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ORIGINAL RESEARCH

Identification of microRNA Signature and Key Genes Between Adenoma and Adenocarcinomas Using Bioinformatics Analysis

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Background: In worldwide, colorectal cancer (CRC) is very common and the mechanisms remain unclear. This study aims to identify between adenomas with epithelial dislocation (false invasion) and adenomas with early adenocarcinoma (true invasion).

Methods: GSE41655 and GSE57965 datasets were obtained in the Gene Expression Omnibus (GEO) database. microRNA expression profiles and clinicopathological data from the TCGA (The Cancer Genome Atlas) database were downloaded to further validate the results in GEO. GEO software and the GEO2R calculation method were used to analyze two gene profiles. The co-expression of differentially expressed microRNAs (DEMs) and genes (DEGs) were identified and searched in the FunRich databases for pathway and ontology analysis. Cytoscape was utilized to construct the mRNA-microRNA network. Validation of gene expression levels was conducted by online databases and qRT-PCR and IHC experiments.

Results: In total, 6 DEMs and 34 DEGs are selected after calculating. KEGG results indicated that genes are enriched in certain tumor associated pathways. Four out of 6 microRNAs had a significant relationship with the overall survival ($P < 0.05$) and showed a good performance in predicting the survival risk of patients with colorectal carcinoma. Furthermore, expression levels of hsa-miR-455 and hsa-miR-125a were then verified by qRT-PCR which all target BCL2L12. IHC results showed that the expression level of BCL2L12 was higher in adenocarcinoma than in adenoma. Based on the selected gene, the top 10 small molecules were screened out as potential drugs.

Conclusion: By using microarray and bioinformatics analyses, DEMs and DEGs were selected and a complete gene network was constructed. To our knowledge, BCL2L12 and related molecules including hsa-miR-455 and hsa-miR-125a were firstly identified as potential biomarkers in the progression from adenoma to adenocarcinoma.

Keywords: microRNA, colorectal cancer, adenoma, prognostic signature

Background

Colorectal carcinoma (CRC) is the second leading cause of malignant cancer and cancer-related death worldwide. Unfortunately, 25% to 40% of people will develop recurrence despite the possibility of surgical cure. Compared with patients with earlier stage, the cure rate and overall survival of patients with advanced colorectal carcinoma are still very poor.[1] Surgical treatment is still the cornerstone of the treatment of locally advanced colorectal carcinoma. At present, effective treatment for unresectable metastatic cancers is lacked, as well as tumors with the worse effect of chemotherapy and radiotherapy.[2] CRC is a multi-step process from normal epithelial cells to adenomas and

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adenocarcinoma, and finally to different organs. In 1990, the development mode of CRC was introduced, among which APC, KRAS, TP53, and DCC were considered as the genes to promote CRC development. Since then, many studies have studied the molecular mechanism of CRC. It suggested that colorectal cancer is caused by the accumulation of genetic and genetic factors (such as Wnt, PIK3CA, and TGF - β) that changed the signaling pathway. In the pathogenesis of CRC, three main recognized pathways are chromosome instability, microsatellite instability, and CpG island methylation phenotype. Many CRC lacks the changes described above, which indicates that there are other mechanisms in the development of CRC.[3] Moreover, there are significant differences in the patterns of gene methylation between the right and left colon. Notably, the prevalence of promoter methylation of the mismatch repair gene hMLH1 and the O-6-methylguanine-DNA methyltransferase (MGMT) is significantly greater in normal right colon mucosa, especially in older women, suggesting epigenetic aberrations in preneoplastic right colon mucosa that may be reflected in subsequent right-sided adenocarcinoma biology. Besides, there is differential prognosis by stage between patients with right- and left-sided CRC. Retrospective studies suggest that right-sided tumors have a slightly better prognosis in stage II CRC, but a slightly worse prognosis in stage III disease, which is probably associated with the higher prevalence of good-prognosis microsatellite unstable (MSI-high) tumors in right-sided stage II cancers.[4]

MicroRNAs are a class of non-coding single-stranded RNA molecules, which are about 22 nucleotides in length and encoded by endogenous genes.[5] In the whole development process of organisms, microRNA may have biological functions such as regulating early cell development, participating in cell differentiation and tissue development, and regulating gene expression, the main role of which is to regulate gene expression.[6] In particular, Wang et al found that microRNA-217 inhibited high glucose induced proliferation and migration of vascular smooth muscle cells by targeting ROCK1.[7] Ma et al found that microRNA-214 was reduced in cutaneous squamous cell carcinoma tissues and cells, and patients with microRNA-214 overexpression had a higher survival rate.[8] Yan et al also reported that microRNA-224, microRNA-147b, and microRNA-31 are related to lymph node metastasis and overall survival for lung cancer by regulating PRPF4B, WDR82, or NR3C2.[9] High expression of microRNA-21 has been correlated with shorter disease-free-survival in stage IIcolorectal cancer patients. Apart from microRNA-21, the classifiers based on microRNAs including microRNA-20a-5p, microRNA-103a-3p, microRNA-106a-5p, and microRNA-143-5p have been reported as novel predictive markers for the recurrence of stage II disease. Furthermore, microRNA-320e has also been recognized as a prognostic biomarker; high level of microRNA-320e is correlated with more advanced disease, recurrence, and dismal prognosis in those with stage II and III colorectal cancer. Moreover, there is some evidence for contribution of microRNA-148a to the carcinogenesis of CRC. It has also been reported that postoperative plasma microRNA-31, microRNA-141, and microRNA-16 are suggested biomarkers of disease recurrence after the surgical resection. Finally, microRNA-429 expression is upregulated in colorectal cancer tissue and as such closely related to tumor size, lymph node involvement, and distant metastases, whereas it leads to shorter survival.[10] However, the regulations of microRNAs in the development of adenoma to carcinoma remain unknown.

In this article, microarray data from the GEO database and colorectal cancer sample data in the TCGA database were used for identifying differently expressed microRNAs (DEMs) between adenoma tissues and adenocarcinoma tissues. By using a variety of mRNA and microRNA-related functional databases and performing verification experiments, several genes associated with adenoma progression to cancer and subsequent pathways were identified. For the study, we selected 2 different sets of data, including adenoma and cancer, 1 of which was not published.

Methods

Microarray Data

GEO database, the full name of gene expression omnibus, is a gene expression database created and maintained by National Biotechnology Information Center NCBI. It was constructed in 2000 and contains high-throughput gene expression data submitted by research institutions around the world. In our study, GSE41655 and GSE57965 datasets were downloaded from GEO.

