The Prognostic Biomarkers and Immunotherapeutic Targets of Septin in Colon Cancer

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Abstract

Background

Colon cancer has the third highest incidence and second highest death rate in the world. Colon cancer poses a huge burden to patients and society in China. More accurate and convenient early diagnosis of colon cancer is a challenge to solve this disease. Some studies have revealed the involvement of Septin family members in colon cancer, but the role of most Septin remains unclear.

Methods

GEPIA, UALCAN, The Human Protein ATLAS database, The Human Disease Methylation Database, MethSurv, SurvivalMeth, cBioPortal, STRING, GeneMANIA, DAVID, Metascape, PASTAA, LinkedOmics, and TIMER were used in our study.

Results

We found that the transcription level of SEPT1/4/5/6/7/10/11 was significantly decreased in colon cancer tissues. The expression level of SEPT2/5/6/7/8/9/10/11 protein was medium to high in colon cancer tissues, while the relative expression level of SEPT2 and SEPT9 was the highest in colon cancer tissues. SEPT7 expression was significantly correlated with pathological stage. For colon cancer patients, high SEPT2/9/10/11 expression was associated with longer OS, and only low SEPT4 expression was obviously associated with longer DSF. Moreover, the methylation level of SEPT2/5/7/8/9/11 was increased in colon cancer tissues, while that of SEPT3/4/6/10 was decreased. SEPT9/10/11 methylation levels were also found to decrease with the progression of colon cancer. 2 CpG SEPT1 2 CpG SEPT3, 5 CpG SEPT4, 3 CpG SEPT5, 8 CpG SEPT6, 3 CpG SEPT7, 5 CpG SEPT8, 20 CpG SEPT9, SEPT10 3 CpG 7 CpG SEPT11 was significantly associated with the prognosis of colon cancer patients. We found that SEPT7 had the lowest level of DNA methylation, while SEPT2 had the highest. For possible mechanisms, we performed interaction analysis, functional enrichment analysis in colon cancer, and identified the transcription factor targets and miRNA targets of septin in colon cancer. We also found a significant association between Septin and immune cell invasion. The results showed that SEPT4 and SEPT5 were significantly associated with clinical outcomes of colon cancer patients.

Conclusions

Septin could be used as a clinical prognostic biomarkers and immunotherapeutic targets for colon cancer.

Introduction

As a disease that affects millions of global people, colon cancer ranked third in incidence and second in mortality worldwide[1]. In China, colon cancer is the second most common cancer after lung cancer, placing a huge burden on patients and society[2, 3]. Given the high cost and unacceptability of

colonoscopy, as well as the general public's poor understanding of the physical examination, it is not surprising that most patients are diagnosed at an advanced stage[4, 5]. In addition, follow-up surgery and chemotherapy in these patients were unsatisfactory[6]. The 5-year survival rate for T1–T2N1a colon tumors was 73.7%, while for T4bN2b tumors was only 12.9%[7]. Both more accurate and convenient early diagnosis of colon cancer, and more effective treatment for it, are the formidable challenge to address this disease. Therefore, it is imperative to develop more early screening targets, therapeutic targets and prognostic biomarkers for colon cancer.

First discovered in Saccharomyces cerevisiae, Septin is an evolutionarily conserved gene family with GTPas activity, widely existing in all eukaryotes except land plants[8, 9]. Septin gene sequences often have high homology among different species, and Septin gene family members are numerous[10]. So far, thirteen Septin genes have been found in humans, named SEPT1 to SEPT12 and SEPT14[11]. Based on the sequences homology, these Setpins can be divided into four subfamilies: SEPT2 subfamily (SEPT1/2/4/5), SEPT3 subfamily (SEPT3/9/12), SEPT6 subfamily (SEPT6/8/10/11/14) and SEPT7 subfamily (SEPT7)[12]. Some studies have shown that Septin protein, as a cytoskeletal component, plays an important role in various physiological processes, participating in intracellular transport, cytokinesis and apoptosis signaling pathways[13, 14]. SEPT9 protein is encoded by the gene located on chromosome 17q25, whose expression is highly correlated with the occurrence and development of cancer[15, 16]. In addition, hypermethylation of CpG island in Septin9-V2 promoter region was detected in peripheral blood samples, which has the potential to be a biomarker for colon cancer[15, 17]. Due to the special structure of Septin gene family, the physiological functions of many members need to be further explored.

Some researches have revealed the expression and specific functions of certain Septin, however, the overall characteristics of most Septin as targets or biomarkers in colon cancer are not clear. A comprehensive analysis of Septin's roles in colon cancer is imminent. In this study, advanced bioinformatics methods were used to achieve this goal. The potential of Septin as clinical markers and therapeutic targets for colon cancer was analyzed based on several large bioinformatics databases. In the future, it is possible to achieve more accurate diagnosis of colon cancer and prognosis assessment of colon cancer patients.

Materials And Methods

GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) includes tumor and normal tissue RNA sequencing data from TCGA and GTEX databases, processed and visualized by R and Perl, mainly providing gene expression analysis, gene correlation analysis, survival analysis, similar gene prediction, dimension reduction analysis. In this study, we used GEPIA to explore the expression level of Septins, the correlation between Septins and the pathological stage of colon cancer, and DFS curve of patients with colon cancer.

UALCAN

UALCAN (http://ualcan.path.uab.edu/index.html) is an effective cancer data online analysis site, mainly based on the related cancer data from TCGA database. It can be used for biomarker identification, expression profile analysis, survival analysis. UALCAN can also be used to query related information in other databases through related links. In our study, we performed UALCAN to detect Septins expression and methylation levels in colon cancer and normal tissues.

The Human Protein ATLAS

The Human Protein ATLAS (HPA, https://www.proteinatlas.org), an online protein analysis site, is divided into three sections, Cell, Tissue and Pathology, showing the expression of proteins in cells, normal tissues and cancerous tissues, respectively. We used HPA to detect the protein expression level of Septins in colon cancer and evaluate their prognostic value.

The Human Disease Methylation Database

The Human Disease Methylation Database (http://bio-bigdata.hrbmu.edu.cn/diseasemeth/), DiseaseMETH Version 2.0, is a web based resource that focuses on abnormal methylomes in human diseases. The database has an ever-increasing volume of data from which more information can be addressed. In addition, the database provides a genome-scale landscape that displays information about human methylation in a scalable and flexible manner. The methylation levels of Septins in colon cancer and normal tissues were explored by HDMD.

MethSurv

MethSurv (https://biit.cs.ut.ee/methsurv/) is a web tool for survival analysis based on CpG methylation patterns. There are many methylation data for different human cancers. MethSurv is able to perform survival analysis of CpG located near or near the query genes. It also provides cluster analysis of the query genes to correlate methylation patterns with clinical features and screen out major biomarkers for each cancer type. In this study, MethSurv was used to evaluate the prognostic value of DNA methylation of Septins in colon cancer.

SurvivalMeth

SurvivalMeth (http://bio-bigdata.hrbmu.edu.cn/survivalmeth/) is a function of DNA methylation related elements affect the prognosis of the database. Prognostic analysis of functional components in the database is mainly divided into three types: genes, combinations of multiple genes or functional components, and enhancers. The prognostic analysis was performed in two steps: proportional risk regression model and survival analysis. In our study, we conducted a comprehensive prognostic analysis of Septins by SurvivalMeth.

cBioPortal

cBioPortal (https://www.cbioportal.org) currently stores DNA copy number data, mRNA and microRNA expression data, non-synonymous mutations, protein level and phosphoprotein level data, DNA methylation data and limited clinical data. It can quickly capture the molecular spectrum and clinical prognosis correlation of large-scale cancer genomics projects, and translate these data sets into visual data for clinical. We used cBioPortal to explore genetic alterations of Septins in colon cancer.

STRING

STRING (http://string.embl.de/) is a biophysical database of predicted protein interaction (PPI) information, containing more than 2,000 species, nearly 10 million proteins and a total of more than 1 billion interactions. Cytoscape, a biological visualization tool built by biomolecular interaction networks, was used to construct PPI networks. Molecular Complex detection (MCODE) algorithm was used to identify the key modules.

GeneMANIA

GeneMANIA (https://genemania.org) is used to generate hypotheses about gene function, analyze gene lists and prioritize genes for functional analysis. Given a list of genes to look up, GeneMANIA uses extensive genomic and proteomic data to find genes with similar functions. In this mode, it weights each functional genomic data set according to the predicted value of the query. Another use of GeneMANIA is for gene function prediction. Given a search gene, GeneMANIA finds genes that are likely to share a function with it, based on how the gene interacts with it.

The GO, DAVID and KEGG

The GO database (http://www.geneontology.org) is the necessary database annotation bioinformatics analysis of species, provide the function of the genome data classification. In general, it is divided into three distinct categories, including biological processes (BP), cellular components (CC) and molecular functions (MF). Kyoto encyclopedia gene and genome (KEGG, http://www.genome.ad.jp/KEGG/) is an integrated genome information database, chemical, and system function, system for gene function analysis, comments, and data visualization. Comments, visualization and comprehensive database (DAVID, http://david.abcc.ncifcrf.gov/) is a gene function classification of online tools, is the important basis of high-throughput genetic analysis. In this study, DAVID bioinformatics resources and Metascape were used for GO enrichment analysis and KEGG enrichment analysis.

PASTAA

PASTAA (http://trap.molgen.mpg.de/cgi-bin/home.cgi) is a new algorithm for detecting transcription factors (TFs) connected with functional categories by predicting the binding affinity of a TF to a promoter. It is mainly applicable to the determination of TF-driven tissue specific expression. In our study, we used PASTAA to predict the potential transcription factor targets of Septins.

LinkedOmics

LinkedOmics (http://linkedomics.org/login.php) is a public web that includes multiomics data from all 32 TCGA cancer types and 10 Clinical Proteomic Cancer Analysis Consortium (CPTAC) cancer coentees. The web application has three analysis modules: LinkFinder, LinkInterpreter, and LinkCompare. Analysis results can be visualized by scatter plots, box plots, or Kaplan-Meier plots. In this study, we explored miRNA targets strongly correlated with Septins by LinkedOmics.

TIMER

TIMER (https://cistrome.shinyapps.io/timer/) database is to use high-throughput sequencing (RNA)-Seq expression profile data analysis infiltration of immune cells in tumor tissue. It mainly provides the infiltration of B cells, CD4 + T cells, CD8 + T cells, Neutrphils, Macrophages and Dendritic cells. Through this software, the expression profile data of tumor samples in TCGA are analyzed, and the data of tumor infiltrating immune cells and gene expression, gene mutation, somatic copy number variation are correlated. We explored the correlation between Septins and immunity by TIMER.

Availability of data and materials

The datasets generated and analysed during the current study are available in the GSE69657 repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi

Results

Expression of SEPT1-SEPT11 in Colon Cancer

First, we intend to investigate the mRNA expression of SEPT1-SEPT11 in colon cancer and normal tissues. The expression levels of SEPT1-SEPT11 in colon cancer and normal tissues were detected by GEPIA. As shown in Fig. 1, we found that compared with normal tissues, the transcriptional levels of SEPT1/4/5/6/7/10/11 in colon cancer tissues were significantly decreased, while the transcriptional levels of the other four Septins were obviously increased. We also assessed the expression levels of SEPT1-SEPT11 in these tissues through UALCAN, which was presented in Fig. 2. Interestingly, SEPT7 expression was significantly elevated in colon cancer tissue compared with normal tissue, the only contrary to the GEPIA results. Protein expression of SEPT1-SEPT11 in various tumor was evaluated by The Human Protein ATLAS (HPA) database (Fig. 3). Combined with Fig. 3 and Supplementary Table 1, it can be found that SEPT2/5/6/7/8/9/10/11 proteins were expressed at medium to high levels in colon cancer. Moreover, after compared the relative expression levels of SEPT1-SEPT11 in colon cancer by GEPIA, we found that of SEPT2 and SEPT9 were the highest in colon cancer tissues (Fig. 4).

