocarcinoma.

Figure 2b: The cell-type-specific expression heatmap for SDC2 and canonical marker genes across colonic and immune cell populations: The heatmap shows the normalized expression intensity (black = high, yellow = low) of SDC2 across the expressed colon resident cell types. SDC2 is highly expressed in colon enterocytes and enteroendocrine cells, as evidenced by its co-expression with EPCAM, PHGR1, and SCG3. In contrast, SDC2 is co-expressed in only slight amounts in mitotic, stromal, and immune cells (T-cells, macrophages, and fibroblasts), suggesting the epithelial specificity of SDC2. The observed expression distribution of SDC2 suggests it may become a sensitive and specific methylation biomarker for the epithelial-origin colon adenocarcinoma, as there was minimal signal potentially from off-target sites in the original cell type.

2b. Expression of SDC2 across colon cell types and immune cells: The y-axis (Figure 2b) displays different cell types in the colon, including epithelial cells, immune cells, and stromal cell types. The x-axis displays reference marker genes for each cell type, such as EPCAM for epithelial cells, ACTG2 for smooth muscle cells, and CD3E for T-cells. The color scale indicates expression levels, with black representing high expression and yellow indicating low expression. SDC2 exhibits strong expression (dark orange to black) in colon enterocytes and other epithelial lineage cells, co-expressed with markers such as EPCAM, SLC44A4, and PHGR1. It is also expressed in enteroendocrine cells, correlating with SCG3 and RTN1. This suggests SDC2 functions as a normal epithelial marker involved in cell-matrix interactions, cytoskeletal anchoring, and maintaining epithelial integrity. Unlike SEPT9, SDC2 is not enriched in mitotic cells and shows a low correlation with cell cycle genes such as TOP2A, HMMR, and RRM2, indicating that it is not actively involved in cell division but rather in maintaining mature cells. In the stromal and immune compartments, SDC2 expression is low or negligible, with fibroblasts, endothelial cells, T cells, macrophages, and plasma cells exhibiting minimal to no expression. Its epithelial-specific expression makes SDC2 a promising tumor biomarker. Its absence in immune and stromal cells reduces background noise in liquid biopsy assays, making plasma methylation targeting SDC2 useful for early detection of colon cancer, especially when combined with SEPT9.

3a. Differential expression of SEPT9 across COAD histological subtypes and SEPT9 promoter hypermethylation: Differential expression of SEPT9 was studied across COAD histological subtypes using TCGA RNA-seq datasets via UALCAN. The dataset contained normal colon tissues (n = 41), COAD tissues (n = 243), and mucinous adenocarcinoma tissues (n = 37). The data is represented in Figure 3a.

3a.1 SEPT9 Expression: Normal Colon vs. Colon Adenocarcinoma: The box plot data (figure 3a) showed significantly higher median SEPT9 expression in adenocarcinoma as compared to normal tissue (p-value = 1.339×10^{-3}). Therefore, it can be said that there is an oncogenic variation in SEPT9 in the case of COAD, likely acting as a driver of abnormal cell cycle division, cell proliferation, and chromosomal instability.

3a.2 SEPT9 Expression: Normal Colon vs. Colon Mucinous Adenocarcinoma: In the case of mucinous adenocarcinoma, there was no significant difference in SEPT9 expression when compared to that of normal colon tissue (p-value = 0.234). A low SEPT9 expression in mucinous subtypes indicates that SEPT9 expression is dependent on histological classification, and it may be expressed preferentially more so (the median SEPT9 expression is highest) in the non-mucinous conventional adenocarcinoma subtypes.

3a.3 SEPT9 Expression: Colon Adenocarcinoma vs. Colon Mucinous Adenocarcinoma: SEPT9 expression was higher in conventional adenocarcinomas as compared to mucinous subtypes (p-value = 0.0844). This may be due to the molecular differences between classical adenocarcinoma and mucinous tumors, which often exhibit distinct methylation landscapes and responses to treatment. The observable increase in SEPT9 expression in adenocarcinoma compared to normal samples provides a compelling argument for its use in diagnostics and early detection. However, lower levels of expression in mucinous subtypes suggest that complementary biomarkers may be needed when using multi-marker panels (such as SDC2, VIM) to enhance the ability to detect all variants of COAD.

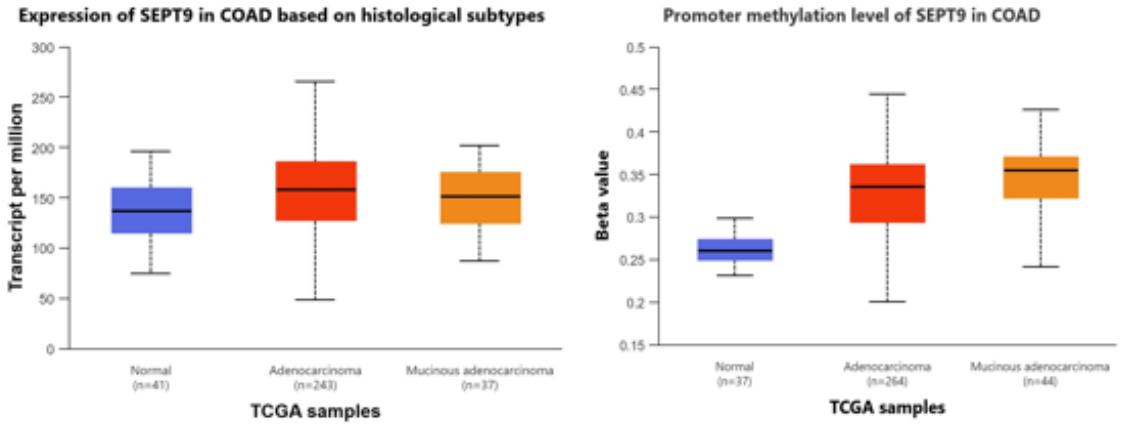

Figure 3a: Differential expression of SEPT9 in colon based on histological subtypes using TCGA data. & Figure 3b: SEPT9 promoter methylation of SEPT9 between normal colon, adenocarcinoma, and mucinous adenocarcinoma using data from TCGA.