GSE41655, including 20 healthy colonics, 20 colorectal adenomas, and 20 colorectal cancer tissue. MicroRNA expression profiling analysis of these tissues was constructed on Affymetrix Human Transcriptome Array 2.0 (GPL17586). Dataset GSE57965, including 5 adenomas and 9 adenocarcinomas, was processed by Affymetrix Human Gene Expression Array (PrimeView) (GPL15207).

DEMs Analysis

GEO2R, an R-associated web application, was applied to filtrate DEMs between adenoma samples and adenocarcinoma samples. We also used R software to analyze two sets of data. The $p < 0.05$ and $|\log FC| \geq 1$ were considered as cutoff criterion.[11]

Functional and Pathway Enrichment Analysis

GO functional analysis and KEGG pathway analysis were performed to predict the potential functions of the DEMs by using the Database for Annotation, Visualization, and Integrated Discovery (FunRich; http://www.funrich.org). Upregulated and downregulated DEMs were submitted to the FunRich online program. The top 10 items of the cellular component (CC), biological process (BP), and molecular function (MF) categories were then sorted and presented in the form of pie graphs. Cytoscape software was utilized to perform KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis. ClueGO is a Cytoscape plug-in, which visualizes non-redundant biological terms for a large number of gene clusters in functional packet networks. By uploading target genes, the network graph according to kappa statistics was established.

Prediction of Potential DEMs Target mRNAs and microRNA-mRNA Regulatory Network

MicroRNAs inhibit the expression of target genes mainly by binding to target mRNA, or promoting the degradation of mRNA, or hindering its translation. Accurate and rapid prediction of microRNA target genes by bioinformatics methods can provide clues for the study of microRNA function. Upregulated and downregulated DEMs were submitted to the FunRich online program to achieve target genes. Besides, GSE57965 was obtained from the GEO database. By combining the target genes from FunRich software and differential expression analysis of GSE57965, the intersection genes between the two results were selected and the microRNA-mRNA regulatory network was constructed by utilizing Cytoscape software.

Analysis of the microRNAs and Their Association with Prognosis of Colorectal Cancer Patients

The Kaplan–Meier plotter can evaluate the effect of genes (mRNA, microRNA, protein) on the survival rate of 21 cancer types (including breast cancer, ovarian cancer, lung cancer, and gastric cancer). The sources of the database include GEO, EGA, and TCGA.[12] Each identified microRNA will be entered into this online tool to assess the survival rate of colorectal cancer patients according to the Kaplan Meier curve.

Independent Prognostic Ability of the microRNA Signature

To explore the effect of DEMs on the prognosis of patients with colorectal cancer, univariate and multivariate Cox proportional hazards regression analysis was performed on the DEMs. The microRNAs associated with the prognosis of colorectal cancer were identified, and a risk model based on TCGA information was built. Five hundred and sixty-nine patients with colorectal cancer obtained from the TCGA database. R software was utilized to divide the samples with complete survival information and DEMs expression profiles into two groups (train group and test group). To reduce the number of microRNAs with similar expression, stepwise Cox regression was performed for microRNAs with $P < 0.005$ to establish a prognostic model. In multivariate Cox regression analysis, we use the function of "Coxph" and "direction = both" in R language survival package.[13,14] According to multivariate Cox regression analysis, these microRNAs were further researched to select important targets and constructed a risk linear model.

To further learn about the association between the identified microRNAs and the prognosis of colorectal cancer patients, we constructed a risk prediction model. The area under the curve (AUC) and receiver operating characteristic (ROC) of 3 and 5 years were calculated by the "survivalROC" software package to evaluate the predictive ability of microRNAs.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Forty participants, including 20 patients with adenomas and 20 patients with adenocarcinomas, were recruited from The Second Affiliated Hospital of Soochow University (Table 1). Before the study began, all the patients agreed. Tissue

Table 1 Clinicopathological Characteristics of the Whole Series

Variable	Number
Patients	40
Sex	
Male	28
Female	12
Age	
Range	30–79
Median ± SD	63.2±12.48
Patients with colorectal adenoma	20
Age	
Range	30–65
Mean ± SD	56.9±11.62
Sex	
Female	9
Male	11
Patients with colorectal carcinoma	20
Age	
Range	35–69
TNM tumor stages	
T1	4
T2	6
T3	2
T4	8
TNM lymph node status	
N0	12
N1	2
N2	6
Metastases	
M0	16
M1	4

Abbreviations: T, tumor; N, lymph node; M, metastases.

samples were collected from each participant before treatment began. After that, Total RNAs of tissues were isolated by TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed to synthesize cDNA using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). qRT-PCR reactions were performed using SYBR reagent (TaKaRa, Otsu, Shiga, Japan) by Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR reactions: hsa-miR-455: 5′-GCAGUCCAUGGGCAUAUACAC-3′ and hsa-miR-125a: 5′-CCGTCCCTGAGACCCTTTAAC-3′.

Immunohistochemical Staining

Genes expression in adenocarcinoma tissues and normal tissues was extracted from the human protein atlas (www.proteinatlas.org). The Human Protein Atlas is a Swedish-based program initiated in 2003 to map all the human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology.

Identification of Candidate Small Molecule Drugs

The Connectivity Map (CMap) (http://www.complement.us/labweb/cmap/) is a database used for predicting potential drugs that may induce or reverse gene expression. The identified genes were input into the CMap database to Search for drugs against the transformation of adenoma to adenocarcinoma, and the enrichment scores were also calculated.

Results

Identification of the microRNAs Between Adenoma Tissues and Adenocarcinoma Tissues

R software was utilized to study the microRNA and mRNA expression profiles from the GSE41655 and GSE57965. Through the cut-off criteria ($P < 0.05$ and $|\log2FC| \geq 1$), 27 DEMs and 692 DEGs were identified. A volcano plot and a heatmap were performed to show upregulated (red) and downregulated (blue) genes between adenomas and adenocarcinomas, respectively (Figure 1).

Screening of Potential Transcription Factors and Enrichment Analysis

For the reason that transcription factors are crucial in microRNA, FunRich is used to research the top 10 enriched transcription factors, namely EGR1, SP1, SOX1, POU2F1, RREB1, TCF3, RORA, FOXA1, SP4, and TEAD1. To further research the function of these microRNAs, we

submitted them to FunRich to perform gene ontology analysis. The result showed that DEMs were most enriched in the nucleus, transcription factor activity, cell communication, and signal transduction (Figure 2). KEGG pathway analysis showed that these potential target genes were mainly enriched in 6 pathways including glycosphingolipid biosynthesis, retinol metabolism, AGE-RAGE signaling pathway in diabetic complications, pancreatic secretion, inflammatory mediator regulation of TRP channels, and renin secretion (Figure 3) (Table 2).

microRNA-mRNA Regulatory Network

By utilizing FunRich software, 1156 potential target genes were obtained and only 34 of them differentially expressed in GSE57965. The Venn Diagram showed the results we screened (Figure 4A). To show the composition and relationship of microRNA and mRNA more clearly, we built a microRNA-mRNA regulatory network by utilizing Cytoscape software. Finally, 41 essential microRNA-mRNA pairs were selected which may play a key role in the transformation of adenoma to adenocarcinoma (Figure 4B).