Then, the correlation between the expression of SEPT1-SEPT11 and the pathological stage of colon cancer patients was evaluated using GEPIA. As shown in Fig. 5, we found a significant correlation between the expression of SEPT7 (P = 0.00211) and pathological stage. Their expression levels increased markedly with the progression of colon cancer. These data suggested that SEPT1-SEPT11 played important roles in the development of colon cancer.

The Prognostic Value of SEPT1-SEPT11 in Colon Cancer Patients

Next, we explored the correlation between SEPT1-SEPT11 and colon cancer by HPA to assess the prognostic value of Septins in colon cancer. As shown by the overall survival curve (Fig. 6), for colon cancer patients, the high expression of SEPT2/9/10/11 indicated longer OS. Meanwhile, patients with high expression of SEPT5 had shorter OS. The data in Table 1 showed that the above correlation was statistically significant. Disease free survival (DFS) curve of colon cancer patients (Fig. 7) suggested that except SEPT2, colon cancer patients with the other 10 low Septins expression had longer DFS. Among them, only SEPT4 was significantly correlated with DSF.

Table 1
The relationship between the expression level of SEPT1-SEPT11 and prognosis in colon cancer patients.

Gene	The Human Protein ATLAS			GEPIA
	OS (Logrank P)	5-year survival high	5-year survival low	DFS (Logrank P)
SEPT1	0.15	60%	**65%**	0.55
SEPT2	**0.033**	**76%**	60%	0.40
SEPT3	**0.037**	57%	**70%**	0.58
SEPT4	**0.0011**	51%	**67%**	**0.017**
SEPT5	**0.00058**	49%	**71%**	0.076
SEPT6	0.24	61%	**64%**	0.44
SEPT7	0.087	55%	**68%**	0.42
SEPT8	0.24	**65%**	63%	0.96
SEPT9	**0.013**	**71%**	56%	0.82
SEPT10	**0.037**	**78%**	58%	0.73
SEPT11	**0.0082**	**71%**	57%	0.88

Methylated Expression of SEPT1-SEPT11 in Colon Cancer Patients

We detected methylation levels of SEPT1-SEPT11 in colon cancer by UALCAN, and the results were shown in Fig. 8. The methylation levels of SEPT2/5/7/8/9/11 were markedly elevated in colon cancer tissues compared with normal tissues, while that of SEPT3/4/6/10 was reduced (average beta value for "SEPT1" is not available for majority of samples in colon cancer), and the results of SEPT2/5/7/9/11 were statistically significant (Supplementary Table 2). Analysis of data from The Human Disease

Methylation Database showed the same results, with only SEPT3 having the opposite methylation level to UALCAN (Supplementary Fig. 1, Supplementary Table 2).

Furthermore, SEPT2-11 methylation levels were measured in different stages of colon cancer by UALCAN, as shown in Supplementary Fig. 2. These results, combined with supplementary Table 2, indicated that the methylation level of SEPT9/10/11 decreased with the progression of colon cancer. In addition, SEPT9 methylation levels were also associated with age stage in colon cancer patients. The older the patients, the lower the methylation level (Supplementary Fig. 3). The above results seem to contradict existing research.

Prognostic Value of Single CpG of SEPT1-SEPT11 Gene

We used MethSurv to evaluate the prognostic value of DNA methylation of SEPT1-SEPT11 in colon cancer, and the heat maps of that were shown in Fig. 9. It was found that cg08916477 of SEPT1, cg13009927 of SEPT2, cg14416575 of SEPT3, cg01806238 of SEPT4, cg13853046 of SEPT4, cg13477510 of SEPT5, cg00911962 of SEPT6, cg08119631 of SEPT6, cg17168428 of SEPT7, cg09527731 of SEPT8, cg05104283 of SEPT9, cg01405751 of SEPT9, cg12203543of SEPT9, cg04142643 of SEPT9, cg15267890 of SEPT9, cg06850467 of SEPT10, cg17864469 of SEPT11, cg14118160 of SEPT11, and cg01124275 of SEPT11 displayed the highest DNA methylation level. Table 2 shown that 2 CpG of SEPT1, 2 CpG of SEPT3, 5 CpG of SEPT4, 3 CpG of SEPT5, 8 CpG of SEPT6, 3 CpG of SEPT7, 5 CpG of SEPT8, 20 CpG of SEPT9, 3 CpG of SEPT10, and 7 CpG of SEPT11 were obviously associated with prognosis in colon cancer patients. The survival curves of these CpG of SEPT1-SEPT11 were presented in Supplementary Figs. 4 and 5.

Table 2
The Prognostic Value of Single CpG of Septins in CRC by MethSurv (P < 0.05).

Gene-CpG	HR	LR Test P-value
SEPT1-Body-Island-cg01081883	1.819	0.024
SEPT1-5'UTR-S_Shelf- cg19663795	1.873	0.019
SEPT3-TSS1500-Island-cg25756166	1.771	0.029
SEPT3-Body-S_Shore-cg19959055	1.847	0.017
SEPT4-Body;5'UTR;1stExon-N_Shelf-cg10575089	1.966	0.028
SEPT4-TSS200;Body-N_Shelf-cg15935247	1.706	0.047
SEPT4-Body;TSS1500-N_Shore-cg01287505	1.661	0.043
SEPT4-3'UTR;Body-S_Shelf-cg13105709	0.566	0.037
SEPT4-Body-Island-cg16178625	2.146	0.0087
SEPT5-Body-N_Shore-cg03432618	2.072	0.003
SEPT5-TSS1500-Island-cg19816290	1.942	0.0062
SEPT5-TSS1500-Island-cg19940065	1.784	0.045
SEPT6-Body-Island-cg04653083	0.576	0.023
SEPT6-Body-Island-cg15845873	0.593	0.033
SEPT6-5'UTR;1stExon-Island-cg06039729	0.513	0.0063
SEPT6-5'UTR;1stExon-Island-cg25553791	0.576	0.023
SEPT6-TSS200-Island-cg07681512	0.448	0.0014
SEPT6-TSS1500-Island-cg08848711	0.606	0.039
SEPT6-Body-N_Shore-cg07449205	1.741	0.023
SEPT6-Body-N_Shelf-cg08119631	1.791	0.017
SEPT7-Body-Island-cg14555127	1.737	0.025
SEPT7-3'UTR-Open_Sea-cg03937717	2.317	0.00095
SEPT7-Body-Open_Sea-cg16284178	2.219	0.0022
SEPT8-TSS200;5'UTR-Island-cg22036694	1.816	0.022
SEPT8-5'UTR;1stExon-Island-cg17025275	0.509	0.0069
SEPT8-Body;5'UTR-N_Shore-cg01422249	1.63	0.045
SEPT8-Body;5'UTR-Open_Sea-cg11865870	0.586	0.035

Gene-CpG	HR	LR Test P-value
SEPT8-Body-Open_Sea-cg09527731	1.66	0.036
SEPT9-TSS1500-N_Shore-cg01733438	1.639	0.047
SEPT9-Body-Open_Sea-cg00910521	1.811	0.014
SEPT9-Body-Open_Sea-cg05503916	1.704	0.035
SEPT9-Body-Open_Sea-cg09420510	2.826	0.001
SEPT9-Body;5'UTR;TSS1500-Open_Sea-cg01513063	1.894	0.019
SEPT9-5'UTR;TSS200;Body-Open_Sea-cg02633924	2.094	0.0057
SEPT9-5'UTR;TSS200;Body-Open_Sea-cg03568017	0.542	0.012
SEPT9-5'UTR;Body;1stExon-Open_Sea-cg15401418	0.592	0.041
SEPT9-5'UTR;Body-Open_Sea-cg11088489	2.174	0.0066
SEPT9-5'UTR;Body-Open_Sea-cg15661536	0.573	0.026
SEPT9-5'UTR;Body-Open_Sea-cg16722931	1.797	0.016
SEPT9-5'UTR;Body-Open_Sea-cg18271897	0.569	0.026
SEPT9-Body;5'UTR-Open_Sea-cg06791979	1.946	0.036
SEPT9-Body;5'UTR-Open_Sea-cg18278424	1.8	0.034
SEPT9-Body;5'UTR-Open_Sea-cg21808045	1.66	0.041
SEPT9-Body;5'UTR-Open_Sea-cg24136318	1.763	0.026
SEPT9-Body;5'UTR-Open_Sea-cg27627381	2.058	0.022
SEPT9-Body;5'UTR-Island-cg14517217	1.777	0.034
SEPT9-5'UTR;Body-S_Shelf-cg16686174	2.008	0.022
SEPT9-Body;TSS1500-N_Shelf-cg20557159	1.851	0.045
SEPT10-TSS200-Island-cg09588752	0.592	0.04
SEPT10-TSS200-Island-cg09588752	0.592	0.04
SEPT10-5'UTR;1stExon;TSS1500-Island-cg06850467	1.937	0.031
SEPT11-TSS200-Island-cg00531823	0.57	0.023
SEPT11-Body-Island-cg02026770	0.454	0.0013
SEPT11-Body-Open_Sea-cg01215215	1.855	0.011
SEPT11-Body-Open_Sea-cg14118160	2.305	0.0075

Gene-CpG	HR	LR Test P-value
SEPT11-Body-Open_Sea-cg17864469	1.849	0.017
SEPT11-Body-Open_Sea-cg21542842	0.501	0.013
SEPT11-Body-Open_Sea-cg27624381	2.205	0.01

Prognostic Value of the DNA Methylation of SEPT1-SEPT11

SurvivalMeth was then used for a comprehensive prognostic analysis of SEPT1-SEPT11 (Fig. 10A-C). It was shown in Fig. 10A that the significant expression patterns of SEPT2/8/9/10 between low-risk and high-risk groups. We found that DNA methylation level of SEPT7 was the lowest, while SEPT2 was the highest (Fig. 10B). However, in the survival curve shown in Fig. 10C, there was no statistically significant association between the low-risk and high-risk groups. Next, we performed prognostic analysis on SEPT1-SEPT11 respectively (Fig. 11). The results in Fig. 11 demonstrated that the low-risk group of SEPT1/3/4/6/9 had a longer survival time for colon cancer patients. While patients with colon cancer in the high-risk group of SEPT5/7/8 survived longer.

Molecular Characteristics Analyses of SEPT1-SEPT11 in Patients with Colon Cancer

We further analyzed the molecular characteristics of SEPT1-SEPT11 (Fig. 12A-D). First, the genetic alterations of SEPT1-SEPT11 were explored by cBioPortal. As shown in Fig. 12A, SEPT1/2/3/4/5/6/7/8/9/10/11 altered in 0%, 2.9%, 1.9%, 2.9%, 0%, 1.9%, 1%, 1%, 9%, 1%, and 0% of colon cancer samples, respectively. Among these alternations, missense mutation of mRNA expression of these Septins were common, as well as splice mutation and truncating mutation. And we analyzed the potential co-expression of SEPT1-SEPT11. It was found that the expressions of SEPT1, SEPT3, SEPT8, SEPT10, and SEPT11 were moderately to strongly correlated (Fig. 12B).

