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The role of CPLX2 and SNAP25 genes in the rehabilitation of colorectal cancer liver metastases

CPLX2, SNAP25 in colorectal cancer liver metastases

Xubin Li, MD[a], Chunbo Kang, MD[a,*]

Abstract

Colorectal cancer liver metastasis generally refers to the process where colorectal cancer cells enter the liver through the bloodstream and form new tumors within the liver. The roles of complexin 2 (CPLX2) and synaptosome-associated protein 25 (SNAP25) in the recovery from colorectal cancer liver metastasis are not yet clear. Data sets GSE147602 and GSE144259 for colorectal cancer liver metastasis were downloaded from the gene expression omnibus database generated from GPL21047 and GPL11154. Batch normalization, differentially expressed genes (DEGs) screening, weighted gene co-expression network analysis, construction and analysis of the protein–protein interaction network, functional enrichment analysis, and Gene Set Enrichment Analysis were conducted. Heatmaps of gene expression were plotted. Immune infiltration analysis and Comparative Toxicogenomics Database analysis were performed. TargetScan was used to screen for miRNAs regulating central DEGs. Through experimental verification, a total of 1215 DEGs were identified. According to gene ontology analysis, they were mainly enriched in cell signaling, G-protein-coupled receptor signaling pathway, signal transduction receptor binding, and cytokine binding. Kyoto Encyclopedia of Genes and Genomes analysis results showed that the target cells were mainly enriched in cholesterol metabolism olfactory transduction. In the enrichment projects of metascape, gene ontology enrichment items included regulation of circulation, muscle structure development, vascular process in the circulatory system, and extracellular matrix organization. The soft-thresholding power for weighted gene co-expression network analysis was set to 4. Four core genes were obtained by intersecting the central genes identified by 5 different algorithms, as shown in a Venn diagram. The heatmap of gene expression showed that the core genes (CPLX2, SNAP25, and Bassoon) were underexpressed in primary colorectal cancer and overexpressed in colorectal cancer liver metastasis. Comparative Toxicogenomics Database analysis showed that 3 genes (CPLX2, SNAP25, and Bassoon) were related to abdominal pain, jaundice, chemical and drug-induced liver injury, and necrosis. The related miRNAs for the CPLX2 gene were hsa-miR-1-3p, hsa-miR-206, hsa-miR-613; for the SNAP25 gene were hsa-miR-181d-5p, hsa-miR-181b-5p, hsa-miR-181c-5p. The results confirmed that CPLX2 and SNAP25 positively regulated the phosphatidylinositol 3 kinase-AKT signaling pathway and promoted the progression of liver metastasis of colorectal cancer. CPLX2 and SNAP25 genes are overexpressed in colorectal cancer liver metastasis and may serve as important molecular targets.

Abbreviations: CPLX2 = complexin 2, CPLXs = complexins, CTD = Comparative Toxicogenomics Database, DEGs = differentially expressed genes, FDR = false discovery rate, GO = gene ontology, GSEA = gene set enrichment analysis, KEGG = Kyoto Encyclopedia of Gene and Genome, PPI = protein–protein interaction, SNAP25 = synaptosome associated protein 25, STRING = search tool for the retrieval of interacting genes, WGCNA = weighted gene co-expression network analysis.

Keywords: colorectal cancer liver metastasis, complexin 2, differentially expressed genes, rehabilitation, synaptosome-associated protein 25

1. Introduction

Colorectal cancer liver metastasis refers to the spread of cancer cells originating from the colon or rectum to the liver, resulting in the formation of metastatic liver tumors.[1] The incidence and mortality rates of colorectal cancer liver metastasis increase with age, particularly in individuals over 60 years old.[2] There is no significant difference in the incidence rates between males and females; however, male patients are more likely to be diagnosed with liver metastases at a younger age.[3] Racially, African Americans and Caucasians are more susceptible to colorectal cancer and its liver metastases than

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

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Asians and Hispanic Americans.[4] The occurrence of colorectal cancer liver metastasis usually indicates that the cancer has progressed to an advanced stage, as liver metastases are often a result of cancer spread.[5] Once cancer cells enter the bloodstream or lymphatic system, they can be transported to sites such as the liver. In the liver, these cancer cells can continue to grow and form new tumors. Treatment options for colorectal cancer liver metastasis typically include surgical resection, chemotherapy, targeted therapy, and radiotherapy, with the specific treatment plan depending on the patient's overall health, tumor size and location, and the extent of metastasis.[6] However, since liver metastases often occur in the late stages of the disease, the cure rate is low. Colorectal cancer liver metastases are usually multifocal, with multiple metastatic tumors appearing simultaneously in different parts of the liver, increasing the complexity and difficulty of treatment.[7] High-fat, low-fiber diets, lack of exercise, and obesity are associated with an increased risk of colorectal cancer and its metastasis. Unhealthy lifestyle habits such as alcohol consumption and smoking are also considered risk factors for colorectal cancer liver metastasis.[8] However, the specific causes of colorectal cancer liver metastasis are not yet clear. Therefore, it is particularly important to further study colorectal cancer liver metastasis and the molecular mechanisms related to its recovery.