Analysis of the DEMs and Their Association with Prognosis of CRC Patients

Kaplan–Meier Plotter was used to analyze the prognosis of patients with colorectal carcinoma. After uploading six microRNAs, we got the corresponding survival curve. The results showed that overexpression of microRNA-199a, microRNA-199b, microRNA-127, and microRNA-125a (Figure 5) were associated with poor overall survival in patients with colorectal carcinoma. However, the expression level of microRNA-455 and microRNA-146b may have no obvious association with the overall survival. This indicated that the identified microRNAs may be potential targets.

Construction of Prognostic Risk Model and Predictability Evaluation

To determine the best prognostic microRNAs, 20 microRNAs were analyzed by LASSO Cox regression via glmnet package in R software. Nine microRNAs were identified to construct risk markers according to the minimum standard (Figure 6A). Then, multivariate Cox proportional risk regression analysis was performed to evaluate the independent prognostic value of 9 candidates prognostic microRNAs. Through Cox model, 7 candidate microRNAs (microRNA-125a-5p, microRNA-377, microRNA-376c, microRNA-455-3p, microRNA-126, microRNA-99a, and microRNA-193b) were identified as independent prognostic factors. Then, we combined the 7 microRNAs to construct a model to predict patient outcomes. The AUC of 3-year survival and 5-year survival of 7-microRNA was 0.809 and 0.981 respectively, which indicated that the model had a good effect in predicting the survival risk of colorectal carcinoma patients (Figure 6B). Through the risk model, CRC patients were divided into a high-risk group and a low-risk group. Results indicated that the model can predict the clinical outcomes of CRC patients. The survival status, risk score, and distribution of 7 microRNAs expressions in every CRC patient were also studied. The survival results indicated that patients with a low-

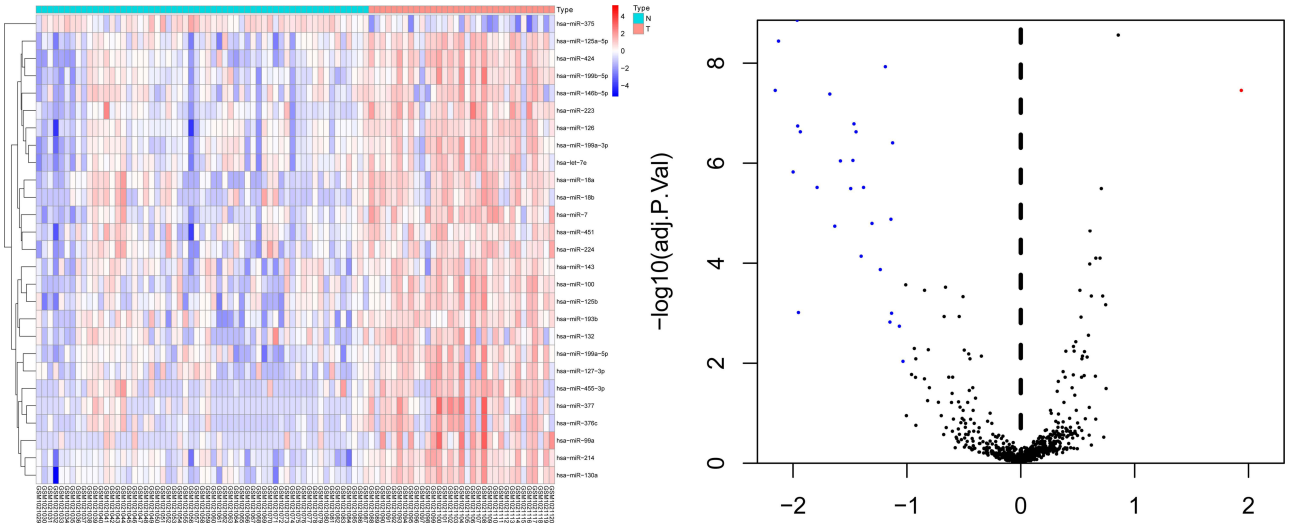

Figure 1 Heat map and volcano plot of differentially expressed microRNAs.
Abbreviations: N, adenoma; T, adenocarcinoma.