Then, STRING was used to perform the PPI network analysis of SEPT1-SEPT11. In Fig. 12C, there were 11 nodes and 55 edges in the PPI network (PPI enrichment p-value: 1.0E-16). The functions of SEPT1-SEPT11 were primarily related to septin cytoskeleton, cell cortex, cytokinesis, cell division, and sperm flagellum, which were conducted by GeneMANIA. Meanwhile, we found that CDC42EP4, ORC5, PCMTD2, SELENOF, CBR4, ZWILCH, H2AP, NRDC, CDC42EP2, PDS5B, ANLN, CEP72, HELB, MOCOS, SLC1A3, MIS18BP1, PRKN, SLC39A14, and SAP18 were closely related to SEPT1-SEPT11 in colon cancer (Fig. 12D).

Functional Enrichment Analysis of SEPT1-SEPT11

We used DAVID and Metascape to perform functional enrichment analysis of SEPT1-SEPT11 (Fig. 13A-C). The top GO biological process (BP) terms, cell component (CC) terms, and molecular function (MF) terms were shown according to the p-value. In the BP category, cell cycle, cell division, cytokinesis, protein heterooligomerization, and positive regulation of nonmotile primary cilium assembly were the top 5 most

enriched terms (Fig. 13A). In the CC category, Septin complex, midbody, cleavage furrow, stress fiber, and spindle were the top 5 most enriched terms (Fig. 13B). In the MF category, GTP binding, protein binding, GTPase activity, structural molecule activity, and cadherin binding involved in cell-cell adhesion were the top 5 most enriched terms (Fig. 13C). The most significantly enriched KEGG pathway was bacterial invasion of epithelial cells (Supplementary Fig. 6). In addition, Metascape was used to confirm the results of functional enrichment analysis (Supplementary Fig. 7–9). Details of the enriched term histogram of SEPT1-SEPT11 can be found in Supplementary Table 3.

Analysis of TFs and miRNA Targets of SEPT1-SEPT11

Then, we analyzed the potential downstream targets of SEPT1-SEPT11. The potential transcription factor targets of SEPT1-SEPT11 were predicted by PASTAA, and the most significant TFs were displayed in Table 3. We found that FosB, PITX2, PAX4a, MyoD, and CRX were the top 5 TFs worthy of our attention. LinkedOmics was used to explore miRNA targets strongly correlated with SEPT1-SEPT11. Supplementary Table 4 showed the miRNAs targets with the strongest positive and negative correlations for each Septin. It was demonstrated that only SEPT2 did not have negatively correlated miRNA target. Furthermore, the volcanic map of miRNAs targets of SEPT1-SEPT11 were shown in Supplementary Fig. 10.

Table 3
The transcription factors of SEPT1-SEPT11 in patients with colon cancer.

Rank	Matrix	Transcription Factor	Association Score	P-Value
1	AP1_01	Fosb, Fra-1	3.184	0.00337
2	LUN1_HAND	N/A	3.123	0.00387
3	LYF1_01	N/A	3.123	0.00387
4	NFKAPPAB50_01	N/A	2.876	0.00701
5	PITX2_Q2	Pitx2, Pitx2	2.822	0.00787
6	PAX4_02	Pax4a	2.646	0.0115
7	MYOD_01	Myod	2.587	0.0123
8	CRX_Q4	Crx, Rx	2.521	0.0152
9	IK2_01	Ik-2	2.521	0.0152
10	P300_01	P300	2.521	0.0152
11	GCNF_01	Gcnf, Gcnf-1	2.431	0.0165
12	BRACH_01	Brachyury	2.392	0.02
13	GATA1_01	Gata-1	2.342	0.0211
14	MAF_Q6_01	Bach1, Bach2	2.342	0.0211
15	AP4_Q6_01	Ap-4	2.282	0.0242
16	E2A_Q2	E12, E47	2.258	0.0245
17	STAT5B_01	Stat5a, Stat5b	2.164	0.0309
18	P53_DECAMER_Q2	Deltanp63alpha, P53	2.124	0.0346
19	MIF1_01	N/A	2.115	0.0356
20	MTF1_Q4	Mtf-1	2.07	0.0383
21	HNF4_Q6_02	Hnf-4, Hnf-4alpha	2.044	0.0383
22	SREBP_Q3	Srebp-1, Srebp-1a	1.977	0.0442
23	STAT3_01	Stat3	1.946	0.0492
24	VJUN_01	V-jun	1.943	0.0492

Immune Cell Infiltration of SEPT1-SEPT11 in Colon Cancer Patients

Finally, we intend to explore the correlation between SEPT1-SEPT11 and immunity. So the TIMER database was used to conduct a comprehensive analysis of the correlation between SEPT1-SEPT11 and immune cell infiltration (Fig. 14). As shown in Figs. 14, the expression of SEPT2/4/6/7/8/10/11 was positively correlated with infiltration of four immune cell types (CD8 + T cells, macrophages, neutrophils, and dendritic cells). Except for SEPT1/3/4, the expression of the other eight Septins was positively correlated with the infiltration of B cells, CD8 + T cells, and CD4 + T cells. Also, we compared the tumor infiltration levels among colon cancer with different somatic copy number alterations for SEPT1-SEPT11 (Supplementary Fig. 11). Additionally, the Cox proportional hazard model was applied for SEPT1-SEPT11 and six tumor-infiltrating immune cells in colon cancer. The results in Table 4 suggested that SEPT4 (P = 0.042) and SEPT5 (P = 0.006) were significantly related to the clinical outcome of patients with colon cancer.

Table 4
The Cox Proportional Hazard Model of SEPT1-SEPT11 and Six Tumor-Infiltrating Immune Cells in colon cancer (TIMER).

	Coef	HR	95% CI_l	95% CI_u	P-value	Sig
B_cell	1.517	4.557	0.043	482.713	0.524	
CD8_T cell	-3.146	0.043	0.001	3.159	0.151	
CD4_T cell	0.502	1.652	0.006	422.082	0.859	
Macrophage	1.478	4.383	0.035	551.333	0.549	
Neutrophil	-1.807	0.164	0.000	262.722	0.631	
DC	1.701	5.477	0.238	126.057	0.288	
SEPT1	0.053	1.054	0.802	1.385	0.705	
SEPT2	-0.195	0.823	0.514	1.316	0.415	
SEPT3	0.229	1.258	0.979	1.616	0.072	
SEPT4	0.302	1.353	1.011	1.809	**0.042**	*
SEPT5	0.418	1.518	1.126	2.407	**0.006**	**
SEPT6	0.173	1.189	0.917	1.544	0.192	
SEPT7	0.145	1.156	0.856	1.563	0.344	
SEPT8	0.007	1.007	0.644	1.574	0.977	
SEPT9	-0.064	0.938	0.623	1.414	0.761	
SEPT10	-0.004	0.996	0.700	1.417	0.981	
SEPT11	-0.094	0.910	0.662	1.252	0.563	

Discussion

Since the Septin family of highly conserved proteins was discovered in budding yeast 40 years ago, homologous proteins have been continuously identified in nearly every eukaryotic lineage except higher plants[18, 19]. The number of Septin isoforms varies from species to species, with two in Caenorhabditis elegans, five in drosophila, and thirteen in humans, respectively, SEPT1-12 and SEPT14[20]. Septin gene is a kind of GTP-binding protein with molecular weight between 30-65kDa, and its GTP-binding domain is very similar to that of Ras protein[12, 21]. The N-terminal sequence of Septin is rich in proline, and the C-terminal is a coiled spiral structure[21, 22]. A single Septin protein monomer can be polymerized into heterologous complexes called septum filaments, which are further assembled to form advanced complex structures such as fiber bundles and participate in the physiological process of cytokinesis[23, 24]. As a non-traditional cytoskeletal component, Septin protein can construct cellular scaffold or diffusion barrier on cell membrane, affecting diverse physiological functions[25]. Septin is involved in the formation of cilia and sperm flagellum structures, neuronal development, and plays an important role in the interaction of pathogenic bacteria invading the body[26–28]. For example, Septin1, which locates to spindle poles in mitosis, was reported to participate in spindle assembly and chromosome congression[29]. Zhang et al. found that Septin4 was a novel PARP1 interaction protein essential in oxidative stress induced vascular endothelial cell injury[30].

In this study, we first explored the expression of SEPT1-11 in colon cancer, and found that compared with normal tissues, the expression of SEPT2/3/7/8/9 was increased while the expression of SEPT1/6 was decreased. Evaluation of the relationship between Septin and pathological stage of colon cancer patients showed that the expression of SEPT7 declined significantly with the progression of colon cancer. Then, we assessed the prognostic value of Septin in colon cancer. It was found that high expression of SEPT2/9/10/11 while low expression of SEPT5 were associated with better OS.

As for the methylation status of SEPT1-11, the results demonstrated that the methylation level of SEPT2/5/7/9/11 was markedly elevated in colon cancer. We also found that the methylation level of SEPT9/10/11 decreased gradually with the development of colon cancer. And SEPT9's methylation expression decreased with the increase of patients' age. These results seem to contradict existing research and deserve further study. Exploring the prognostic value of the DNA methylation of Septin, we found that 2 CpG of SEPT1, 2 CpG of SEPT3, 5 CpG of SEPT4, 3 CpG of SEPT5, 8 CpG of SEPT6, 3 CpG of SEPT7, 5 CpG of SEPT8, 20 CpG of SEPT9, 3 CpG of SEPT10, and 7 CpG of SEPT11 were significantly associated with prognosis of colon cancer patients. In addition, the results presented that patients with colon cancer in the low-risk group of SEPT1/3/4/6/9 or in the high-risk group of SEPT5/7/8 survived longer.

Next, the molecular characteristics of SEPT1-11 in colon cancer were the focus of our investigation. In the exploration of genetic alteration, SEPT1-11 was found to have 0%, 2.9%, 1.9%, 2.9%, 0%, 1.9%, 1%, 1%, 9%, 1%, and 0% alterations in colon cancer samples, respectively. For the correlation analysis of Septin, we found that the expression of SEPT1, SEPT3, SEPT8, SEPT10 and SEPT11 were moderately to strongly

correlated, indicating that they may function synergistically in the occurrence and development of colon cancer.

Then, the function of Septin was performed with GO and KEGG pathway enrichment analyses. Not surprisingly, the functions of SEPT1-11 genes were mainly related to cell cycle, cell division, cytokinesis, GTPas activity, and bacterial invasion of epithelial cells. We also analyzed the downstream targets of SEPT1-11 in depth. Potential key transcription factors (such as FosB, PITX2, PAX4a) and miRNA targets of Septin were identified. Furthermore, we assessed the correlation between SEPT1-11 and immune cell infiltration in colon cancer. The results showed that SEPT2/6/7/9/10/11 was significantly correlated with B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils and dendritic cells. The Cox proportional hazard model for SEPT1-11 and tumor-infiltrating immune cells showed that SEPT4 and SEPT5 were significantly correlated with the clinical outcome of colon cancer patients. The above results suggested that Septin may participate in the progression of colon cancer by influencing immune status.