Bioinformatics is an interdisciplinary field that utilizes knowledge from computer science, mathematics, statistics, and biology to address problems of data analysis, simulation, prediction, and understanding in biology.[9] It involves studying the genomic structure, function, and evolution of organisms; the transcription process; the composition, structure, and function of proteins; as well as the composition and metabolic pathways of metabolites.[10] Bioinformatics techniques are widely applied in basic research, drug development, medical diagnosis, and agricultural production, providing powerful tools and methods for life science research. The application of bioinformatics in the medical field is increasingly involved in personalized medicine and precision treatment. By analyzing individual genomic information, epigenomic information, and other data, more precise diagnostic, preventive, and treatment plans can be provided for diseases.[11]

Currently, the relationship between complexin 2 (CPLX2), synaptosome-associated protein 25 (SNAP25) genes, and colorectal cancer liver metastasis and its recovery is not clear. Therefore, this study aims to use bioinformatics techniques to explore the core genes between colorectal cancer liver metastasis and primary colorectal cancer and to perform enrichment analysis and pathway analysis. Public datasets will be used to verify the significant role of CPLX2 and SNAP25 genes in the recovery from colorectal cancer liver metastasis.

2. Methods

2.1. Colorectal cancer liver metastasis dataset

In this study, the colorectal cancer liver metastasis datasets GSE147602 and GSE144259 were downloaded from the gene expression omnibus database (https://www.ncbi.nlm.nih.gov/geo/) generated from GPL21047 and GPL11154 platforms. GSE147602 includes 10 primary colorectal cancer and 10 colorectal cancer liver metastasis samples. GSE144259 includes 3 primary colorectal cancer and 3 colorectal cancer liver metastasis samples. These were used to identify differentially expressed genes (DEGs) in colorectal cancer liver metastasis.

2.2. Remove batch effect

For the merging and batch normalization of multiple datasets, the R packages were first utilized to merge the datasets GSE147602 and GSE144259. The datasets were merged using the R package in SilicoMerging (DOI: 10.1186/1471-2105-13-335), resulting in a combined matrix. Further, we used the remove batch effect function from the R package limma (version 3.42.2) to remove batch effects, obtaining a batch effect-removed matrix for subsequent analysis.

2.3. Selection of differentially expressed genes

The R package "limma" was used for probe summarization and background correction of the combined matrix from GSE147602 and GSE144259. The Benjamini-Hochberg method was employed to adjust the raw *P* values. Fold change (FC) was calculated using the false discovery rate (FDR). The cutoff for DEGs was set at $P < .05$ and $FC > 1.2$. Volcano plots were generated for visual representation.

2.4. Weighted gene co-expression network analysis

Initially, the median absolute deviation of each gene's expression profile was calculated, and genes in the lowest 50% of median absolute deviation values were discarded. The R package weighted gene co-expression network analysis (WGCNA's) good Samples Genes method was used to remove outlier genes and samples. A scale-free co-expression network was then constructed using WGCNA. CIBERSORT To further analyze the modules, we calculated the differences between the module eigengenes, selected a cutline for the module dendrogram, and merged some of the modules. Additionally, modules with a distance of less than 0.25 were merged. Notably, the gray module was considered to be a collection of genes that could not be assigned to any module.

2.5. Construction and analysis of the protein–protein interaction network

The Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/) aims to collect, score, and integrate all publicly available sources of protein–protein interaction (PPI) information, and to complement these sources with computational predictions. In this study, the list of differentially expressed genes was input into the STRING database to construct a PPI network of predicted core genes (confidence > 0.4). Cytoscape software provides biologists with analysis and two-dimensional visualization of biological networks. This study visualized and predicted core genes through the PPI network formed by the STRING database using Cytoscape software. First, we imported the PPI network into Cytoscape software, calculated the most relevant genes using 5 algorithms (maximal clique centrality, maximum neighborhood component, edge permeability component, closeness, and stress) separately, and took their intersection. The core gene list was then exported after visualization.