Box plot of SEPT9 mRNA expression (transcripts per million, TPM) in normal colon tissues (n=41), COAD tissues (n=243), and mucinous adenocarcinoma tissues (n=37). There is significant upregulation of SEPT9 in conventional adenocarcinoma compared to normal tissue ($p = 1.34 \times 10-3$), indicating transcriptional activation as a result of transformation to a malignant phenotype. No significant differences were observed between normal and mucinous adenocarcinoma, suggesting potential subtype-specific expression. Data were extracted from RNA-seq profiles in the TCGA COAD cohort via UALCAN analysis.

Figure 3b: SEPT9 promoter methylation of SEPT9 between normal colon, adenocarcinoma, and mucinous adenocarcinoma using data from TCGA: The box plot we show represents beta values (0-1 scale), thus reflecting DNA methylation in the promoter region of SEPT9 in normal subtype (n=37), COAD tissues (n=264) and mucinous adenocarcinoma tissues (n=44). There is significant hypermethylation of SEPT9 in adenocarcinoma and mucinous subtypes of colon compared to normal ($p<1 \times 10^{-12}$), and a mild but statistically significant difference was observed between adenocarcinoma and mucinous adenocarcinoma subtypes (p=0.018). These differences in promoter methylation of SEPT9 signify a reliable and statistically substantial epigenetic alteration in COA. Data source: UALCAN, Using TCGA. The beta-value cut-off values were adapted from PMIDs 29027401, 23291739.

3b. SEPT9 Promoter Hypermethylation Is a Hallmark of COAD Subtypes: SEPT9 promoter hypermethylation patterns were studied in COAD subtypes, and box plot data are presented in Figure 3b. The dataset consisted of normal colon (n = 37), colon adenocarcinoma (n = 264), and mucinous adenocarcinoma (n = 44).

3b.1 SEPT9 Methylation Patterns: Normal Colon vs. Colon Adenocarcinoma: In the case of adenocarcinoma, higher median beta values and highly significant hypermethylation of the SEPT9 promoter ($p = 1.62 \times 10^{-12}$) were observed compared to normal colon tissue. Hypermethylation may affect transcriptional regulation or alternative splicing, leading to disease and carcinogenesis.

3b.2 SEPT9 Methylation Patterns: Normal Colon vs. Colon Mucinous Adenocarcinoma: The mucinous subtype showed significantly higher methylation than the normal group ($p = 1.62 \times 10^{-12}$), suggesting that hypermethylation of the SEPT9 promoter is a common trait of COAD.

3b.3 SEPT9 Methylation Patterns: Colon Adenocarcinoma vs. Colon Mucinous Adenocarcinoma: In the case of adenocarcinoma and mucinous adenocarcinoma, a slight difference in mean values was observed (p = 0.0188), which is statistically significant.

This suggests that the differences in SEPT9 methylation are relatively small, and both cancer subtypes exhibit increased methylation; specifically, the conventional adenocarcinomas have a slightly higher level of methylation.

Hypermethylation of the SEPT9 promoter is a signature epigenetic alteration in colon cancer. The consistency of this signature across subtypes suggests that SEPT9 can serve as a non-invasive plasma biomarker. It may represent early tumorigenic changes, appropriate for screening purposes (i.e., the Epi proColon® test). The combination of methylation and gene expression data can provide a more comprehensive understanding of the epigenetic regulation of COAD pathogenesis.

Expression of SDC2 in COAD based on histological subtypes and Promoter methylation levels

4a. Expression of SDC2 in COAD Based on Histological Subtypes: Like SEPT9, the expression of SDC2 was studied in histological subtypes of COAD. The data (Figure 4a) showed that all the subtypes (normal, adenocarcinoma, and mucinous adenocarcinoma) have similar median SDC2 expression (~14 to 15 TPM), but the spread (ranges) and variability (standard deviation) of expression are greater in mucinous adenocarcinoma.

4a.1 SDC2 Expression and Statistical Significance: In the case of the normal colon, a moderate expression of SDC2 was observed with a tighter range. Adenocarcinoma showed a slightly lower median compared to normal tissue, but the overall range is wider, with some outliers exhibiting very low expression. The highest SDC2 expression was observed in mucinous adenocarcinoma tissue, with overall variability suggesting heterogeneity in SDC2 expression within this subtype. Although there appears to be a slight visual difference, the medians are very similar, and overall, there does not seem to be any dramatic shift across the groups. This comparison provided no statistically significant differences, suggesting that SDC2 expression alone was insufficient for distinguishing between normal, adenocarcinoma, and mucinous adenocarcinoma subtypes.

4a.2 Biological Implication of SDC2 Expression and Statistical Significance: SDC2 is expressed in normal and cancerous colon, which emphasizes its endogenous role in maintaining epithelial integrity and signaling. The absence of significant differential expression suggests that epigenetic modification (i.e. methylation) and not just transcriptional upregulation may be more informative of its use as a biomarker. Therefore, methylation-specific detection of SDC2 offers improved diagnostic sensitivity compared to expression analysis alone.