More and more evidences show that Septin plays an indispensable role in the growing tumors. For example, the down-regulated miR-744-5P in colon cancer lost its inhibitory effect on SEPT2, which functions as an oncogene and promotes the proliferation of colon cancer cells[31]. Notch target gene SEPT4 is involved in the Notch signaling pathway, and inactivation of this pathway has been confirmed to be associated with the development of cancers[32]. Recent studies revealed that SEPT6 was the upstream gene of UBC in prostate cancer and inhibited tumor growth by regulating UBC[33]. Other evidences showed that SEPT6 was also participated in the pathogenesis of breast cancer and hepatocellular carcinoma, which suggested that targeting SEPT6 may be a new therapeutic strategy for cancers[34, 35]. Overexpression of SEPT7 plays a crux role in inhibiting the growth and migration of glioma cells[36, 37]. And there was reported that SEPT7 and SEPT2 could synergically promote the proliferation, migration and invasion of breast cancer cells[38]. SEPT9, the most well-known member of the Septin family, has been studied for many years, with much emphasis on methylation and cancer biomarkers[39–41]. SEPT9 methylation analysis has great potential and usefulness as a non-invasive marker for early colon cancer, and might be used in combination with other conventional markers to detect disease recurrence[42, 43]. Other functions of SEPT9 are also worth discussing and exploring, which is what we did in our study.

The data in this study came from multiple online bioinformatics databases, it is understandable that there are biases or conflicts in the analysis results. These contradictions instead become the focus of our study. Next, we plan to verify the analysis results in vitro and in vivo, and further explore the specific mechanism of the Septin family in the occurrence and development of colon cancer.

In conclusion, our research provides valuable information on the role of septin family members as biomarkers, clinical therapeutic targets, and immune targets for colon cancer. It is hoped that these results will help in early screening and clinical treatment of colon cancer, and contribute to the development of novel targets or drugs.

Conclusions

We found that methylation of Septin was closely associated with colon cancer. Septin have great potential for clinical value, and may function as prognostic biomarkers and immunotherapy target in colon cancer.

Abbreviations

SEPT: Septin

GEPIA: Gene Expression Profiling Interactive Analysis

HPA: The Human Protein ATLAS

PPI: protein-protein interaction

DAVID: Databases for annotation, visualization, and integrated discovery

GO: gene ontology

BP: biological processes

CC: cellular components

MF: molecular functions

KEGG: Kyoto Encyclopedia of Genes

TF: transcription factors

TIMER: Tumor Immune Estimation Resource

Declarations

Acknowledgments

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Contributions

ZZ, CY, and YZ designed the study and analyzed the data; HY, JY drafted the paper; HZ, YB reviewed and revised the paper. All authors read and approved the final manuscript.

Corresponding author

Correspondence to Haiyang Zhang and Yi Ba.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All participants were informed and gave written consent.

Competing interests

The authors declare that they have no competing interests.

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Figures

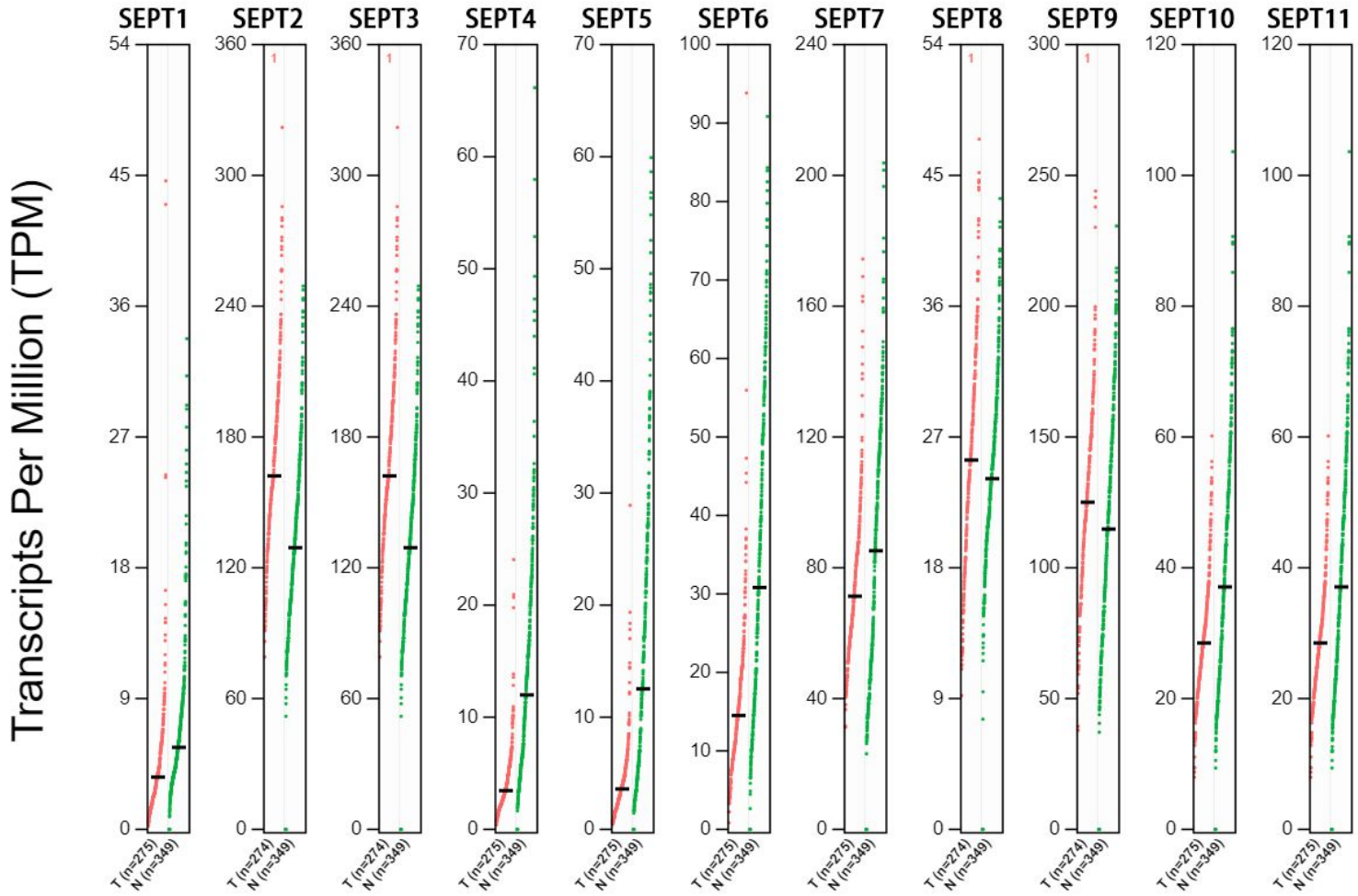

Figure 1

The mRNA levels of SEPT1-SEPT11 in colon cancer and nornal tissues (GEPIA).

Compared that in normal tissue, the transcription levels of SEPT2/3/8/9 in colon cancer tissues were significantly elevated, while the transcription levels of SEPT1/4/5/6/7/10/11 were significantly reduced. T for colon cancer tissue (red), N for normal tissue (green).

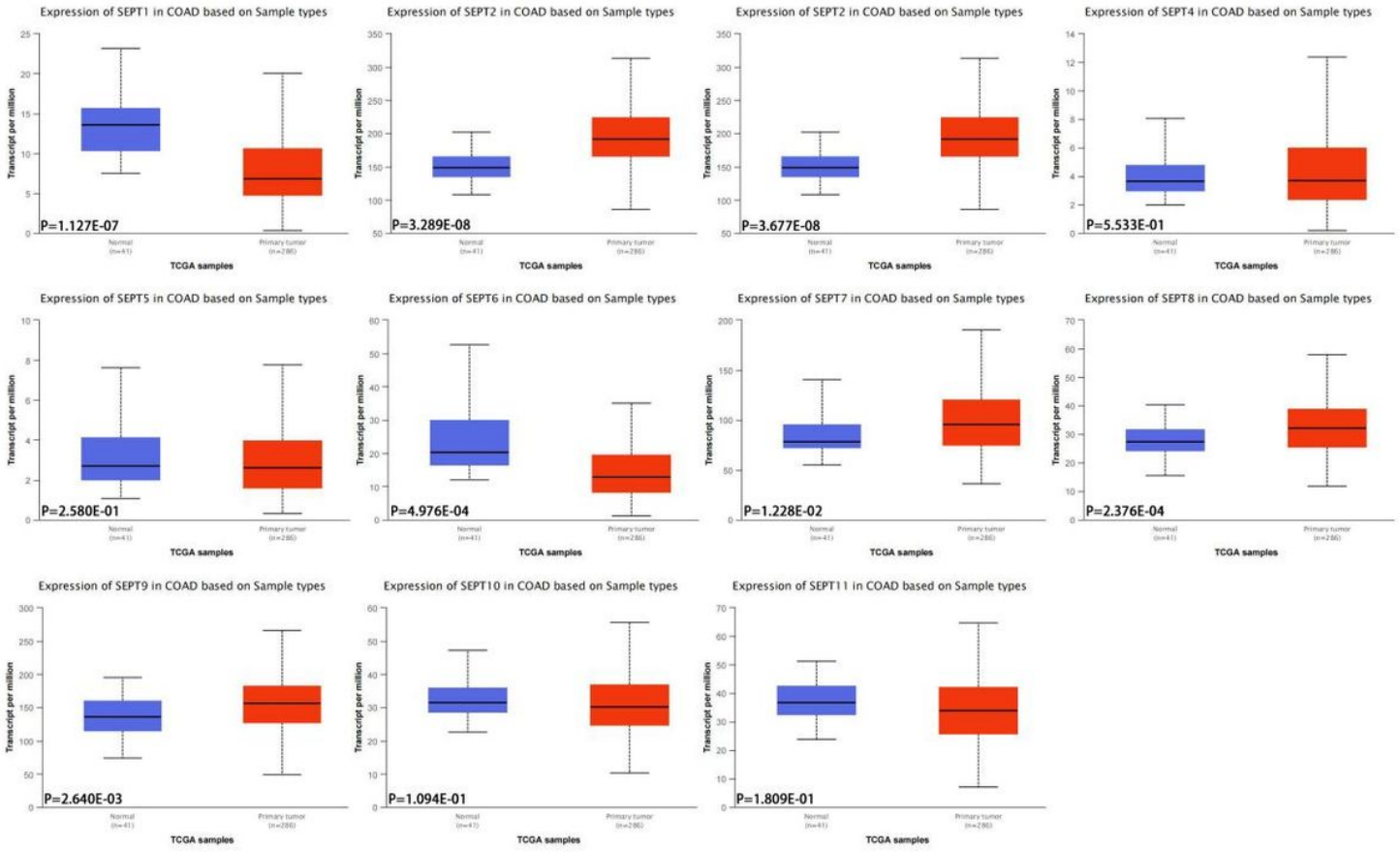

Figure 2

The transcription levels of SEPT1-SEPT11 in colon cancer (UALCAN).

Compared with normal tissue, the transcriptional levels of SEPT1 and SEPT6 in colon cancer tissues were significantly decreased, while the transcriptional levels of SEPT2/3/7/8/9 were significantly increased in primary tumor (P □ 0.05).