2.6. Functional enrichment analysis

Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis are computational methods for evaluating gene functions and biological pathways. This study input the list of differentially expressed genes into the KEGG API (https://www.kegg.jp/kegg/rest/keggapi.html), obtained the latest gene annotations of the KEGG pathway, and used this as a background to map genes to the background set. The R package clusterProfiler (version 3.14.3) was used for enrichment analysis to obtain the results of gene set enrichment. The GO annotations of genes in the R package org.Hs.eg.db (version 3.1.0) were also used as a background to map genes to the background set, setting the minimum gene set to 5 and the maximum gene set to 5000. A *P* value of < 0.05 and an FDR of < 0.25 were considered statistically significant measures.

Moreover, the Metascape database provides comprehensive gene list annotation and analysis resources and allows for visualization export. We used the Metascape database (https://metascape.org/gp/index.html) for functional enrichment analysis of the aforementioned list of differentially expressed genes and exported the results.

2.7. Gene set enrichment analysis

For gene set enrichment analysis (GSEA), we obtained the GSEA software (version 3.0) from the GSEA website (DOI:10.1073/pnas.0506580102, https://software.broadinstitute.org/gsea/index.jsp). The samples were divided into 2 groups according to primary colorectal cancer and colorectal liver metastasis, and the c2.cp.kegg.v7.4.symbols.gmt subset was downloaded from the Molecular Signatures Database (DOI:10.1093/bioinformatics/btr260, https://www.gsea-msigdb.org/gsea/downloads.jsp) to evaluate related pathways and molecular mechanisms based on gene expression profiles and phenotypic grouping. With a minimum gene set of 5 and a maximum of 5000, one thousand permutations, a P value of < 0.05, and an FDR of < 0.25 were considered statistically significant. GO and KEGG analyses were conducted on the whole genome by GSEA.

2.8. Gene expression heatmap

We used the R package heatmap to create a heatmap of the expression levels of core genes found by the 5 algorithms in the PPI network in GSE147602 and GSE144259, visualizing the expression differences between primary colorectal cancer and colorectal liver metastasis among the core genes.

2.9. Immune infiltration analysis

CIBERSORT (https://CIBERSORT.stanford.edu/) is a commonly used computational method for estimating immune cell infiltration. The LM22 gene file is used to define 22 immune cell subgroups. We applied an integrated bioinformatics approach and used the CIBERSORT software package to analyze the batch-corrected combined matrix of GSE147602 and GSE144259. Using the principle of linear support vector regression, the expression matrix of immune cell subtypes was deconvolved to estimate the abundance of immune cells. A confidence level of $P < .05$ was used as a cutoff criterion to filter samples with sufficient confidence.

2.10. Comparative Toxicogenomics Database analysis

The Comparative Toxicogenomics Database (CTD) integrates a large amount of data on interactions between chemicals, genes, functional phenotypes, and diseases, providing great convenience for research on disease-related environmental exposure factors and potential mechanisms of drugs. We input the core genes into the CTD website, found the diseases most related to the core genes, and used Excel to draw a radar chart of the expression differences for each gene.

2.11. miRNA

TargetScan (www.targetscan.org) is an online database for predicting miRNAs and their target genes. In our study, TargetScan was used to screen for miRNAs regulating the central DEGs.

2.12. Real-time quantitative polymerase chain reaction

This study validated the regulatory roles of CPLX2 and SNAP25 in colorectal cancer liver metastasis through in vitro experiments. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to analyze 4 cell models: primary colorectal cancer group (SW480), high-metastasis group (LoVo), SW480 dual-gene overexpression group (SW480-CPLX2&SNAP25_OE), LoVo dual-gene knockdown group (LoVo-CPLX2&SNAP25_KO).

2.12.1. RNA extraction and qPCR analysis. Discard cell culture medium, wash cells with phosphate buffered saline, and lyse cells using RNA extraction reagent (Servicebio, Cat# G3013). Transfer lysate to a centrifuge tube and incubate for 5 minutes. Add chloroform, vortex vigorously, and centrifuge at 12,000 rpm, 4°C for 15 minutes. Collect the aqueous phase, mix with isopropanol for RNA precipitation, and centrifuge at 12,000 rpm, 4°C for 10 minutes to pellet RNA. Wash the RNA pellet with 75% ethanol, air-dry, and dissolve the RNA in DEPC-treated water.

2.12.2. RNA quality control. Measure RNA concentration and purity using a Nanodrop 2000 spectrophotometer (A260/A280 ratio).

2.12.3. cDNA synthesis. Remove genomic DNA and perform reverse transcription using a cDNA synthesis kit (Servicebio, Cat# G3331-50) following the manufacturer's protocol.

2.12.4. qPCR amplification. Prepare reactions with 2× qPCR MasterMix (Servicebio, Cat# G3326-01), cDNA template, and gene-specific primers. Each sample was run in technical triplicates.