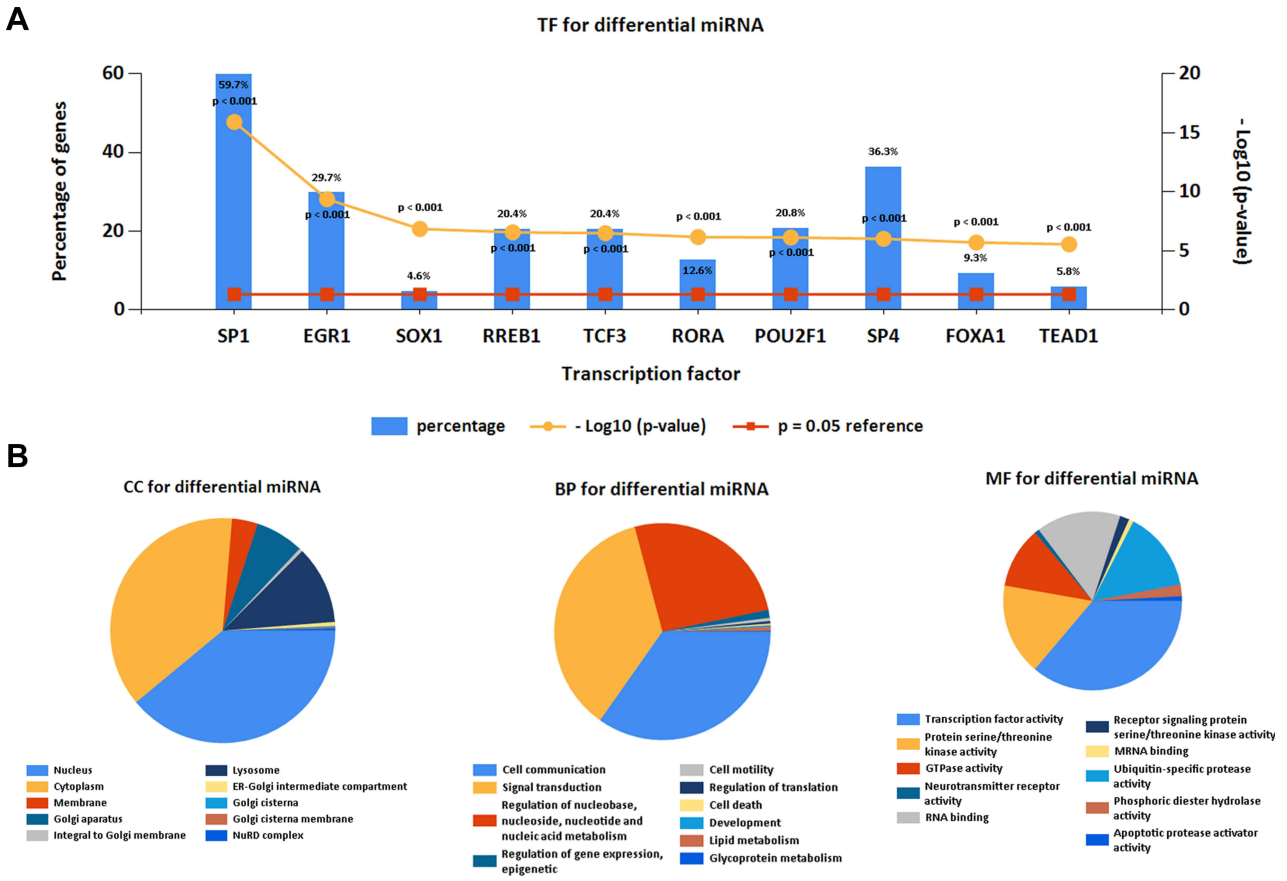

Figure 2 Screening of potential TFs and target mRNAs of differentially expressed microRNAs. (**A**) Identification of the potential TFs of differentially expressed microRNAs by FunRich software. (**B**) The top 10 of biological process, cellular component, and molecular function of the target genes of microRNAs.

risk score had a greater survival time than patients with a high-risk score (P < 0.0001) (Figure 6C and D).

Verification of Potential Biomarker Expression by qRT-PCR and IHC

According to the combination of the GEO and TCGA analysis results, 2 microRNAs were identified for further research (Figure 7A). Then, the selected biomarkers including microRNA-125a-5p and microRNA-455-3p were validated in colorectal cancer tissue samples using qRT-PCR analysis. Consistent with the prediction, the results showed that the expression levels of microRNA-125a (P-value = 0.015) and microRNA-455 (P-value = 0.013) in the tissue of adenoma patients were lower than that of adenocarcinoma patients (Figure 7B). By using FunRich software, we also found that microRNA-125a-5p and microRNA-455-3p had high reliability, which all target BCL2L12. The results of immunohistochemistry also revealed that BCL2L12 was downregulated in adenoma tissues compared with adenocarcinoma tissues (Figure 7C). This mRNA expression was detected by the polyclonal antibody using immuno-histochemistry and sections were counterstained lightly with hematoxylin. Statistics analysis have shown that higher BCL2L12 protein expression in colorectal carcinomas than overall colorectal adenoma (Table 3).

Screening of Small Molecule Drugs

By using CMap software, Top ten molecular drugs significantly correlated molecules with BCL2L12 were achieved including navitoclax, maraviroc, HIC, and imiquimod. The result is shown in Table 4. The molecular chemical structure diagram of navitoclax is also shown in Figure 8.

Discussion

Colorectal adenoma is closely related to colorectal cancer. Studies suggest that at least 80% of colorectal cancer evolved from colorectal adenoma, which lasted for more than 5 years, with an average of 10–15 years.