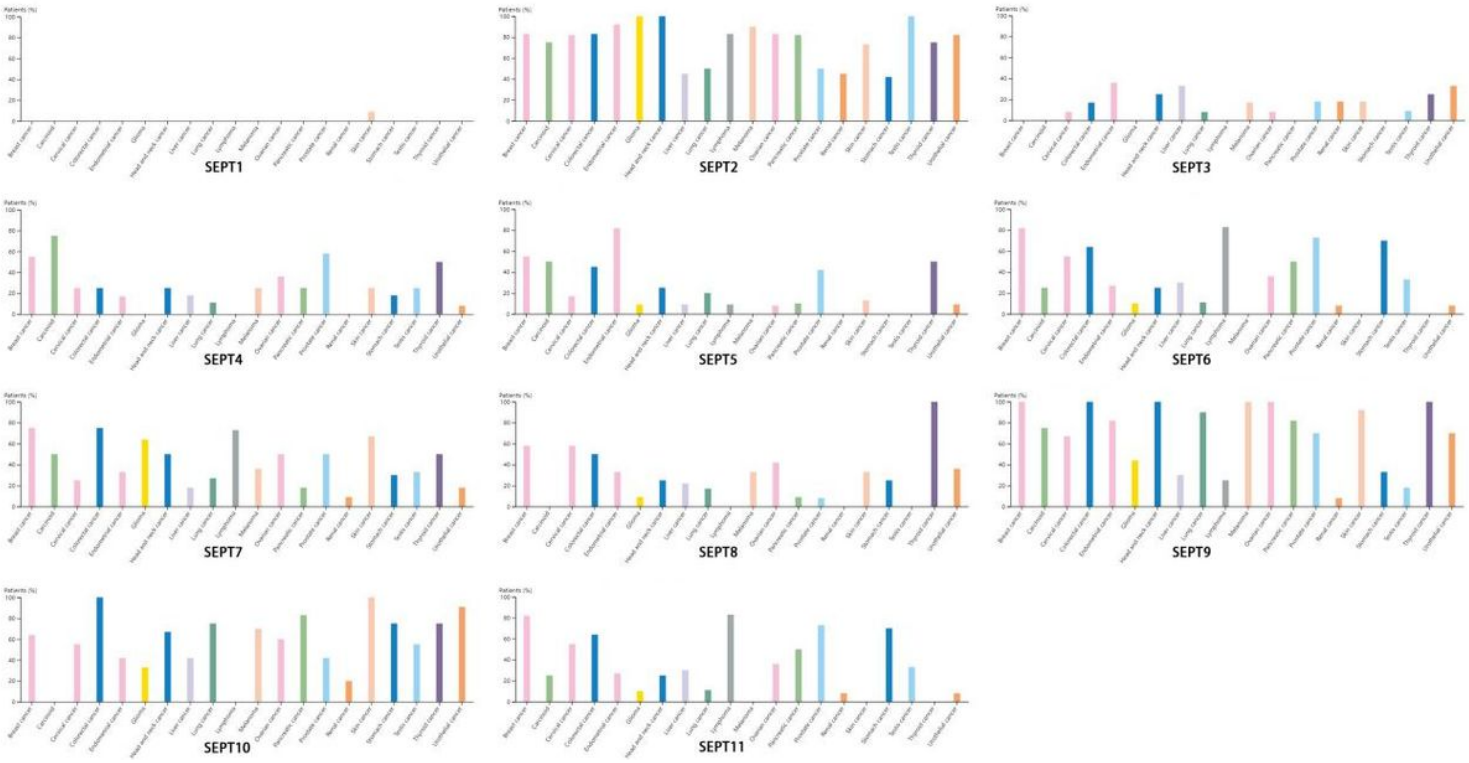

Figure 3

Protein expression of SEPT1-SEPT11 in various tumors (The Human Protein ATLAS).

SEPT2/5/6/7/8/9/10/11 proteins were expressed at medium to high levels in colon cancer.

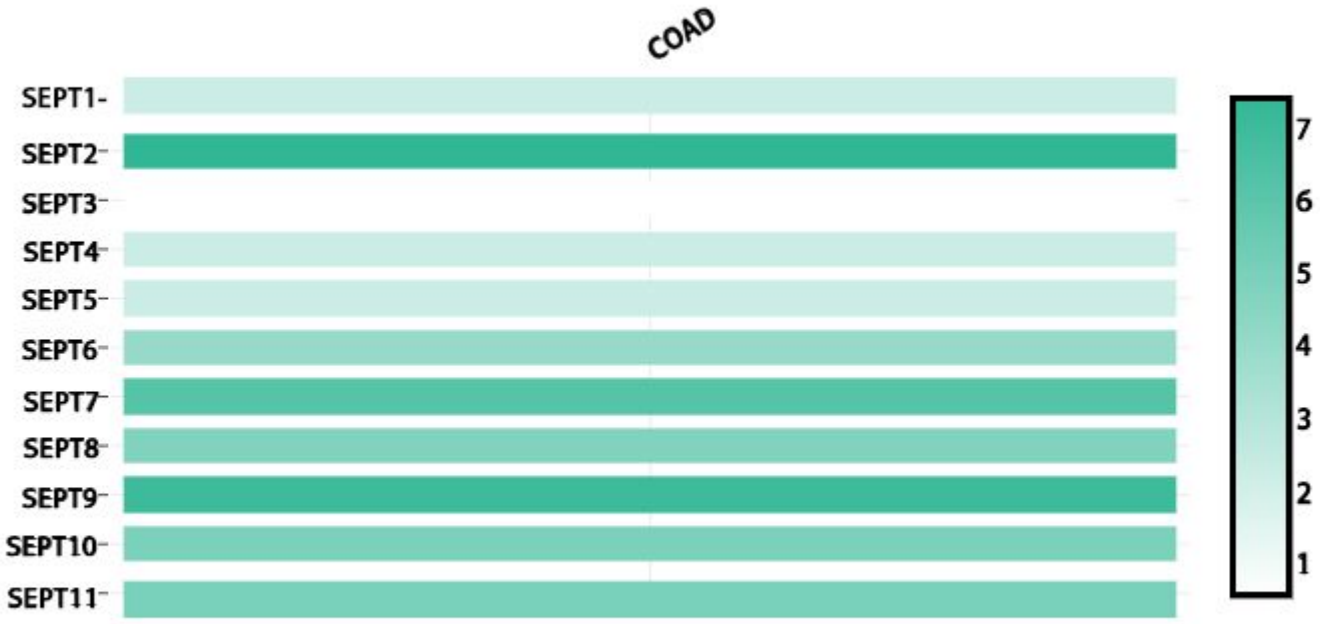

Figure 4

The relative expression level of SEPT1-SEPT11 in colon cancer patients (GEPIA).

The darker the bar, the higher the relative expression. SEPT2 and SEPT9 showed the highest expression in colon cancer among SEPT1-SEPT11.

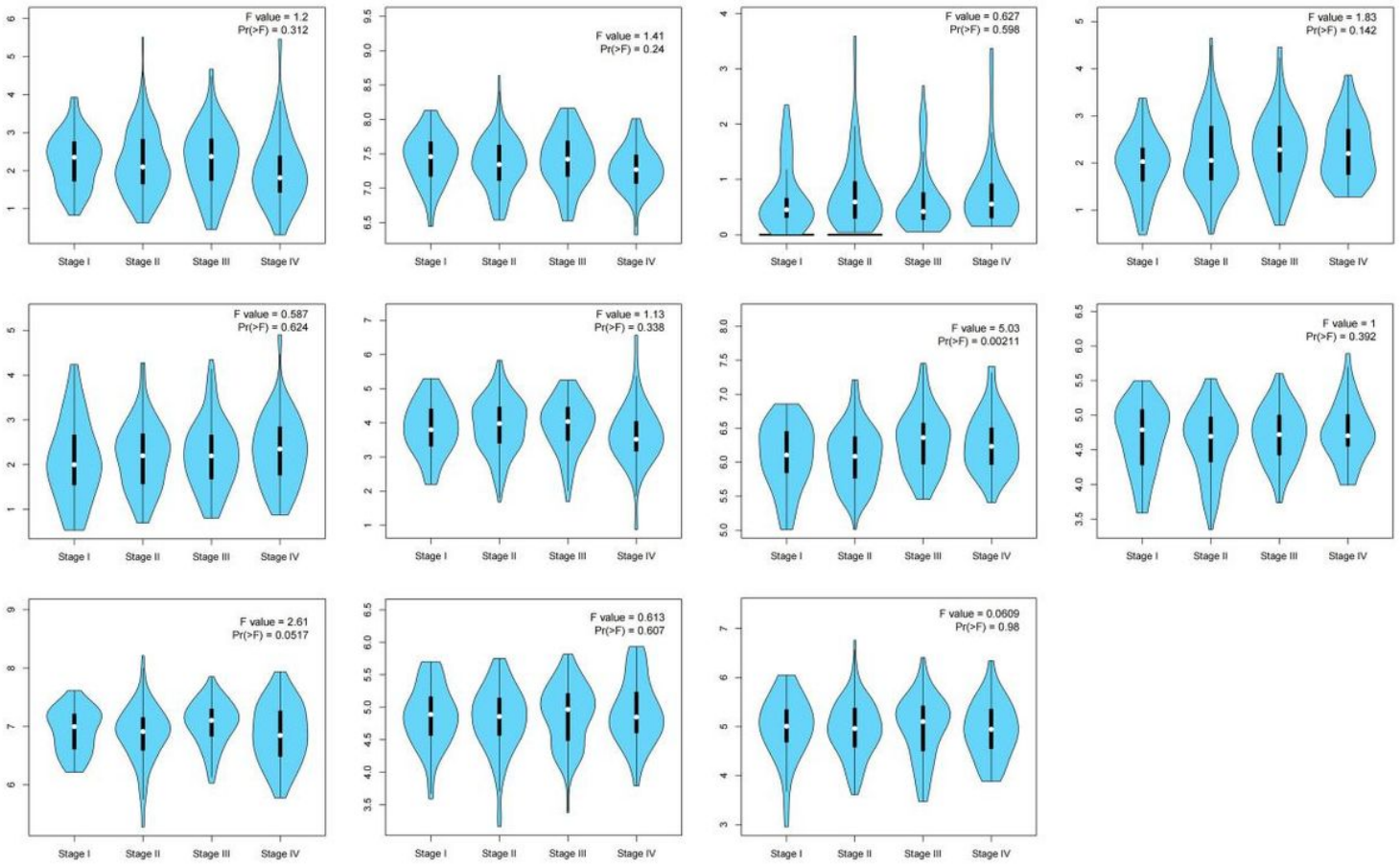

Figure 5

Correlation between SEPT1-SEPT11 and the pathological stage of patients with colon cancer (GEPIA).

SEPT7 expression level was significantly correlated with pathological stage of patients with colon cancer.