2.12.5. Cycling conditions. Initial denaturation: 95°C for 5 minutes,40 cycles: 95°C for 10 sec, 60°C for 30 sec. Fluorescence data were collected using a StepOne Real-Time PCR System, and melt curve analysis was performed to validate product specificity.

3. Results

3.1. Analysis of differentially expressed genes

In this study, differential gene expression analysis was conducted based on the predefined cutoff values, and a total of 1215 DEGs were identified from the batch-corrected merged matrices of GSE147602 and GSE144259 (Fig. 1).

3.2. Functional enrichment analysis

3.2.1. Differentially expressed genes. The identified DEGs underwent GO and KEGG pathway analysis. According to the GO analysis, they were mainly enriched in cell signaling, G-protein-coupled receptor signaling pathway, signal transduction receptor binding, and cytokine binding (Fig. 2A–C). KEGG analysis revealed enrichment in cholesterol metabolism and olfactory transduction pathways (Fig. 2D).

3.2.2. Gene set enrichment analysis. GSEA was performed on the entire genome to identify potential enrichments among nondifferentially expressed genes and validate the results of differential gene expression. The intersection of enrichment terms with GO and KEGG enrichment terms of DEGs showed significant enrichment in cell signaling, G-protein-coupled receptor signaling pathway, signal transduction receptor binding, and cytokine binding (Fig. 2E–H).

3.2.3. Metascape enrichment analysis. Metascape enrichment analysis revealed GO enrichment terms including blood circulation regulation, muscle structure development, vascular processes in the circulatory system, and extracellular matrix organization (Fig. 3A). Additionally, enrichment networks colored by enrichment terms and P values were visualized

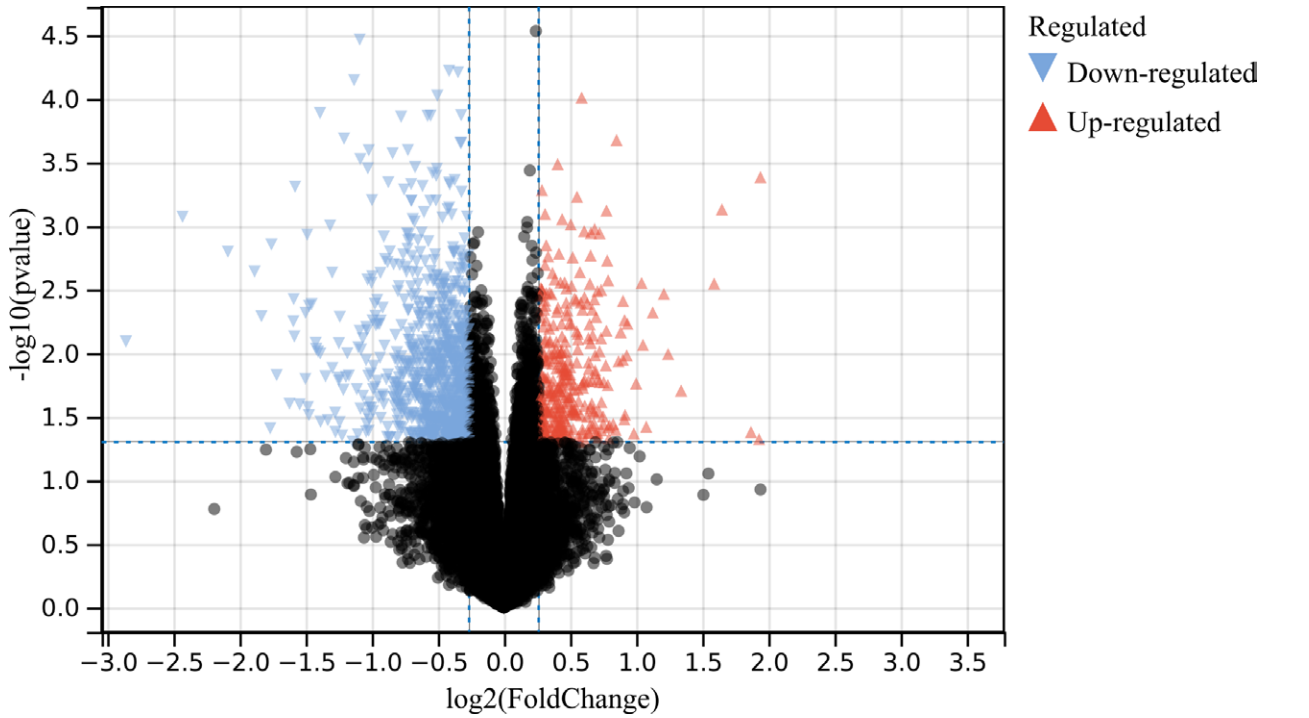

Figure 1. Analysis of differentially expressed genes. A total of 1215 DEGs were identified. DEG = differentially expressed genes.