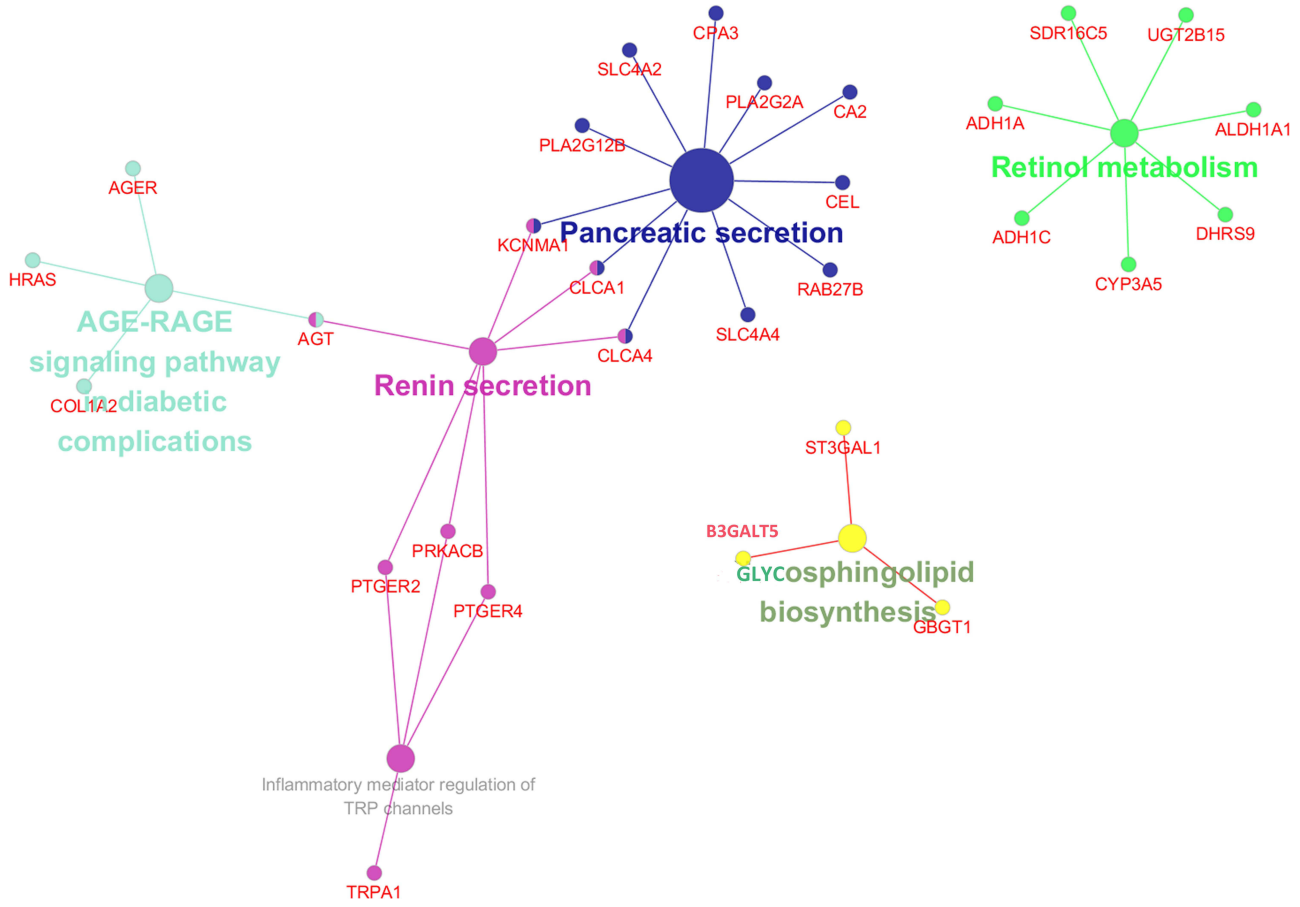

Figure 3 KEGG pathway enriched by potential target mRNAs.

Early detection and complete endoscopic resection of adenoma are the keys to survival, and there is almost no chance of cancer development.[15–17] In this present study, GSE41655 and GSE57965 were obtained from the GEO. Five hundred and sixty-nine colorectal carcinoma patients' information was downloaded from the

Table 2 KEGG Enrichment Analysis of Potential Target mRNAs

GOID	GOTerm	Ontology Source	Nr. Genes	Associated Genes Found
KEGG:00603	Glycosphingolipid biosynthesis	KEGG_27.02.2019	3	[B3GALT5, GBGT1, ST3GAL1]
KEGG:00830	Retinol metabolism	KEGG_27.02.2019	7	[ADH1A, ADH1C, ALDH1A1, CYP3A5, DHRS9, SDR16C5, UGT2B15]
KEGG:04933	AGE-RAGE signaling pathway in diabetic complications	KEGG_27.02.2019	4	[AGER, AGT, COL1A2, HRAS]
KEGG:04972	Pancreatic secretion	KEGG_27.02.2019	11	[CA2, CEL, CLCA1, CLCA4, CPA3, KCNMA1, PLA2G12B, PLA2G2A, RAB27B, SLC4A2, SLC4A4]
KEGG:04750	Inflammatory mediator regulation of TRP channels	KEGG_27.02.2019	4	[PRKACB, PTGER2, PTGER4, TRPA1]
KEGG:04924	Renin secretion	KEGG_27.02.2019	7	[AGT, CLCA1, CLCA4, KCNMA1, PRKACB, PTGER2, PTGER4]

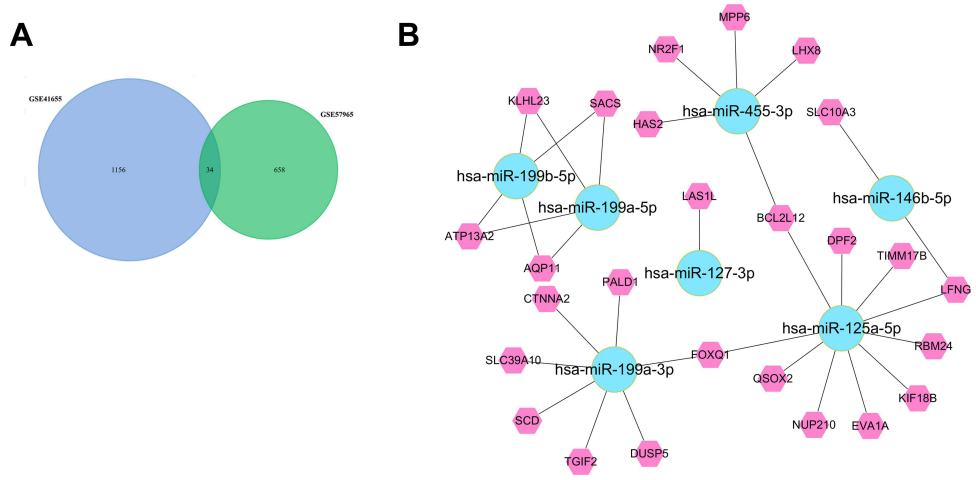

Figure 4 Identified target mRNAs and microRNA-mRNA regulatory network. (**A**) Venn Diagram of GSE41655 and GSE57965. (**B**) microRNA-mRNA regulatory network.