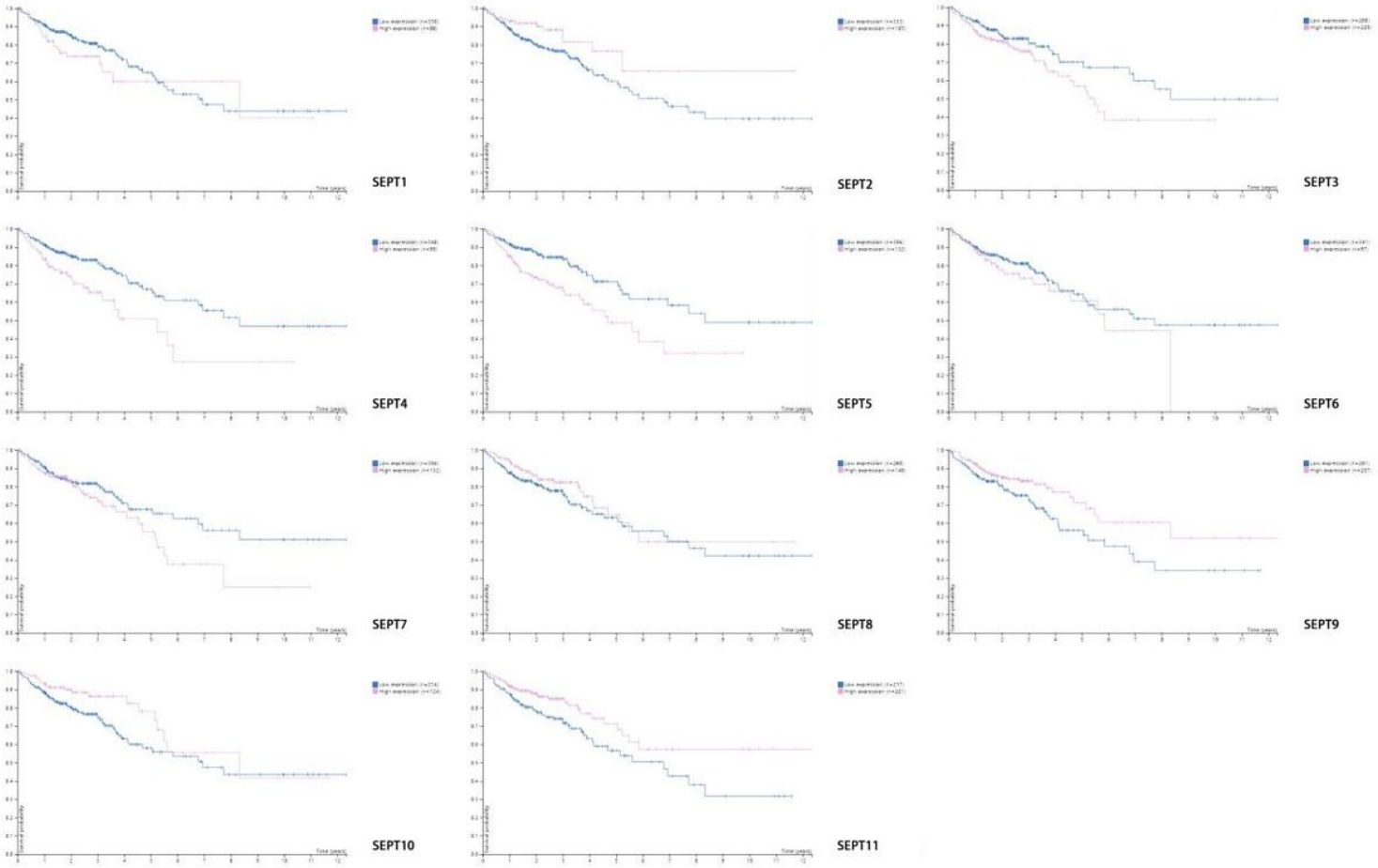

Figure 6

Overall survival curves for the expression of SEPT1-SEPT11 in patients with colon cancer (The Human Protein ATLAS).

Low expression level of SEPT1/3/4/5/6/7 indicated longer OS, while high expression level of SEPT2/8/9/10/11 suggested longer OS in colon cancer cases.

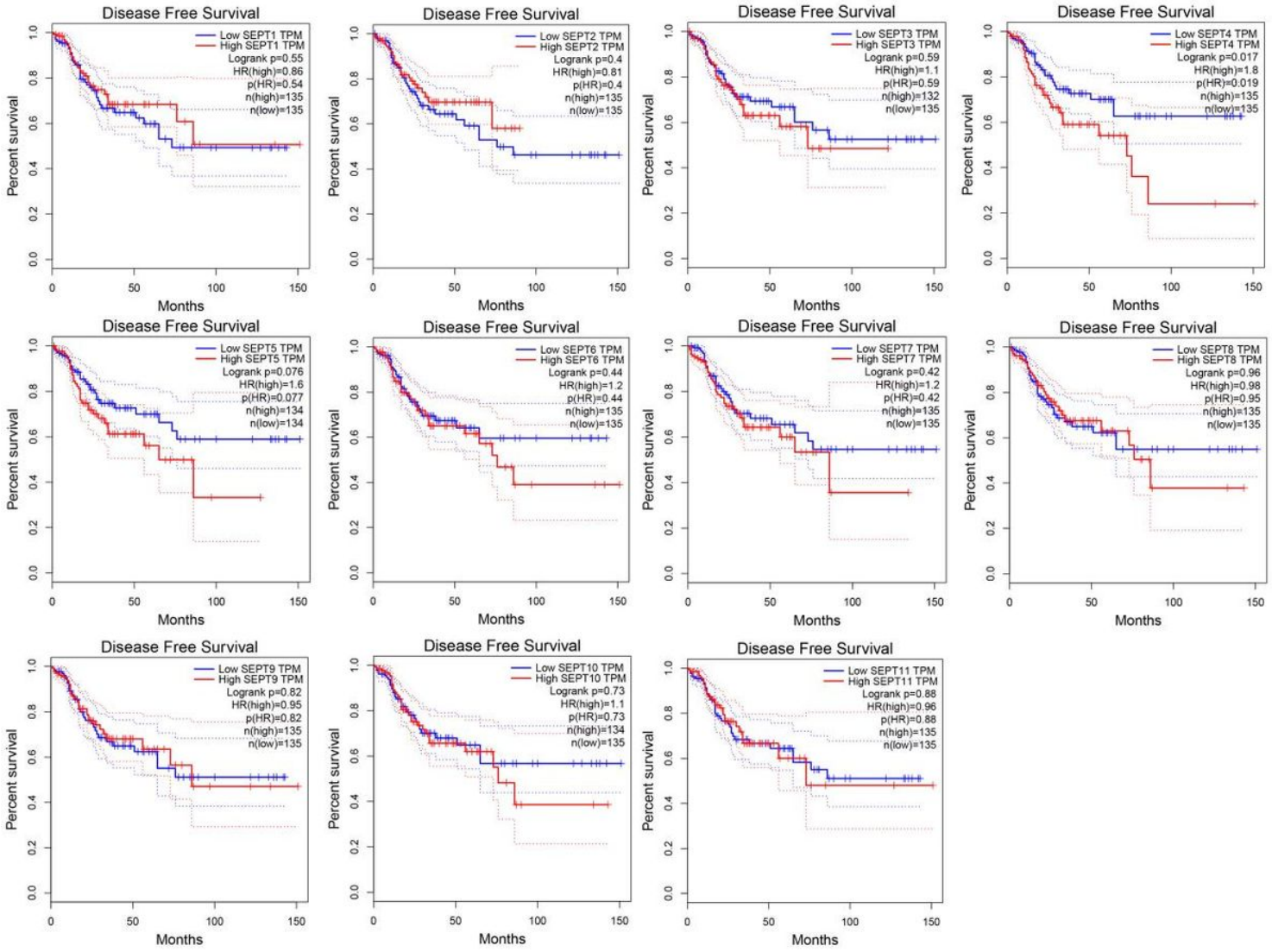

Figure 7

Disease free survival curves for the expression of SEPT1-SEPT11 in colon cancer patients (GEPIA).

Low expression level of SEPT1/3/4/5/6/7/8/9/10/11 indicated longer DFS, while high expression level of SEPT2 suggested longer OS in colon cancer cases.

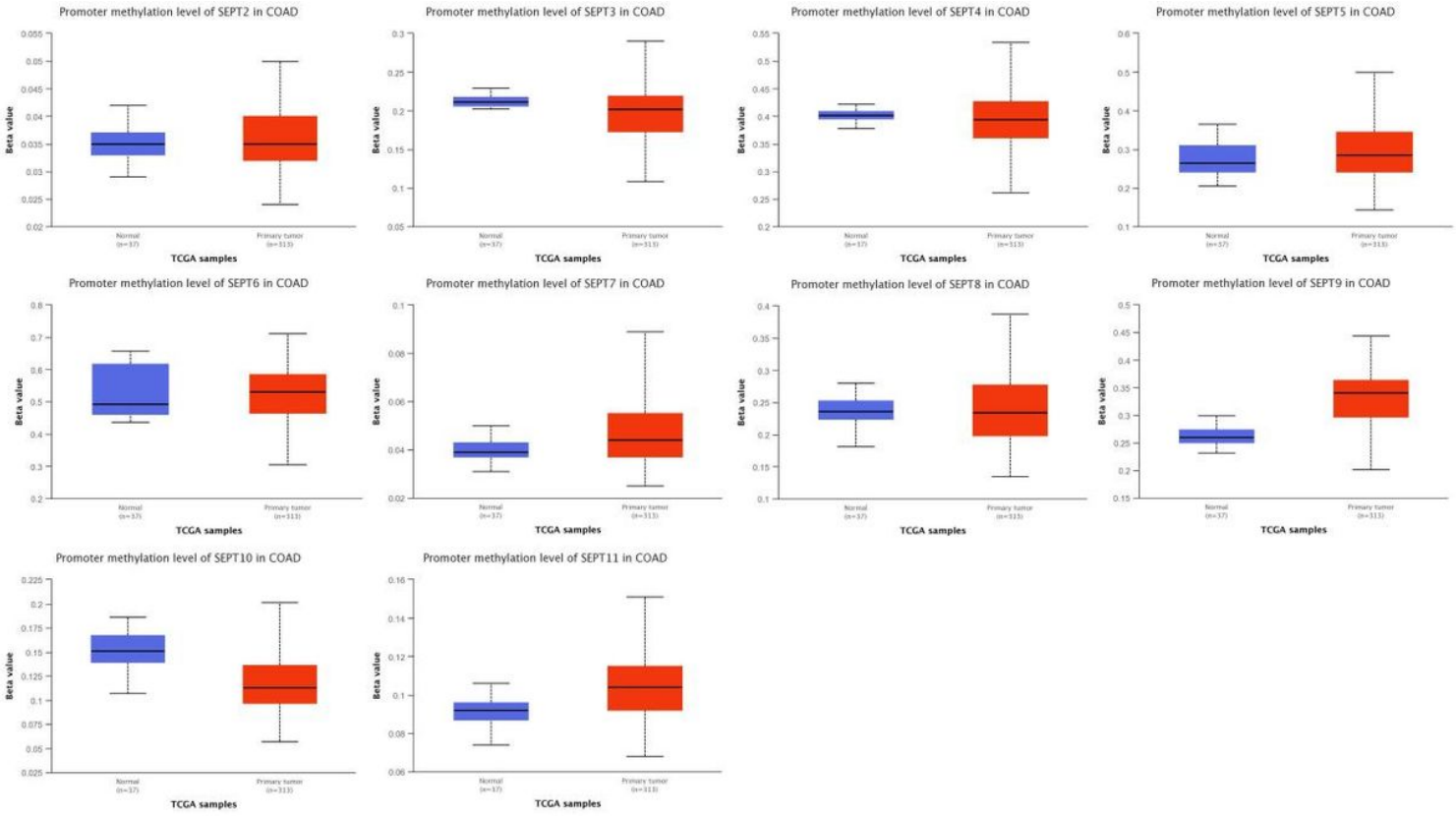

Figure 8

The methylation levels of SEPT2-11 (UALCAN).

Compared with normal tissues, the methylation levels of SEPT3/4/6/10 was decreased in colon cancer tissues , while that of SEPT2/5/7/8/9/11 were increased (average beta value for "SEPT1" is not available for majority of samples in colon cancer).