(Fig. 3B,C), representing the associations and confidence levels of various enrichment terms.

3.3. Weighted gene co-expression network analysis

The selection of soft-thresholding power is a crucial step in WGCNA. A soft-thresholding power of 4 was set for WGCNA, as determined by network topology analysis (Fig. 4A,B). Hierarchical clustering trees of all genes were constructed, important modules were identified, and interactions between these modules were analyzed (Fig. 4C,D). Heatmaps of module-trait correlations (Fig. 5A) and scatterplots of correlations between module eigengenes and gene significance with clinical traits (Fig. 5B–D) were generated. Based on a cutoff criterion ($|MM| > 0.8$), 474 highly interconnected genes were identified as hub genes in clinically significant modules. A Venn diagram was generated to show the intersection of WGCNA and DEGs, which was used for the creation and analysis of PPI networks (Fig. 5E).

3.4. Construction and analysis of protein–protein interaction network

The PPI network of DEGs was constructed using the STRING online database and analyzed using Cytoscape software (Fig. 6A). Five different algorithms were utilized to identify hub genes, and their intersection was visualized using a Venn diagram (Fig. 6B–G), resulting in the identification of 4 core genes (SNAP25, SYT1, CPLX2, and Bassoon [BSN]).

3.5. Gene expression heatmaps

Expression heatmaps of core genes in samples were visualized (Fig. 7A), revealing low expression of core genes (CPLX2, SNAP25, and BSN) in primary colorectal cancer and high expression in colorectal cancer liver metastasis, indicating potential regulatory roles in the recovery of colorectal cancer liver metastasis.

3.6. Comparative Toxicogenomics Database analysis

In this study, core gene lists were input into the CTD website to search for diseases associated with core genes, enhancing understanding of gene-disease associations. Three genes (CPLX2, SNAP25, and BSN) were found to be associated with abdominal pain, jaundice, chemical and drug-induced liver injury, and necrosis (Fig. 7B–D).

3.7. Immune infiltration analysis

We used the CIBERSORT software package to analyze the debatch-based merging matrices of GSE147602 and GSE144259 and obtained the proportion results of immune cells in the full gene expression matrix (Fig. 8A) and the heat map of immune cell expression in the dataset (Fig. 8B) with 95% confidence interval. The co-expression correlation analysis of infiltrating immune cells was also performed, and the co-expression patterns among immune cell components were obtained (Fig. 8C). The results showed that T_cells_CD4_memory_resting accounted for a higher proportion, and Mast_cells_activated was more common in colorectal cancer liver metastases than in primary colorectal cancer liver metastases. Mast_cells_activated and T_cells_regulatory_(Tregs) had a strong correlation.

3.8. Prediction and functional annotation of miRNAs related to hub genes

In this study, we input the list of hub genes into TargetScan to identify related miRNAs, thereby enhancing our understanding of gene expression regulation (Table 1). We found that the miRNAs related to the CPLX2 gene are hsa-miR-1-3p, hsa-miR-206, and hsa-miR-613; the miRNAs related to the SNAP25 gene are hsa-miR-181d-5p, hsa-miR-181b-5p, and hsa-miR-181c-5p; the miRNAs related to the BSN gene are hsa-miR-140-3p.1, hsa-miR-140-3p.1.

3.9. Real-time quantitative polymerase chain reaction

RT-qPCR results revealed that CPLX2 and SNAP25 exhibited basal low expression in low-metastatic SW480 cells but were