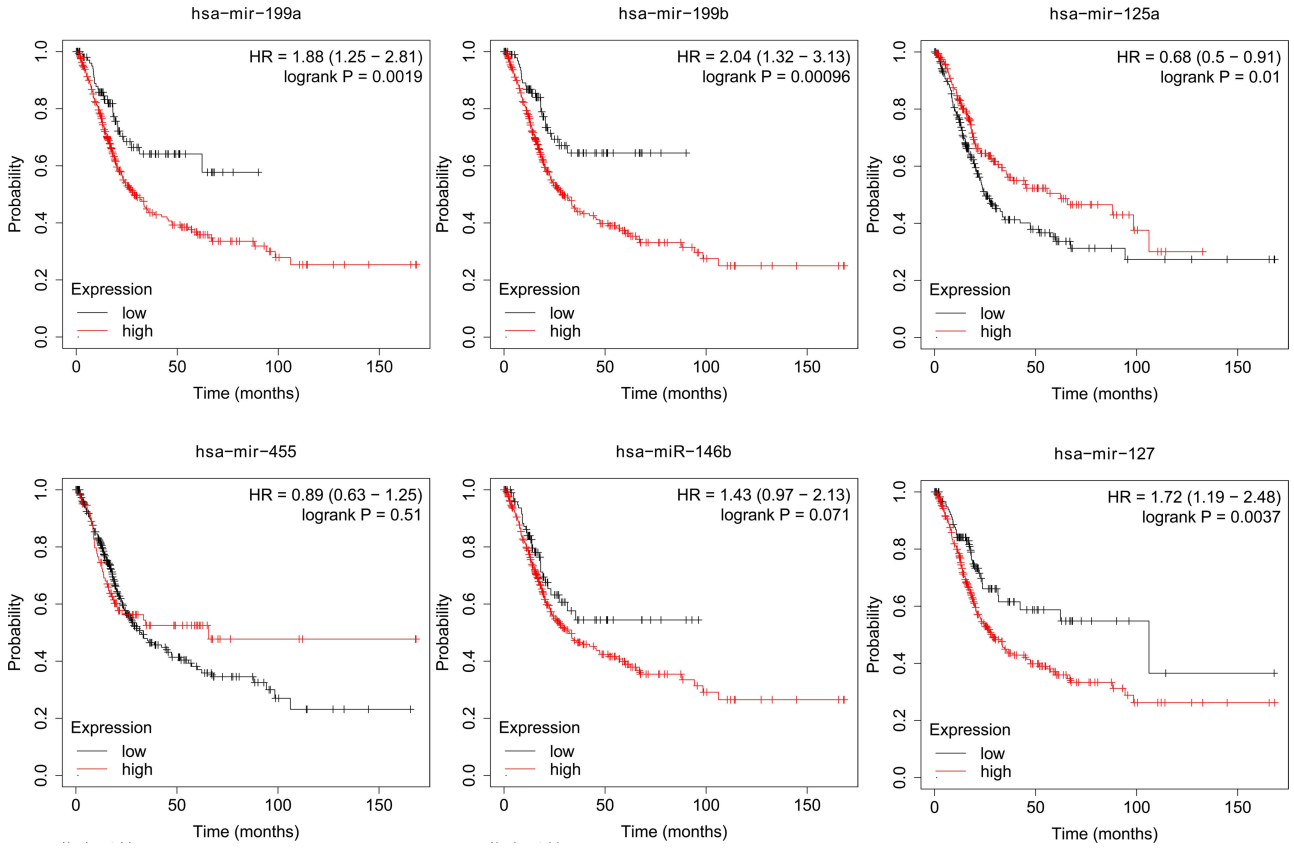

Figure 5 The association between microRNAs and colorectal cancer prognosis.

TCGA database. Twenty-seven CRC-related microRNAs were identified and may play a vital role in promoting the development of adenomas into adenocarcinomas. To further study the regulatory mechanism of the 27 microRNAs in colorectal cancer, we used FunRich for further study. Gene ontology enrichment analysis showed that these microRNAs were primarily associated with the nucleus, transcription factor activity, cell communication, and signal transduction. This is consistent with the recognition that cell cycle and cell proliferation regulator function defects are the key reasons for cancer occurrence and development.[18,19] The ion transport in

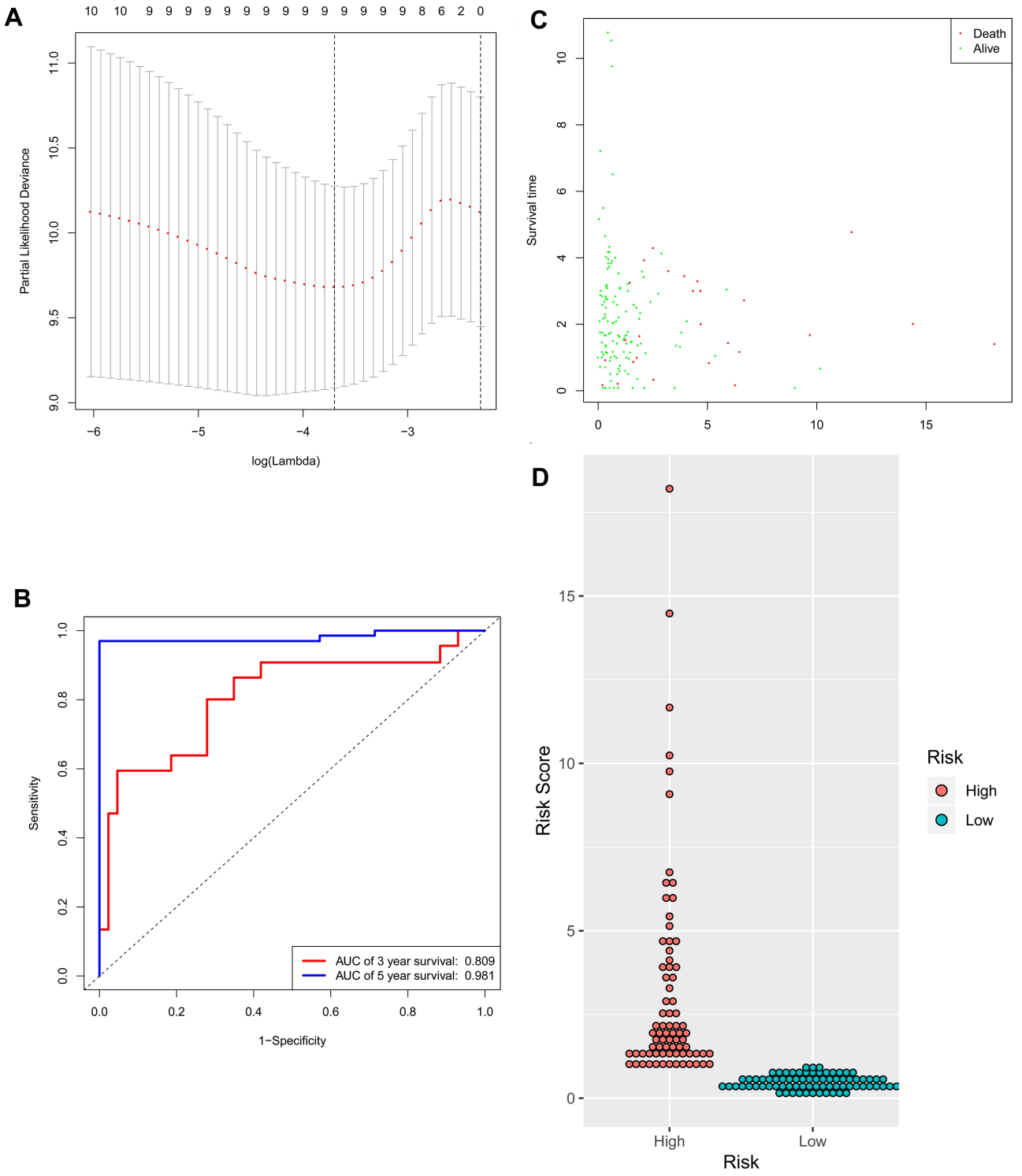

Figure 6 Identification of the signature significantly associated with the survival of patients with CRC in the training group. (**A**) LASSO Cox regression algorithm was used to reduce the scope. (**B**) Time-dependent ROC curves analysis. (**C** and **D**) Risk score distribution and survival status for patients in high- and low-risk groups by the signature. LASSO, least absolute shrinkage and selection operator; ROC, receiver operating characteristic.

tumor cells is essentially different from that in normal cells.[20] KEGG enrichment pathway analysis showed that these selected microRNAs were mainly enriched in 6 pathways including glycosphingolipid biosynthesis, retinol metabolism, AGE-RAGE signaling pathway in diabetic complications, pancreatic secretion, inflammatory mediator regulation of TRP channels, and renin secretion. Several studies on cancer-related