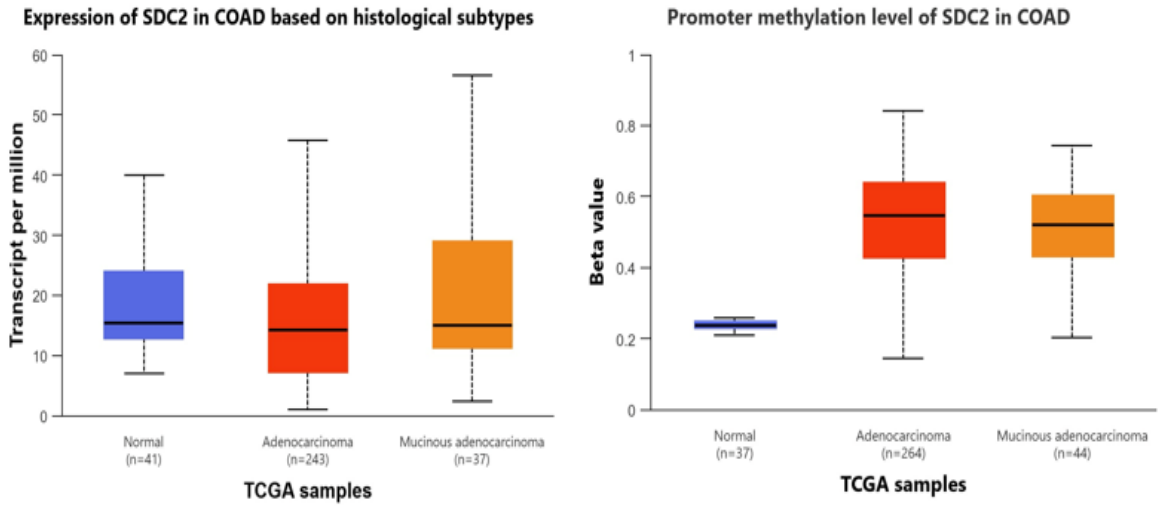

Figure 4a: SDC2 expression in COAD by histological subtypes & Figure 4b: Promoter methylation levels of SDC2 in COAD (Colon Adenocarcinoma) among the histological subtypes. & Figure 4b: Promoter methylation levels of SDC2 in COAD (Colon Adenocarcinoma) among the histological subtypes.

Box plot of SDC2 mRNA expression (transcripts per million, TPM) in three types, as compared to those from TCGA (The Cancer Genome Atlas) with UALCAN. Normal colon (n=41), COAD (n=243), and mucinous adenocarcinoma (n=37). The centre line in the box is the median expression, boxes represent interquartile range (IQR), and whiskers represent the range of the data without outliers; the whiskers show the expression central line in the column.

Figure 4b: Promoter methylation levels of SDC2 in COAD (Colon Adenocarcinoma) among the histological subtypes: Box plot displaying DNA methylation levels of the SDC2 gene promoter across normal colon (n=37), COAD (n=264), and mucinous adenocarcinoma (n=44). Methylation values are represented as beta values, which range from 0 (unmethylated) to 1 (methylated), and were extracted from TCGA through the UALCAN portal.

The middle line represents the median, the ends of the boxes indicate the interquartile range (IQR), and the "whiskers" illustrate the spread of the data.

4b. Promoter Methylation Levels of SDC2 in COAD Among the Histological Subtypes: Like SEPT9, promoter methylation levels of SDC2 in COAD were studied in histological subtypes of COAD. The data is represented in Figure 4b.

4b.1 Hypermethylation of SDC2 in Normal Tissue vs. Various COAD Subtypes: The data (Figure 4b) demonstrated very low promoter methylation (median β ≈ 0.25) in the normal subtype, which is consistent with a hypomethylated state. Adenocarcinoma and mucinous adenocarcinoma tissue exhibit strong promoter hypermethylation (median β ≈ 0.60–0.65) and are well within the hypermethylated boundary (0.50–0.70). All those differences are highly statistically significant ($p < 1 \times 10^{-12}$) but demonstrate an extremely strong epigenetic alteration in COAD. Both disease subtypes exhibit hypermethylation to some extent, although adenocarcinoma shows a slightly greater median value.

4b.2 Biological Implications of Promoter Hypermethylation of SDC2: Promoter hypermethylation of SDC2 is likely to lead to transcriptional repression, specifically during early-stage transformations. This point remains critically important, noting that although there is no appreciable degree of altered mRNA expression, it is likely that these epigenetic alterations lead to repression prior to and independent of mRNA reduction, which may impact potential applications as diagnostic biomarkers.

5. Protein-Protein Interaction (PPI) network of SEPT9 and SDC2

5a. Protein-Protein Interaction (PPI) network of SEPT9: The protein-protein interaction (PPI) network of SEPT9 and its functional partners was investigated using the STRING database to elucidate the interactions of SEPT9 within the septin family (Figure 5a).

5a.1 Pivotal Role of SEPT9: SEPT9 depicted a prominent centrality within the network, displaying multiple direct interactions with many members of the septin family (i.e., SEPT2, SEPT6, SEPT7, and SEPT11). This is consistent with the notion that SEPT9 plays a central role in septin complex assembly, which is linked to cellular processes such as cytokinesis, cell polarity, and membrane dynamics.

Interconnected Septin Family Cluster: The clustering observed among the three-dimensional representations of SEPT1–14 illustrated the conserved, multimeric structure of septins. They are filamentous proteins that modulate mitotic divisions to regulate the cytoskeleton. This also supports the concept that SEPT9 itself regulates tumour cell division and chromosomal stability.

Interactions Aside from Septins: ANLN (Anillin): Essential for cytokinesis and known to interact with actin and myosin, linking SEPT9 to the contractile ring at a time of cellular division; AURKA (Aurora Kinase A) and HIF1A: both are indicated in oncogenesis and pathways linking the cell cycle to hypoxia; this may suggest broad involvement of SEPT9 in tumor progression.