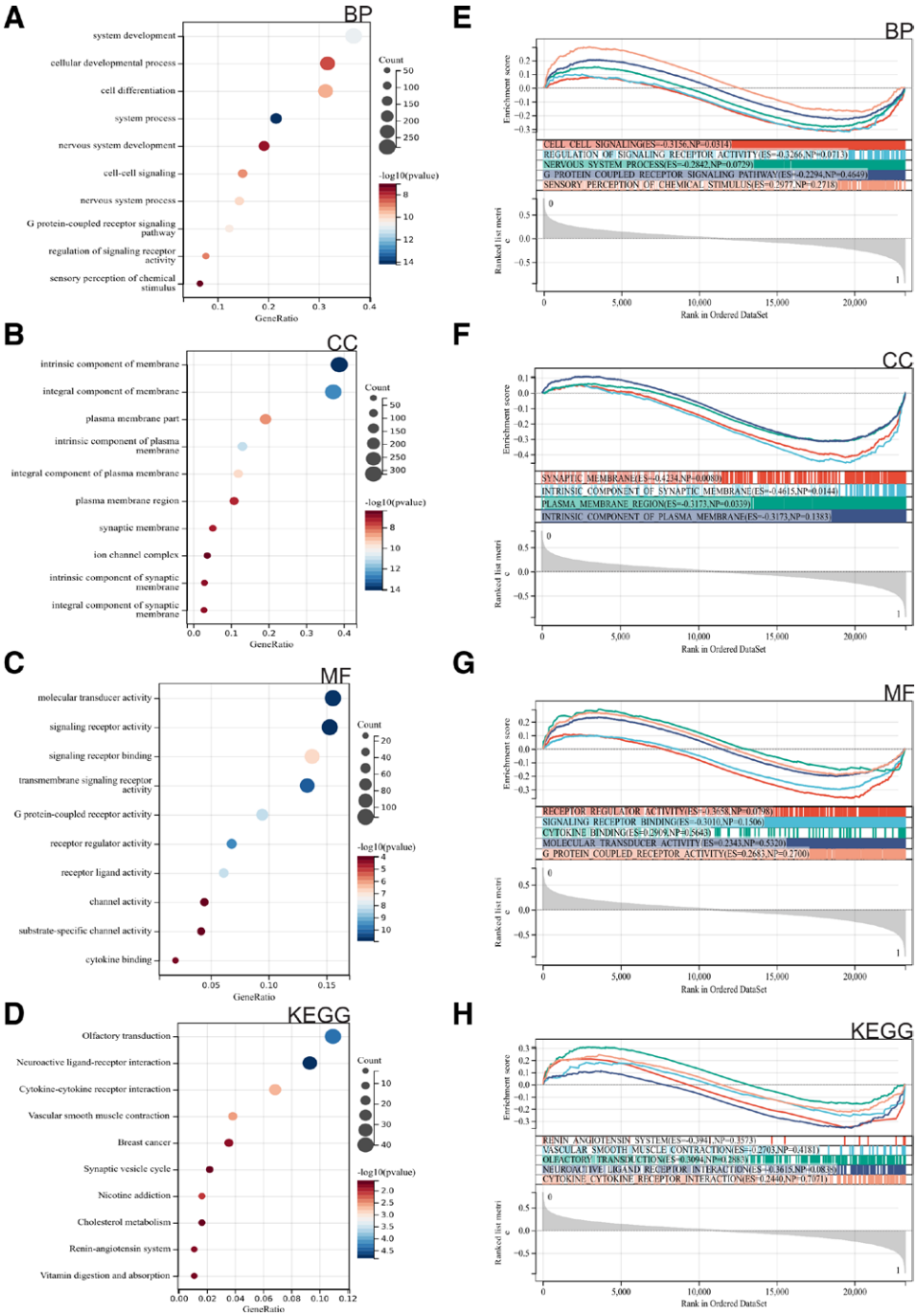

Figure 2. Functional enrichment analysis. (A–C) GO analysis. (D) KEGG analysis. (E–H) GSEA. GSEA = gene set enrichment analysis, KEGG = Kyoto Encyclopedia of Gene and Genome.

highly expressed in high-metastatic LoVo cells. Compared with the SW480 group, the expression of metastasis-related molecules in LoVo cells showed significant changes: epithelial marker E-cadherin was markedly downregulated ($P < .0001$), mesenchymal markers vimentin and matrix metalloproteinase 9 (MMP9) were significantly upregulated ($P < .0001$). The key molecule phosphatidylinositol 3 kinase (PI3K) in the PI3K-AKT signaling pathway was also significantly elevated in the LoVo group compared with SW480 ($P < .0001$). These findings align with the bioinformatics analysis results.

Furthermore, in the SW480 overexpression group: overexpression of CPLX2 and SNAP25 led to significant upregulation of PI3K, MMP9, and vimentin ($P < .001$), E-cadherin was notably downregulated ($P < .001$). Conversely, in the LoVo knockdown group (LoVo-CPLX2&SNAP25_KO): Dual-gene silencing significantly decreased the expression of PI3K, MMP9, and vimentin ($P < .001$), E-cadherin expression was restored to basal levels ($P < .001$). These results confirm that CPLX2 and SNAP25 positively regulate the PI3K-AKT signaling pathway, promote metastatic progression, and act as key drivers of colorectal cancer liver metastasis (Fig. 9).