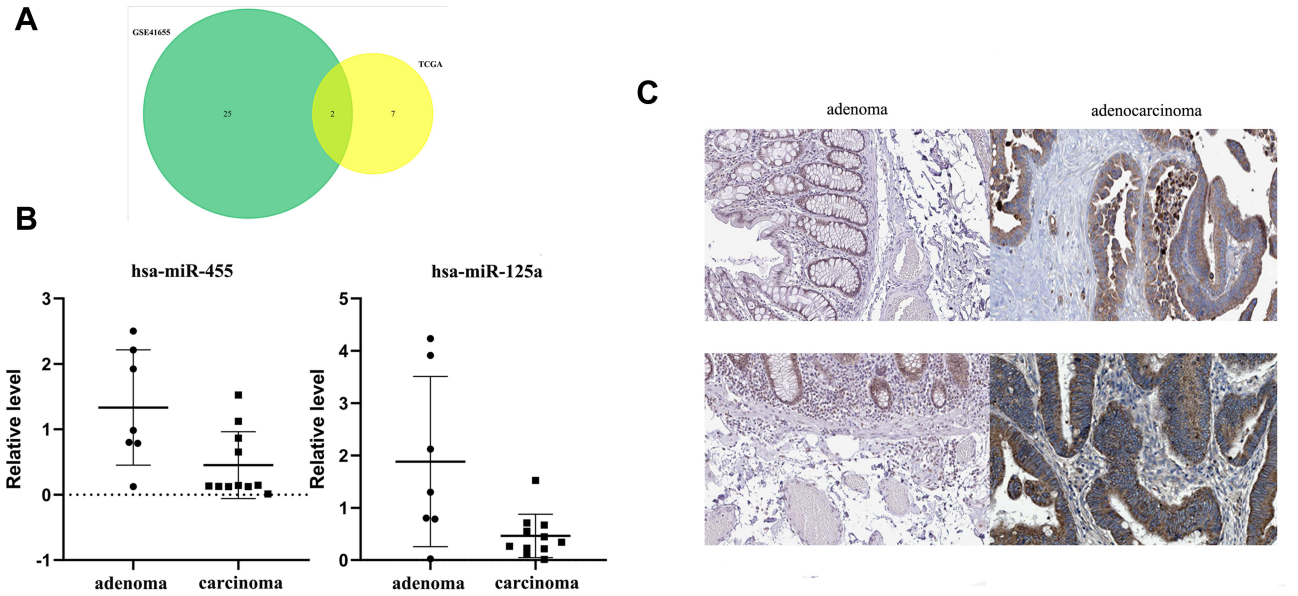

Figure 7 Genes expression in human colorectal carcinoma specimens and adenomas. (**A**) Venn Diagram of GSE41655 and TCGA. (**B**) QRT-PCR results indicate that the expression levels of microRNA-455 and microRNA-125a in tissues of adenoma patients and cancer patients. (**C**) BCL2L12 expression in adenoma patients and colorectal carcinoma patients.

glycosylation have shown that abnormal glycosylation is a common feature of various stages of malignant transformation and tumor progression.[21,22] It is important to note that the glycosylation changes observed so far are relatively specific to the type and stage of cancer, making glycans potential biomarkers and targets for drug therapy. As for the AGE-RAGE signaling pathway in diabetic complications, previous studies have shown that glycine may protect diabetic macrovascular complications by improving Glo1 function, inhibiting AGE/RAGE pathway and subsequent oxidative stress.[23] TRP channels are regulated by proinflammatory mediators, neuropeptides, and cytokines. Antagonists or agonists targeting these receptors have made great progress in the treatment of pain.[24] However, the regulation mechanism of these pathways in colorectal cancer has not been discovered yet.

Table 3 Expression of BCL2L12 Protein in Colorectal Adenoma and Carcinoma

	BCL2L12 Expression		
	Case No.	Low	High
Colorectal adenoma	20	18	2
Colorectal carcinoma	20	1	19

Note: p<0.0001.

The microRNA-mRNA regulatory network was constructed based on FunRich and Cytoscape. Six DEMs (microRNA-199a, microRNA-199b, microRNA-127, microRNA-125a, microRNA-455, and microRNA-146b) and 34 potential genes were selected. Previous studies have shown that they are involved in the development of cancer. For instance, a previous study reported down-regulation of microRNA-199a-5p in NSCLC altered the expression level of GRP78 and spliced XBP1 through UPR pathway.[25] As for hsa-miR-127, a previous study reported it affects the survival of glioma patients by regulating replication initiation factor 1 and promotes the proliferation, migration, and invasion of tumor cells. The hsa-mir-127/REPIN1 pathway is involved in gliomas and could be a potential therapeutic target.[26] Onnis et al also demonstrated that combined expression of Epstein–Barr nuclear antigen 1 and microRNA-127 affects the expression of major B cell regulatory factors in memory B cells.[27] As for hsa-miR-125a, a previous study reported upregulated microRNA-125a obviously enhanced cell proliferation, migration and invasion in HSCC, with upregulation of C–C Chemokine Receptor Type 7.[28] It also activates p53 and Induces Apoptosis in Lung Cancer Cells.[29] Besides, microRNA-125a may play an anticancer role by regulating BRCA1 signaling pathway, and reintroduction of microRNA-125a analogues may be a potential adjuvant therapy for advanced/chemoresistant

Table 4 Results of Connectivity Map Analysis

Rank	Score	Type	ID	Name	Description
1	99.98	cp	BRD-K82746043	Navitoclax	BCL inhibitor
2	99.93	cp	BRD-A04352665	Maraviroc	CC chemokine receptor antagonist
3	99.93	kd	CGS001-23119	HIC2	BTB/POZ domain containing
4	99.86	cp	BRD-K26657438	Imiquimod	TLR agonist
5	99.79	cp	BRD-K01638814	Rilmenidine	Adrenergic receptor agonist
6	99.75	kd	CGS001-8771	TNFRSF6B	Tumour necrosis factor (TNF) receptor family
7	99.75	cp	BRD-K48923948	BMS-641988	Androgen receptor antagonist
8	99.68	kd	CGS001-6925	TCF4	Basic helix-loop-helix proteins
9	99.65	kd	CGS001-286530	P2RY8	GPCR/Class A: Purinergic receptors, P2Y
10	99.63	cp	BRD-A67373739	AICA-ribonucleotide	AMPK activator
11	99.61	cp	BRD-K16554956	PTB1	AMPK activator

breast carcinoma.[30] Survival analysis indicated that overexpression of microRNA-199a, microRNA-199b, microRNA-127, and microRNA-125a were associated with poor OS in patients with colorectal cancer by utilizing km-plot software.