SH3KBP1, LMO7, and CDC42-related proteins provide evidence for SEPT9 in membrane trafficking, signaling pathways, and actin cytoskeleton remodeling mechanisms that are often deregulated in cancers.

5a.2 Biological Repercussions in COAD: The vast range of interactions of SEPT9, associated with cell division, signalling, spatial regulators, and structural components, supports the relevance of SEPT9 as a switch in tumorigenesis. The PPI data support the transcriptomic and methylation findings in this study, strengthening the claim that SEPT9 is a viable prognostic and diagnostic biomarker in colon adenocarcinoma.

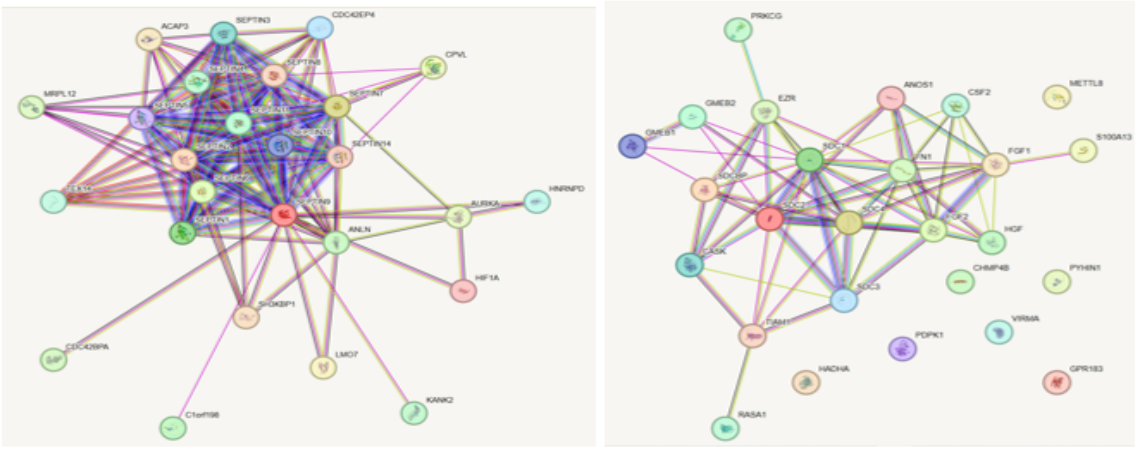

Figure 5a: Protein–Protein Interaction (PPI) Network of SEPT9 and Its Functional Partner & Figure 5b: Protein-Protein Interaction (PPI) Network of SDC2 and Its Functional Partners.

To create a high-confidence PPI network that shows interactions with the SEPT9 (Septin 9) protein (highlighted in red), the STRING database was used. The nodes represent proteins, while the edges

represent various types of evidence-based interactions from experimental evidence, co-localization, co-expression, curated databases, and text mining methods. The color of the lines represents: Green: gene neighbourhood, Blue: co-occurrence, Black: co-expression, Pink: experimentally determined, Yellow: text mining, Purple: protein homology, Sky blue: curated database evidence.

Figure 5b: Protein-Protein Interaction (PPI) Network of SDC2 and Its Functional Partners: This PPI network is generated using the STRING database and serves to illustrate the interactions of SDC2 (Syndecan-2) (red) with its known and predicted interacting proteins. The nodes represent proteins, and the edges represent evidence-based interactions that can take many forms, including experimental data, curated databases, co-expression, and beyond. The edge color codes are listed below: Pink = experimentally determined Green = gene neighbourhood, Blue = co-occurrence, Black = co-expression, Light blue = curated database, Yellow = text mining, Purple = protein homology.

5b. Protein-Protein Interaction (PPI) network of SDC2: Like SEPT9, the PPI network was studied for SDC2 and its known and predicted interacting proteins using the STRING database (Figure 5b).

5b.1 Essential Role of SDC2: SDC2 serves as a central point of integration, with multiple syndecan family components (i.e., SDC1, SDC3, and SDC4) and several critical signalling modulators in close proximity.

This denotes the key role of SDC2 in cell adhesion, migration, angiogenesis, and organization of extracellular matrix (ECM), as these are the major processes of tumor progression. SDC2 Interaction Partners FN1 (Fibronectin 1), HGF (Hepatocyte Growth Factor), FGF1/FGFG2 (Fibroblast Growth Factors): These relationships suggest involvement in ECM remodeling, growth factor signaling, and epithelial-to-mesenchymal transition (EMT) signaling events associated with metastasis. SDCBP (syntenin-1) and EZR (Ezrin): Syntenin-1 and ezrin are both associated with connecting the plasma membrane to the actin cytoskeleton, indicating their importance in cell shape and polarity. TIAM1, CASK: Scaffold proteins involved with signal transduction and possibly cytoskeleton organization. These are additional considerations for SDC2's role in invasive behaviour.

5b.2 Biological Importance to COAD: Dysregulation and cooperation and/or mutual isolation of the SDC2-interacting proteins will result in loss of epithelial integrity, creating opportunities for tumor invasion and migration. This interaction network indicates the potential downstream consequences of SDC2 promoter hypermethylation, which may represent a disconnect in signaling pathways or interaction with the tumor microenvironment. Because SDC2 is expressed in epithelial cells, this may make these interactions epithelial-tumor specific, presenting SDC2 as a clinically relevant epigenetic biomarker. The STRING network image of SDC2 supports its biological viability as a colon cancer biomarker by its vast interaction with pro-tumorigenic pathways, such as ECM modulation, growth factor signaling, and cytoskeletal dynamics. The findings suggest the potential value of solidifying SDC2 methylation status as an avenue for early detection and prognosis of COAD.