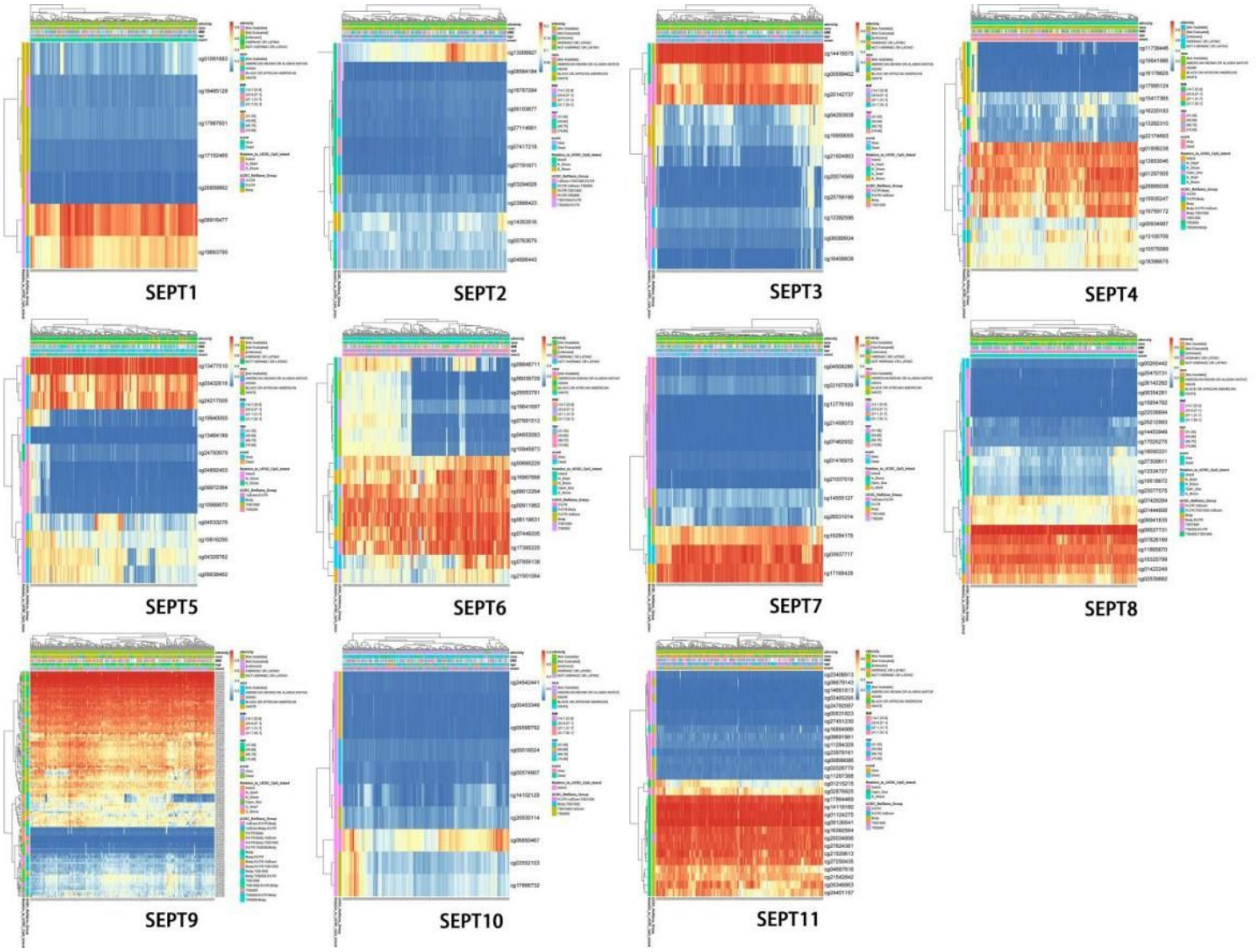

Figure 9

The heat map of DNA methylation clustered expression level of SEPT1-SEPT11 (MethSurv).

cg08916477 of SEPT1, cg13009927 of SEPT2, cg14416575 of SEPT3, cg01806238 of SEPT4, cg13853046 of SEPT4, cg13477510 of SEPT5, cg00911962 of SEPT6, cg08119631 of SEPT6, cg17168428 of SEPT7, cg09527731 of SEPT8, cg05104283 of SEPT9, cg01405751 of SEPT9, cg12203543of SEPT9, cg04142643 of SEPT9, cg15267890 of SEPT9, cg06850467 of SEPT10, cg17864469 of SEPT11, cg14118160 of SEPT11, and cg01124275 of SEPT11 displayed the highest DNA methylation level.

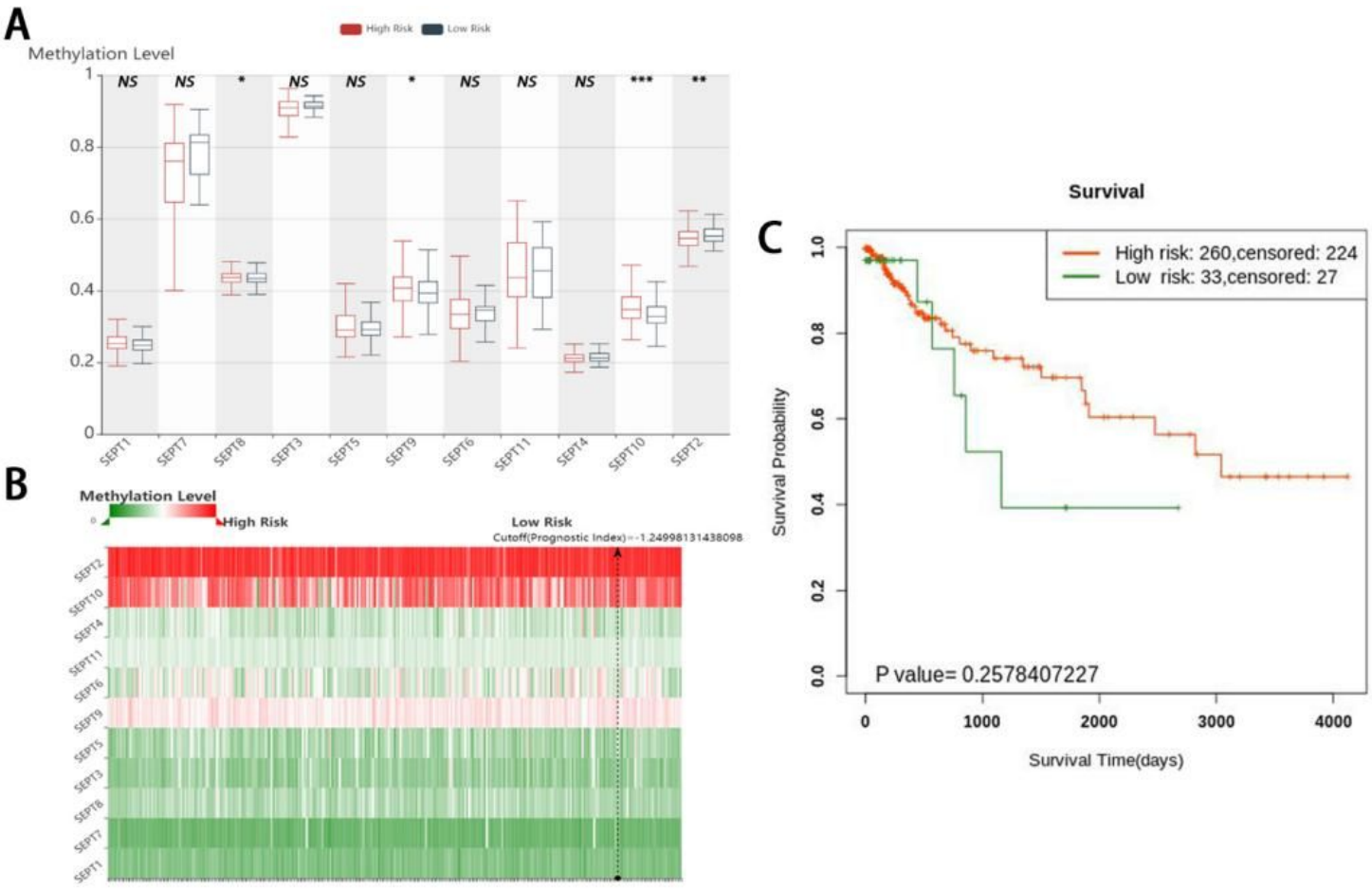

Figure 10

The prognostic value of the DNA methylation of SEPT1-SEPT11 in colon cancer (SurvivalMeth).

(A) The methylation level of CpGs in high-risk group and low-risk group. (B) The heat map of CpG methylation level. (C) The survival curve of Kaplan–Meier plot. NS > 0.05, *P < 0.05; **P < 0.01; ***P < 0.001.

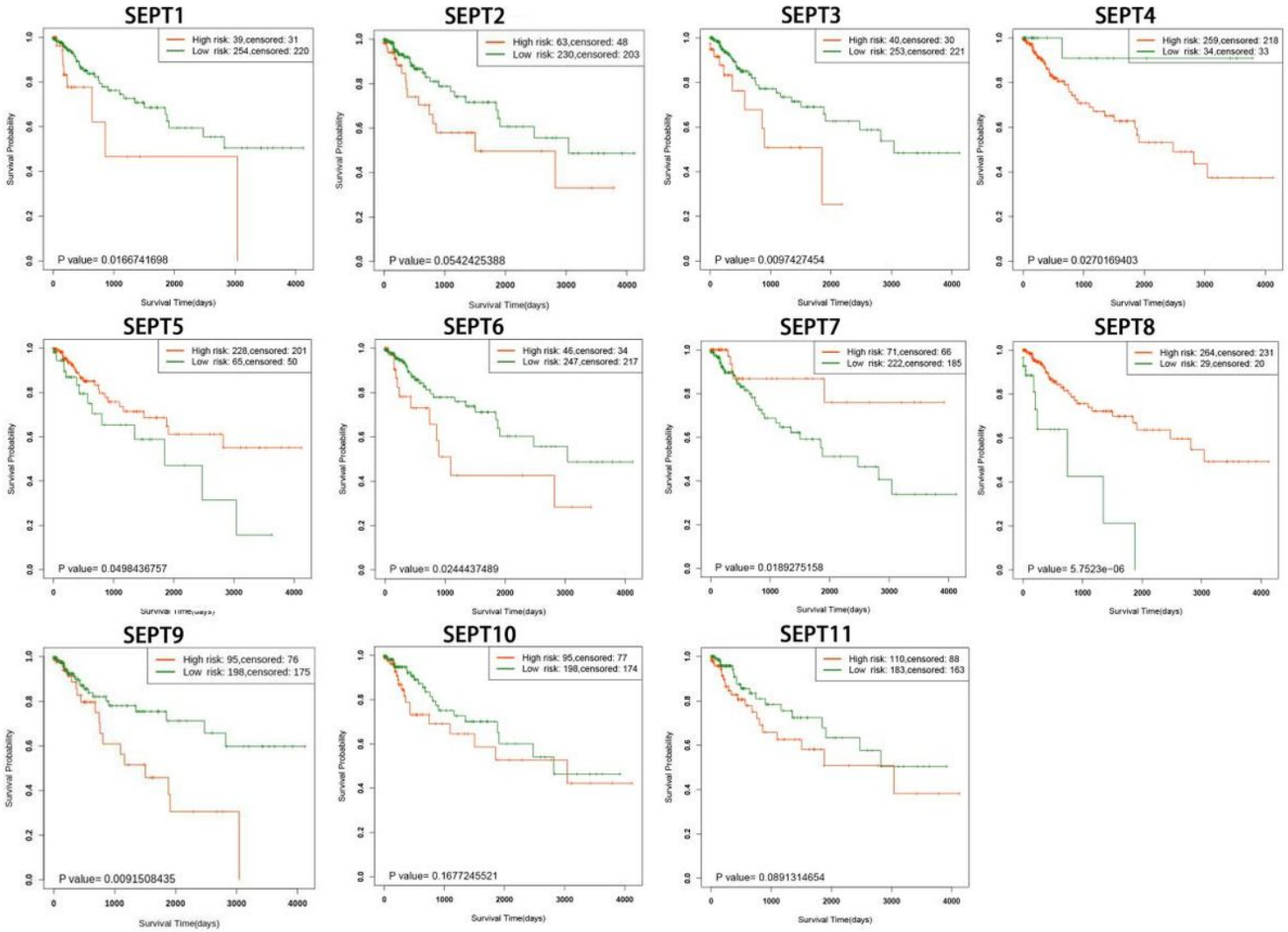

Figure 11

Overall survival curves for the methylated expression of SEPT1-SEPT11 in colon cancer patients (SurvivalMeth).