Moreover, 34 potential genes including AQP11, ATP13A2, KLHL23, BCL2L12, DPF2, EVA1A, FOXQ1 were further investigated in our research. KLHL23 (Kelch-like Family Member 23) has been studied a lot in recent decades. Overexpression of KLHL23 may affect the treatment of gastric cancer.[31] DPF2 (Double plant homeodomain finger 2) is a highly conserved member of the D4 protein family, which is widely expressed in human tissues. Recently, it has been found that DPF2 can inhibit myeloid differentiation of hematopoietic stem/progenitor cells and acute myeloid leukemia cells.[32] FOXQ1, fork-head box q1, recent studies have found that FOXQ1 and other gene changes may be involved in the mechanism of secondary resistance to anti EGFR antibody therapy in colorectal carcinoma metastasis.[33] It has also been found to be expressed in breast cancer, ovarian cancer, and endometrial cancer, in which the receptor, through its ligand, is produced by tumor cells or matrix elements to stimulate the invasion and metastasis of tumor cells.[34] Besides, another study also reported that FOXQ1 is up-regulated in colorectal cancer cells and clinical specimens, which may be of great significance for the diagnosis of colorectal carcinoma.

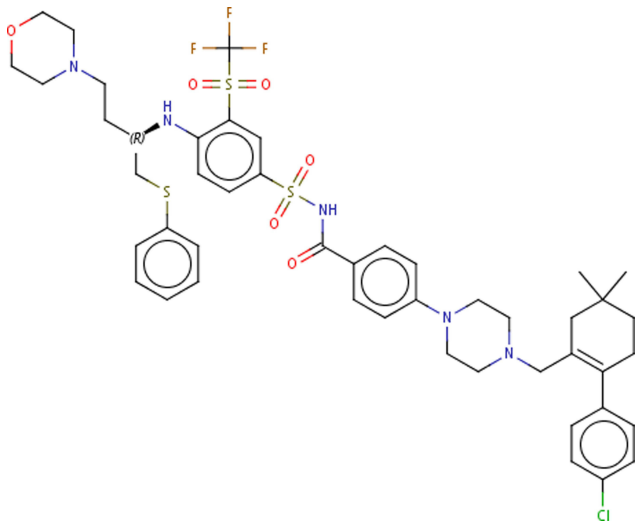

Figure 8 Chemical structure diagram of navitoclax.

Besides, a 7-microRNAs signature (microRNA-125a-5p, microRNA-377, microRNA-376c, microRNA-455-3p, microRNA-126, microRNA-99a, and microRNA-193b) of 569 patients with colorectal cancer were constructed by using single variable and multivariate Cox and risk scoring methods. CRC patients were divided into a high-risk group and a low-risk group. The results showed that the signal had good repeatability and robustness in predicting the prognosis of CRC patients.

According to the combination of the GEO and TCGA analysis results, 2 microRNAs (microRNA-455 and microRNA-125a) were identified for further research. After

using qRT-PCR analysis, we found that microRNA-455 and microRNA-125a were differently expressed in the tissue of adenoma patients and cancer patients, and we also found that microRNA-455 and microRNA-125a had strong effects, which all target BCL2L12. By using IHC, we also found it has a positive strong expression in colorectal cancer tissues and a negative weak expression in adenoma tissues. The BCL2L12 gene, a member of the BCL2 family, was discovered and cloned in 2001.[35]

The BCL2L12 gene is located on chromosome 19q13.3, between IRF3 and PRMT1/hrmt1l2. It is composed of 7 coding exons and 6 inserted introns. It spans 8.8kb genome region and encodes a proline rich protein with a BH2 and a putative BH3 domain. It is obviously related to apoptosis. BCL2L12 mRNA was expressed in many tissues.[36] In addition to the BH2 domain, BCL2L12 protein contains five consistent PXXP tetrapeptide motifs and a proline rich region, indicating that it interacts with protein tyrosine kinases such as phospholipase, RAS guanosine triphosphatase activating protein and SRC like tyrosine kinases.[37] The prognostic significance of BCL2L12 expression in several tumor types has been assessed.[38,39] Many preclinical epithelial ovarian cancer studies correlate Bcl-2-regulated apoptosis to metformin's chemo-sensitizing effects. The chemo-sensitizing effect of metformin seems to be correlated with p53 function. In the presence of p53, metformin suppresses hexokinase II (glycolytic enzyme) and pyruvate dehydrogenase kinase (anti-apoptotic serine/threonine kinase). As a result, epithelial ovarian cancer cells are sensitized to metformin.[40] Besides, BCL2L12 protein expression could be utilized as a significant prognostic tissue biomarker in patients with primary advanced-stage Laryngeal squamous cell carcinoma.[41] For colorectal cancer, the expression of BCL2L12 mRNA gradually increased during the treatment, similar to the expression of other BCL-2 family genes that are beneficial to apoptosis or specific pro-apoptotic transcripts, so it is suggested that BCL2L12 can promote apoptosis in colorectal cancer cells treated with chemotherapy.[42] By using CMap software, several small molecules with potential anti-BCL2L12 effects were identified. Navitoclax, a targeted high-affinity inhibitor of BCL-2, was found to enhance the activity of AML patients combined with other chemotherapy drugs in early clinical trials.[43] However, researches about navitoclax Inhibiting the transformation of adenoma to adenocarcinoma has not been performed yet, and more clinical trials are needed to understand this relationship.

At present, with the development of cancer treatment, individual difference therapy has been paid more and more attention. Therefore, it is important to discover novel therapeutic targets and methods for cancer patients. Our conclusions demonstrated that many DE mRNAs and microRNAs taken part in the transformation from adenoma to adenocarcinoma and had prognostic worth. Because all of our data are obtained from the GEO and TCGA database through R software, further data analysis and basic experiments are needed to verify.

Conclusion

Our research summarized some mechanisms of the progression of colorectal cancer. Plenty of DEMs and DEGs were selected between adenomas and adenocarcinomas by utilizing bioinformatics methods. Also, microRNA-455, microRNA-125a and BCL2L12 were identified as potential targets for inhibiting the transformation of adenoma to adenocarcinoma. However, these findings may be considered for future investigation.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The present study was conducted by the Declaration of Helsinki. The Ethics Committee of the Second Affiliated Hospital of Soochow University approved the study. The participants approved the use of clinical samples by providing written informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors have declared that no competing interest exists.

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