6. RNA expression overview of SEPT9 and SDC2

6a. RNA Expression Overview of SEPT9 across Multiple Cancer Types: RNA expression of SEPT9 was studied through TCGA in multiple types of cancer, and the data are presented in Figure 6a.

6a.1 General Trends: Variability in SEPT9 expression increases with the type of cancer, indicating a functional implication in a variety of tissues and malignancies. We lumped cancer types into categories (e.g., hematological, gastrointestinal, and genitourinary), and each box plot is the following:

The median expression (represented by a horizontal line),
The IQR (the range of the box),
And the whiskers and outliers that account for variability by patient samples.

6b.2 Importance of Colon Adenocarcinoma: In the "Colon adenocarcinoma (TCGA)" group, SEPT9 has a medium level of expression, with its TPM levels mostly falling in the middle range of all cancer types. This further supports our finding from Figure 3a, which shows that SEPT9 levels are significantly higher in the COAD tumor tissue compared to normal tissue. This lends credence to the functional role of SEPT9 in colon tumour biology, considering its role in regulating the cytoskeleton, mitosis, and cytokinesis, which are all processes disrupted during oncogenesis and thus render similarities in normal versus tumor biological processes necessary.

6a.3 Low Cancer-Specificity but Strong Diagnostic Utility: The headline "RNA cancer category: low specificity cancer" means that SEPT9 is expressed in many cancers, but so what? That does not reduce its utility.

In fact, the opposite is true: it emphasises the potential value of SEPT9 for use in liquid biopsy panels. SEPT9's promoter hypermethylation should be more tissue-specific (based on Figure 3b). While SEPT9 exhibits multiple cancer expressions, it can serve as a more tissue-specific diagnostic tool through methylation than through mRNA

expression. The figure confirms that SEPT9 is expressed in COAD and other tissues, which supports its biological significance.

However, when combined with COAD-specific hypermethylation, as illustrated in earlier figures, there is a high degree of specificity and sensitivity for SEPT9 detection in colon cancer. It reinforces the argument that SEPT9 expression and promoter methylation offer a powerful dual parameter for the early, non-invasive detection of COAD.

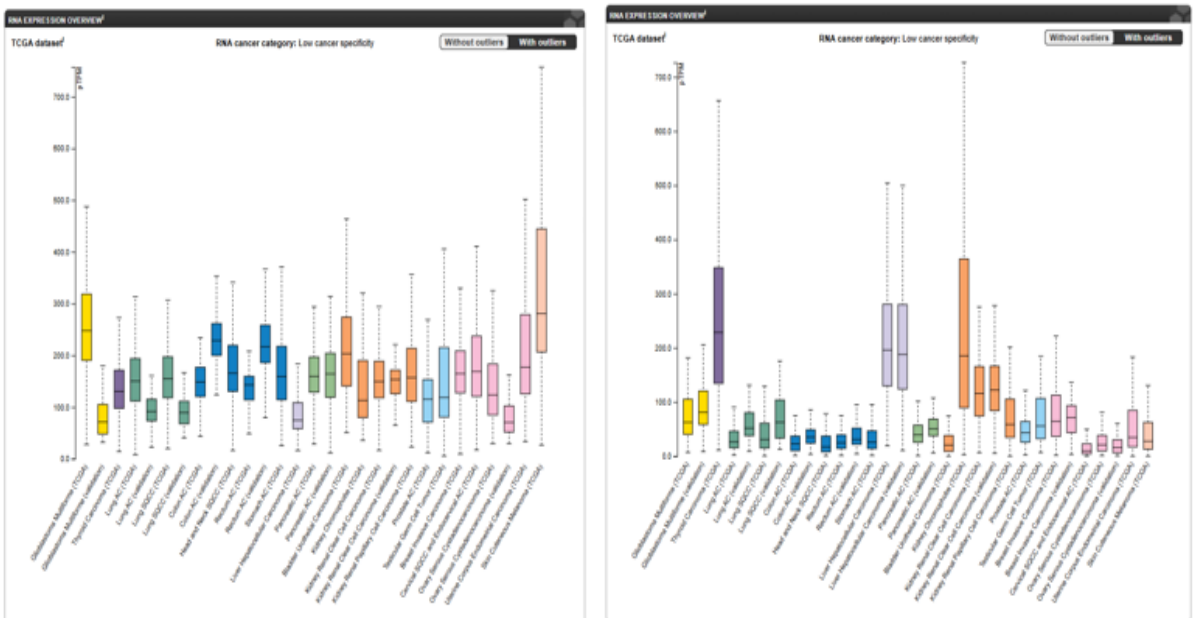

Figure 6a: A box plot of the RNA expression of the SEPT9 gene from transcriptomic data accessed through TCGA in multiple types of cancer. & Figure 6b: The box plot presents RNA expression levels of SDC2 across different tumor types using TCGA transcriptomic data.

The Y-axis represents the expression level in transcripts per million (TPM), and the X-axis displays various types of cancer. The colored box plots represent the median, the interquartile range, and any outliers for the gene of interest as gene expression levels. The results show that SEPT9 has low specificity for any one type of cancer, with several tumors exhibiting moderate to high expression levels, including colon adenocarcinoma. This indicates its biological importance and medical significance as a pan-cancer and COAD biomarker.

Figure 6b: The box plot presents RNA expression levels of SDC2 across different tumor types using TCGA transcriptomic data: The Y-axis represents transcripts per million (TPM), indicating the intensity of gene expression, while the different colors on the X-axis correspond to distinct tumor types. The vertical whiskers indicate a range of differences in the variability of the patient samples, including outliers. Overall, SDC2 exhibits low tissue specificity for expression, with moderate levels observed in COAD and higher levels in many other tumor types. Therefore, SDC2 should be considered a potential sensitive and specific biomarker with methylation status for colon cancer.