The colon cancer patients in the low-risk group of SEPT1/3/4/6/9 survived longer, whereas patients with colon cancer in the high-risk group of SEPT5/7/8 had a longer survival time.

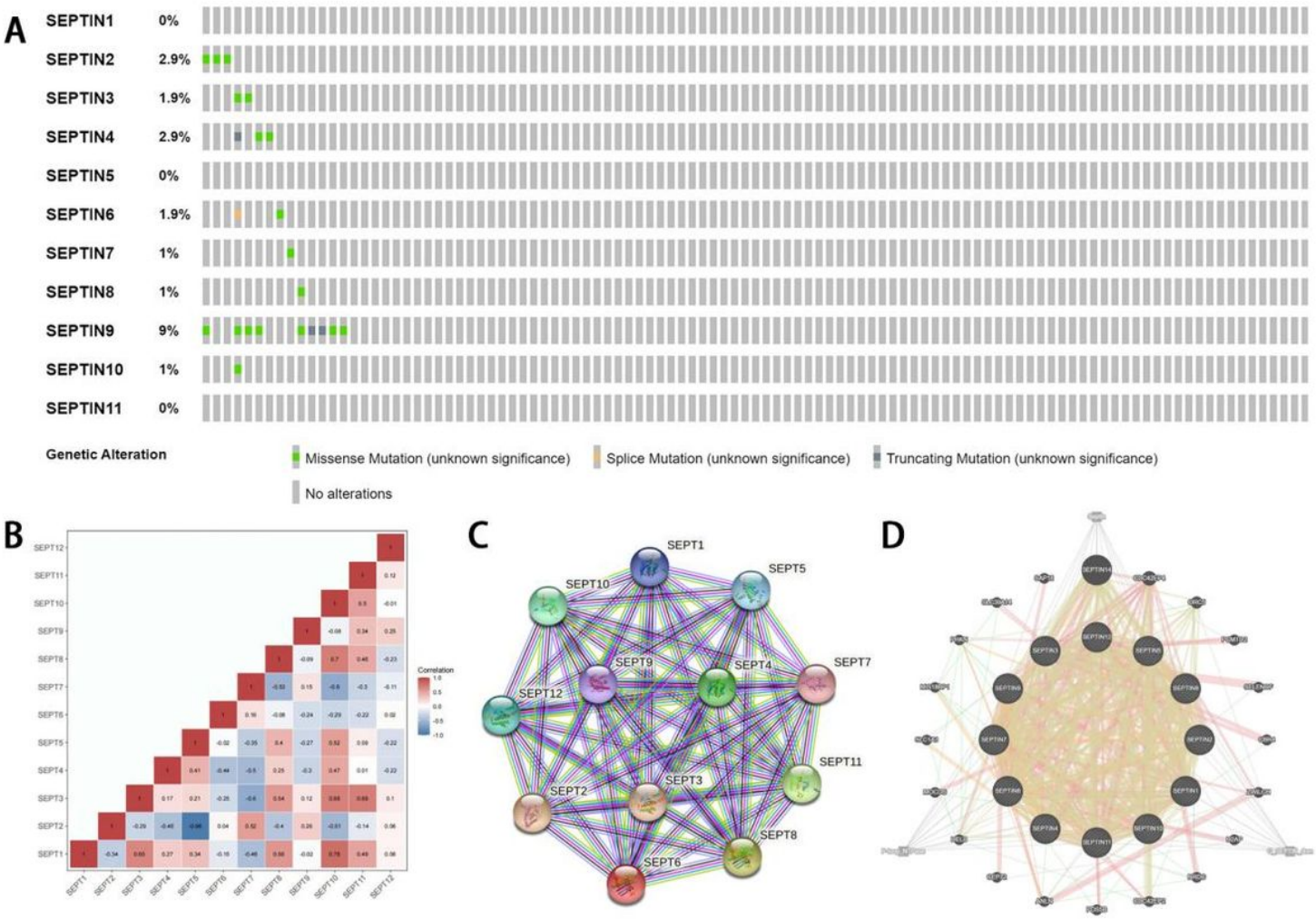

Figure 12

Molecular characteristics analyses of SEPT1-SEPT11 in CRC Patients (cBioPortal, STRING, and GeneMANIA).

(A) The genetic alterations in SEPT1-SEPT11 in colon cancer. (B) The correlation heat map of SEPT1-SEPT11 in colon cancer. (C and D) The PPI network of SEPT1-SEPT11.

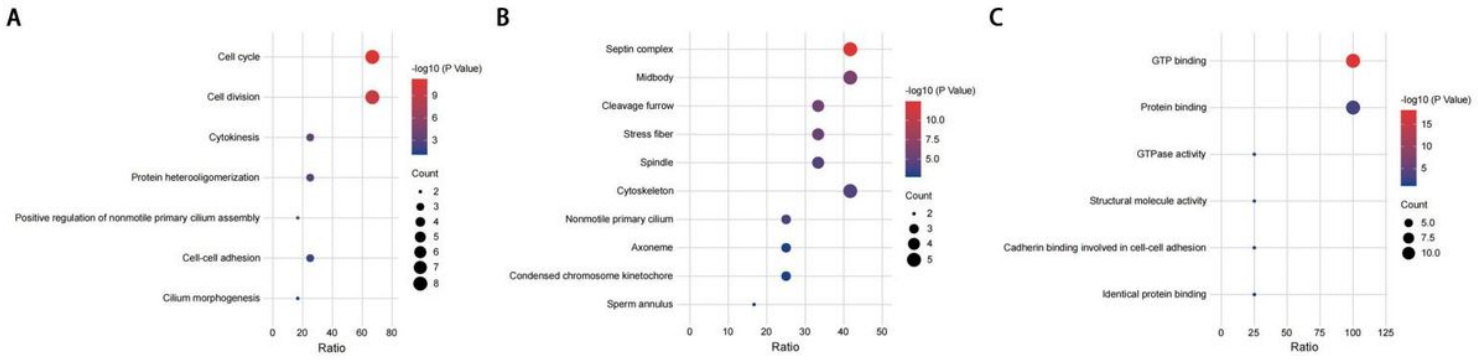

Figure 13

The enrichment analysis of SEPT1-SEPT11 in colon cancer (David 6.8).

(A) Bubble plot of GO enrichment in BP enriched terms. (B) Bubble plot of GO enrichment in CC enrichederms. (C) Bubble plot of GO enrichment in MF enriched terms.

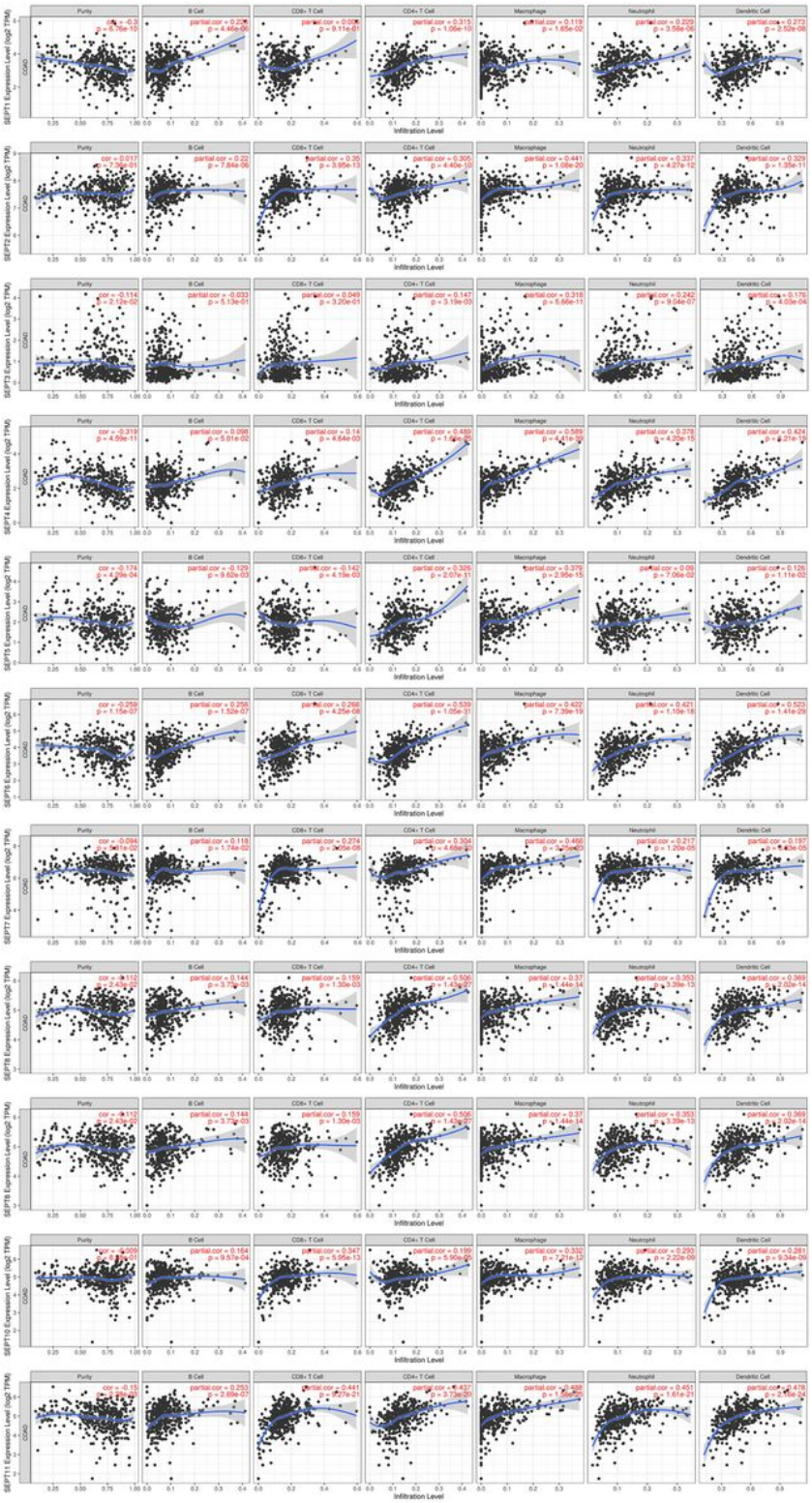

Figure 14

The correlation between SEPT1-SEPT11 and immune cell infiltration in colon cancer (TIMER).

Analysis of the correlation between SEPT1-SEPT11 and six immune cell infiltrations (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells).

Supplementary Files

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- Supplementarymaterial.docx