4. Discussion

Colorectal cancer liver metastasis refers to the process where malignant tumors originating from the colon grow

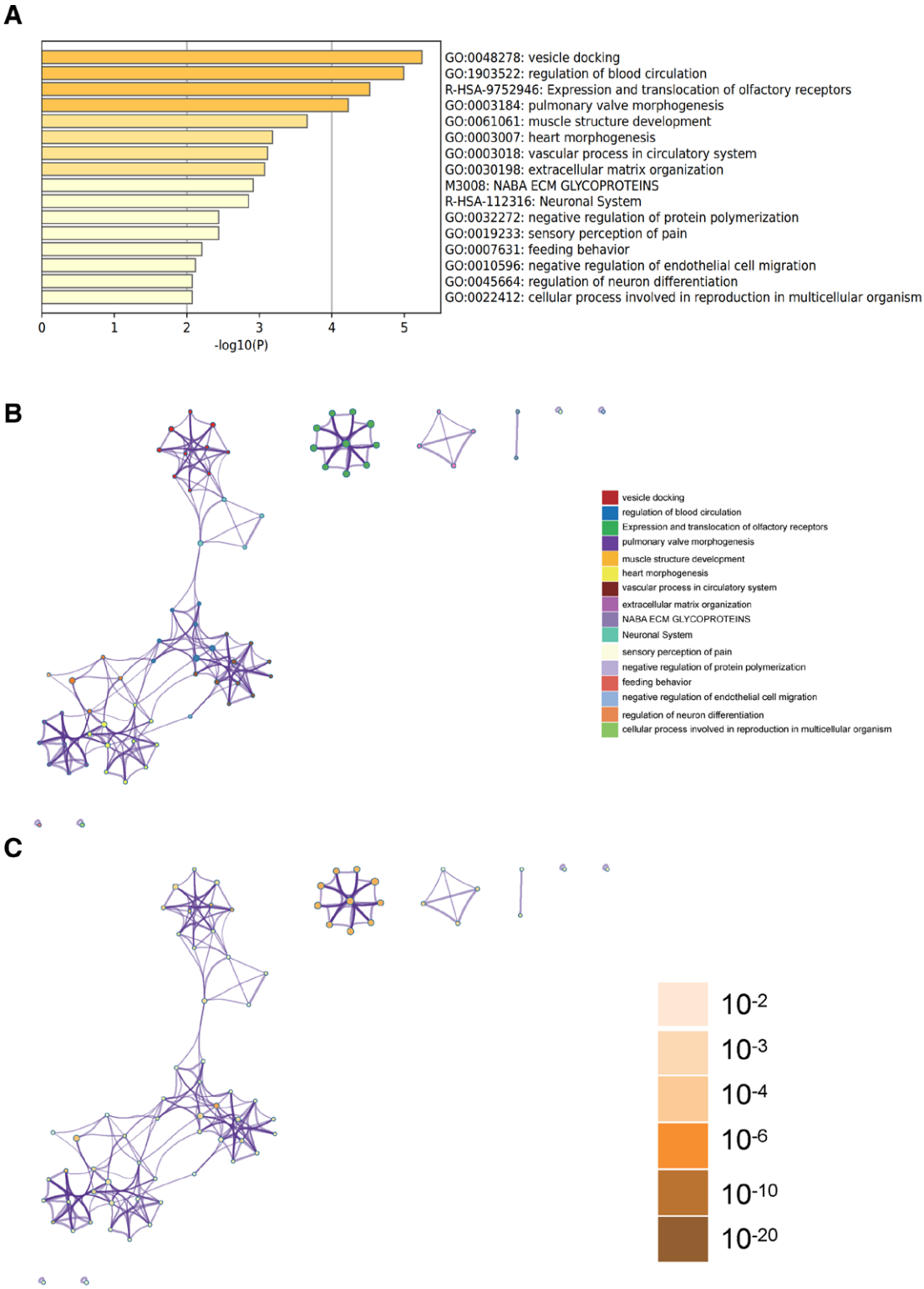

Figure 3. Metascape enrichment analysis. (A) Metascape enrichment analysis revealed GO enrichment terms including blood circulation regulation, muscle structure development, vascular processes in the circulatory system, and extracellular matrix organization. (B) The enrichment networks colored by enrichment terms were visualized. (C) The enrichment networks colored by *P* values were visualized. GO = gene ontology.

and metastasize to the liver. Patients with colorectal cancer liver metastasis typically have poorer prognosis and lower survival rates, posing serious health hazards.[12] Exploring the molecular mechanisms related to colorectal cancer liver metastasis and its recovery is crucial, particularly for targeted drug research. The main findings of this study indicate that the CPLX2 and SNAP25 genes are upregulated in colorectal cancer liver metastasis, and higher expression of CPLX2 and SNAP25 genes is associated with worse prognosis.