6b. RNA Expression Overview of SDC2 across Multiple Cancer Types: RNA expression of SDC2 was studied through TCGA in multiple types of cancer, and the data are presented in Figure 6b.

6b.1 Expression Pattern of SDC2: COAD, as indicated midway along the X-axis, shows moderate RNA expression of SDC2. This is consistent with the in-silico data from UALCAN and HPA, in which SDC2 was found to be present in the normal colon and often hypermethylated and transcriptionally silenced in COAD tumors.

6b.2 Low Cancer-Type Specificity: The heading "Low cancer specificity" indicates that SDC2 has normal expression across multiple cancer types but is increased in one. Importantly, expression alone does not imply diagnostic utility. It is the promoter methylation pattern, discussed in the prior figure (4b), that makes SDC2 highly specific to colon cancer in plasma-based methylation assays. SDC2 likely has low levels of transcription under non-tumor conditions in COAD but experiences promoter hypermethylation as tumorigenesis initiates, resulting in transcriptional silencing. This is why plasma DNA methylation (and not mRNA expression) is the most reliable detection locus, coinciding with the clinical use of ColoDefense and other methylation-based screening technologies. This figure illustrates that SDC2 exhibits some level of expression in multiple tissues, which is consistent with its involvement in important biological

processes related to adhesion, cytoskeletal organization, and extracellular matrix signaling. The importance of SDC2 as a biomarker lies in its methylation profile, which offers high selectivity and sensitivity for COAD. This further confirms the proposition that methylated SDC2 would be a reliable epigenetic biomarker for the early detection of COAD when combined with SEPT9, and ideally, in multi-gene panels of selected biomarkers.

7. Interactive survival scatter plot and survival analysis of SEPT9 and SDC2 in COAD: This outlines the relationship between gene expression levels and patient survival in COAD. For SDC2 (Fig. 7a), prolonged life is positively correlated with higher SDC2 expression; the peak expression in deceased patients was around 130 TPM, whereas in survivors, it was higher at approximately 150 TPM.

Density plots support this, indicating that low SDC2 expression correlates with shorter survival, and epigenetic suppression via promoter hypermethylation further emphasizes its role as a protective and prognostic biomarker. Combining expression and methylation data strengthens the potential for SDC2 in early detection and therapy stratification.

Similarly, SEPT9 shows (Fig. 7b) that decreased expression is associated with decreased survival outcomes. Deceased patients cluster on the left of the cut-off (23 TPM), while survivors are more dispersed, with some higher expression levels (24 TPM). Although overall expression is modest, slight variations appear significant, suggesting that residual SEPT9 expression may have a significant influence on prognosis. Its methylation status, linked to early carcinogenesis pathways, supports its use as both a diagnostic and prognostic marker. Together, SEPT9 and SDC2 could form a complementary biomarker panel for the early detection and prediction of survival in colon cancer.

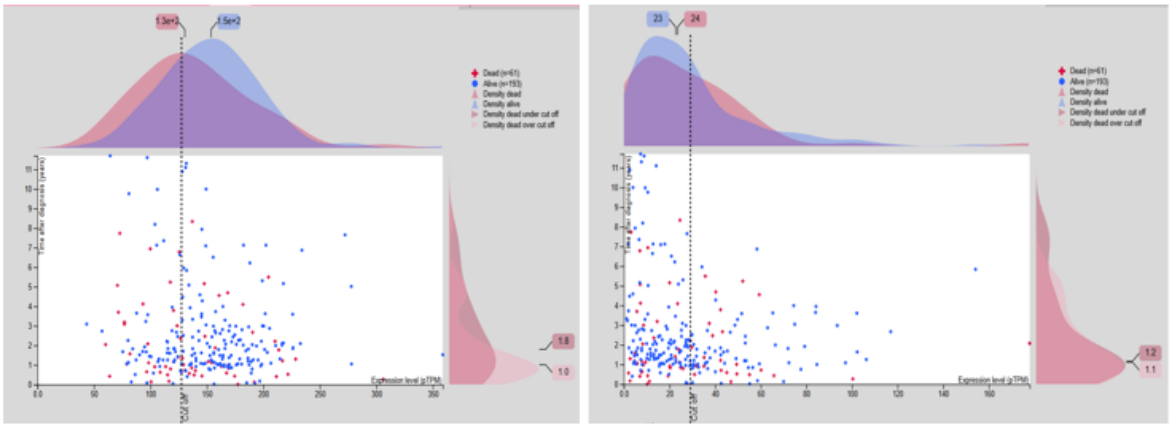

Figure 7a: Interactive Survival Scatter Plot of SDC2 expression in COAD (TCGA, cohort) & Figure 7b: Interactive Survival Scatter Plot of SEPT9 Expression in COAD (TCGA Cohort)

This scatter plot maps SDC2 expression (transcripts per million, TPM) against the overall survival time (OS, in years) for COAD patients, using data from TCGA.

Each point is a patient: Red '+ ' = deceased patients (n = 61), Blue dots = living patients (n = 193). SDC2 expression is presented on the x-axis, while survival time (in years, OS) after diagnosis is displayed on the y-axis.

The density plots at the top and to the right illustrate the distribution of SDC2 expression (x) and survival times (y) for both groups (alive and deceased). For interpretation purposes, a cut-off (dotted line) for low and high expression (≈130 TPM) is shown.

Figure 7b: Interactive Survival Scatter Plot of SEPT9 Expression in COAD (TCGA Cohort): This scatter plot illustrates the relationship between SEPT9 expression (in transcripts per million (TPM)) and years of overall survival in COAD patients from the TCGA dataset. Each point is an individual patient: Red '+' = dead (n = 61), Blue dots = alive (n = 193). The x-axis describes SEPT9 expression, and the y-axis details the survival time from the date of diagnosis. We also provided a cut-off (~24 TPM), which separates patients into low and high-expression groups. The density plots (above and to the right) visualize the distribution of expression and survival for the two groups.

8. Comparative overview of SEPT9 and SDC2 gene profiles across tissues, subcellular localizations, and cancer contexts: The observations (Figure 8) reveal that both SEPT9 and SDC2 are expressed in colon tissues, undergo promoter hypermethylation during colon adenocarcinoma, and serve as non-invasive biomarkers (detectable in the plasma of patients with methylation-specific assays). This emphasizes the molecular compatibility of SEPT9 and SDC2.

Both are involved in maintaining the organization of the cell and tumor evolution, and their epigenetic regulation (rather than expression) is behind the strength of their diagnostics.

Interestingly, SDC2 exhibits no detectable expression in blood; therefore, it may be a potential methylation-based biomarker in plasma.

They combine as a solid dual marker panel for early detection and non-invasive screening for colon adenocarcinoma.

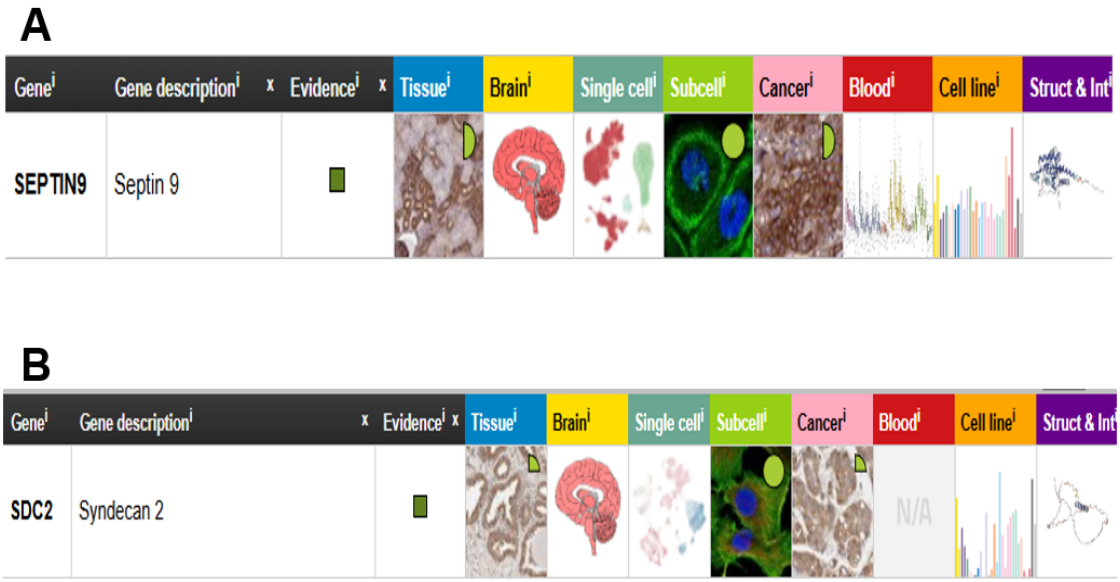

Figure 9: The integrated visualization of the Human Protein Atlas (HPA) presents the expression and localization of SEPT9 (Septin 9) and SDC2 (Syndecan-2) in some tissues, i.e., brain, scRNA-seq, subcellular localization, cancer, blood, cell lines, and molecular interactions.

Discussion

Colorectal cancer, specifically COAD, presents a significant global health issue, particularly in low- and middle-income nations such as India, where late diagnosis leads to elevated mortality rates.

Despite advancements in imaging and screening techniques, challenges such as invasiveness, cost, and limited sensitivity underscore the critical necessity for minimally invasive and highly specific molecular biomarkers aimed at early diagnosis, prognosis, and monitoring.

This research evaluated two epigenetically regulated genes, SEPT9 and SDC2, using a comprehensive in silico analysis. SEPT9, a member of the septin GTPase family involved in cytoskeletal organization and cell division, showed moderate expression in normal colon tissue. It was observed that there is an overexpression in non-mucinous COAD, with promoter hypermethylation leading to silencing in tumor tissues. A study of single cells revealed that SEPT9 expression was predominantly limited to dividing epithelial cells, making it less likely that immune or stromal cells would yield false positives. Furthermore, increased levels of SEPT9 were correlated with reduced survival rates, highlighting its importance in predicting outcomes. SDC2, a transmembrane proteoglycan implicated in cell-matrix interactions and Wnt signaling, exhibited moderate expression in normal colon epithelium. In tumor tissues, on the other hand, it was significantly hyper methylated and silenced, with very little expression in blood cells. This suggests that it is very specific for detecting epithelial tumors. The methylation status of SDC2 correlated with a diminished overall survival rate, indicating its potential as a diagnostic and prognostic biomarker. The distinct functions of SEPT9 and SDC2 in regulating cell division and epithelial matrix signaling indicate divergent tumor mechanisms. The outcome offers a multi-marker approach to improve early detection, prognosis, and treatment surveillance.

Conclusion

This study employed a wide range of in silico techniques to investigate the diagnostic and prognostic relevance of two genes, SEPT9 and SDC2, regulated by epigenetic mechanisms, in colon adenocarcinoma (COAD). The analysis of large data repositories commonly employed in the field (TCGA, UALCAN, GEPIA, and Human Protein Atlas) enabled the evaluation of SEPT9 and SDC2 regarding differential expression, promoter methylation, protein localization, and their correlation with survival outcomes.

Promoter hypermethylation was observed for both genes in COAD tissue compared to normal controls;

however, this was accompanied by aberrant expression and poor survival. Single-cell studies revealed that epithelial cell-specific expression was observed, with virtually no contribution from the immune system, making these epithelial cells ideal for use in plasma-based methylation studies.

These findings suggest that SEPT9 and SDC2 serve as additional epigenetic biomarkers for early diagnosis, prognosis, and potential applications in colon adenocarcinoma screening, particularly within the Indian population.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA a Cancer Journal for Clinicians [Internet]. 2024 Apr 4;74(3): 229–63. Available from: https://doi.org/10.3322/caac.21834
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA a Cancer Journal for Clinicians [Internet]. 2023 Jan 1;73(1):17–48. Available from: https://doi.org/10.3322/caac.21763
3. Pancione M, Remo A, Colantuoni V. Genetic and epigenetic events generate multiple pathways in colorectal cancer progression. Pathology Research International [Internet]. 2012 Jul 24;2012:1–11. Available from: https://doi.org/10.1155/2012/509348
4. Shen Y, Wang D, Yuan T, Fang H, Zhu C, Qin J, et al. Novel DNA methylation biomarkers in stool and blood for early detection of colorectal cancer and precancerous lesions. Clinical Epigenetics [Internet]. 2023 Feb 17;15(1):26. Available from: https://doi.org/10.1186/s13148-023-01443-7
5. Grobbee EJ, Wisse PH, Schreuders EH, Van Roon A, Van Dam L, Zauber AG, et al. Guaiac-based faecal occult blood tests versus faecal immunochemical tests for colorectal cancer screening in average-risk individuals. Cochrane Database of Systematic Reviews [Internet]. 2022 Jun 6;2022(6):CD009276. Available from: https://doi.org/10.1002/14651858.cd009276.pub2
6. Lopez N, Peterson C. Advances in biomarkers: going beyond the carcinoembryonic antigen. Clinics in Colon and Rectal Surgery [Internet]. 2016 Aug 17;29(03):196–204. Available from: https://doi.org/10.1055/s-0036-1584289
7. Adhit KK, Wanjari A, Menon S, K S. Liquid Biopsy: An Evolving paradigm for Non-invasive disease diagnosis and monitoring in Medicine. Cureus [Internet]. 2023 Dec 8;15(12):e50176. Available from: https://doi.org/10.7759/cureus.50176
8. Gao Q, Zeng Q, Wang Z, Li C, Xu Y, Cui P, et al. Circulating cell-free DNA for cancer early detection. The Innovation [Internet]. 2022 May 6;3(4):100259. Available from: https://doi.org/10.1016/j.xinn.2022.100259
9. Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harbor Perspectives in Biology [Internet]. 2016 May 18;8(9):a01 9505. Available from: https://doi.org/10.1101/cshperspect.a019505
10. Sun J, Zheng MY, Li YW, Zhang SW. Structure and function of Septin 9 and its role in human malignant tumors. World Journal of Gastrointestinal Oncology [Internet]. 2020 Jun 15;12(6):619–31. Available from: https://doi.org/10.4251/wjgo.v12.i6.619
11. Xie L, Jiang X, Li Q, Sun Z, Quan W, Duan Y, et al. Diagnostic value of methylated septin9 for colorectal cancer detection. Frontiers in Oncology [Internet]. 2018 Jul 2;8. Available from: https://doi.org/10.3389/fonc.2018.00247
12. Oh T, Kim N, Moon Y, Kim MS, Hoehn BD, Park CH, et al. Genome-Wide identification and validation of a novel methylation biomarker, SDC2, for Blood-Based detection of colorectal cancer. Journal of Molecular Diagnostics [Internet]. 2013 Jun 6;15(4):498–507. Available from: https://doi.org/10.1016/j.jmoldx.2013.03.004
13. Zhao G, Liu X, Liu Y, Li H, Ma Y, Li S, et al. Aberrant DNA methylation of SEPT9 and SDC2 in stool specimens as an integrated biomarker for colorectal cancer early detection. Frontiers in Genetics [Internet]. 2020 Jun 18;11:643. Available from: https://doi.org/10.3389/fgene.2020.00643
14. Muthamilselvan S, Raghavendran A, Palaniappan A. Stage-differentiated ensemble modeling of DNA methylation landscapes uncovers salient biomarkers and prognostic signatures in colorectal cancer progression. PLoS ONE [Internet]. 2022 Feb 24;17(2):e0249151. Available from: https://doi.org/10.1371/journal.pone.0249151
15. Zhang H, Sun X, Lu Y, Wu J, Feng J. DNA-methylated gene markers for colorectal cancer in TCGA database. Experimental and Therapeutic Medicine [Internet]. 2020 Feb 27;19(4):3042–50. Available from: https://doi.org/10.3892/etm.2020.8565
16. Network CGA. Comprehensive molecular characterization of human colon and rectal cancer. Nature [Internet]. 2012 Jul 17;487(7407):330–7. Available from: https://doi.org/10.1038/nature11252
17. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia [Internet].

2017 Jul 18;19(8):649–58. Available from: https://doi.org/10.1016/j.neo. 2017.05.002

18. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research [Internet]. 2017 Apr 5;45(W1):W98–102. Available from: https://doi.org/10.1093/nar/gkx247
19. Luo Y, Sun F, Peng X, Dong D, Ou W, Xie Y, et al. Integrated Bioinformatics analysis to identify abnormal methylated differentially expressed genes for predicting prognosis of human colon cancer. International Journal of General Medicine [Internet]. 2021 Aug 1; Volume 14:4745–56. Available from: https://doi.org/10.2147/ijgm.s324483