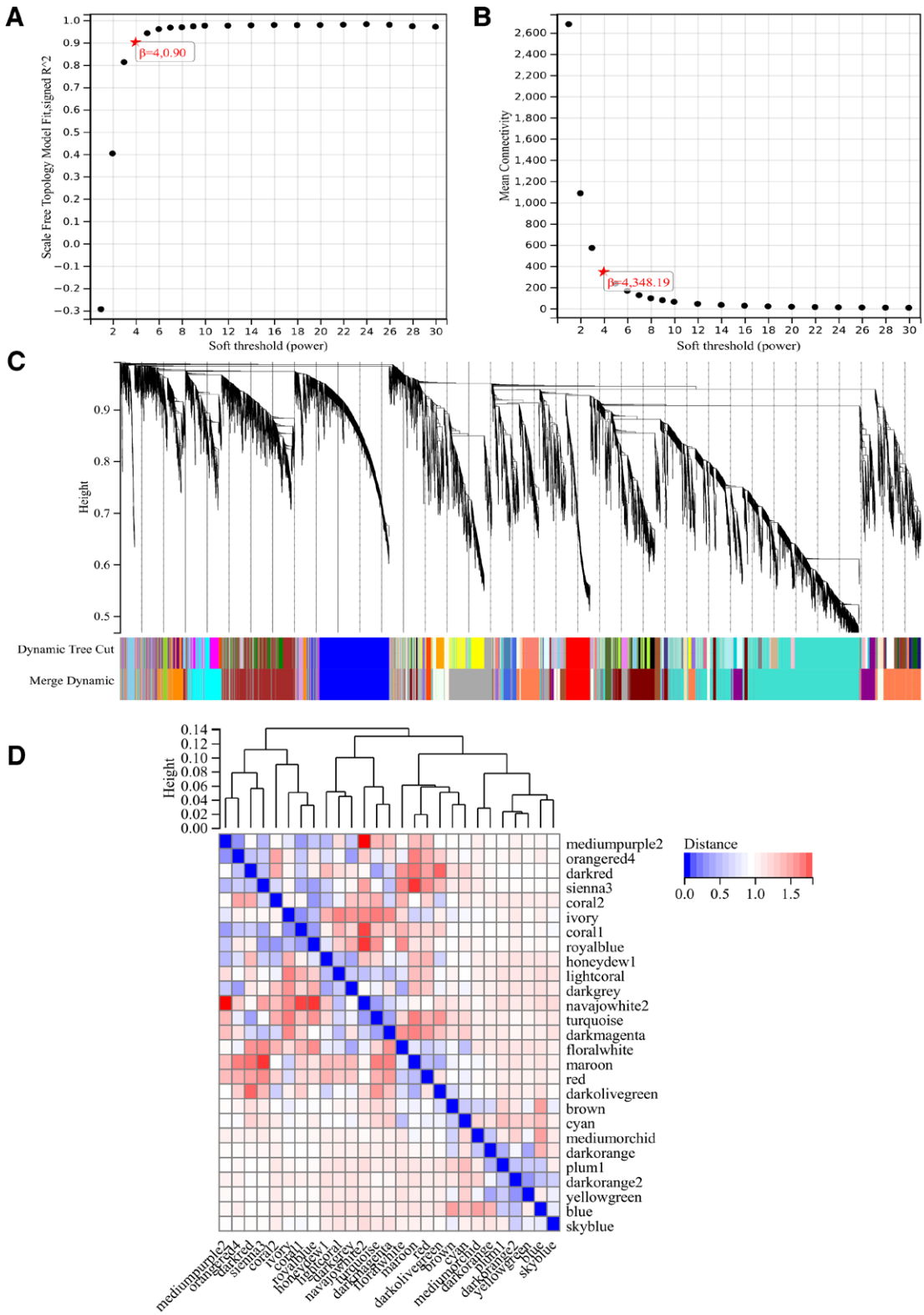

Figure 4. WGCNA. (A and B) A soft-thresholding power of 4 was set for WGCNA. (C) Hierarchical clustering trees of all genes were constructed, and important modules were identified. (D) The interactions between these modules. WGCNA = weighted gene co-expression network analysis

The CPLX2 gene encodes a synaptic protein called CPLX2, which is crucial for synaptic vesicle fusion and participates in the regulation of neurotransmitter release processes.[13] Neurotransmitter release is vital for normal neuronal functioning and synaptic communication.[14] Through interaction with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, CPLX2 regulates the timing and rate of synaptic vesicle release, closely linking neurotransmitter release to neuronal signaling.[15]

CPLXs are a group of proteins that regulate calcium-triggered fusion between vesicles and the plasma membrane by interacting with SNARE complexes. CPLXs have 4 subtypes;

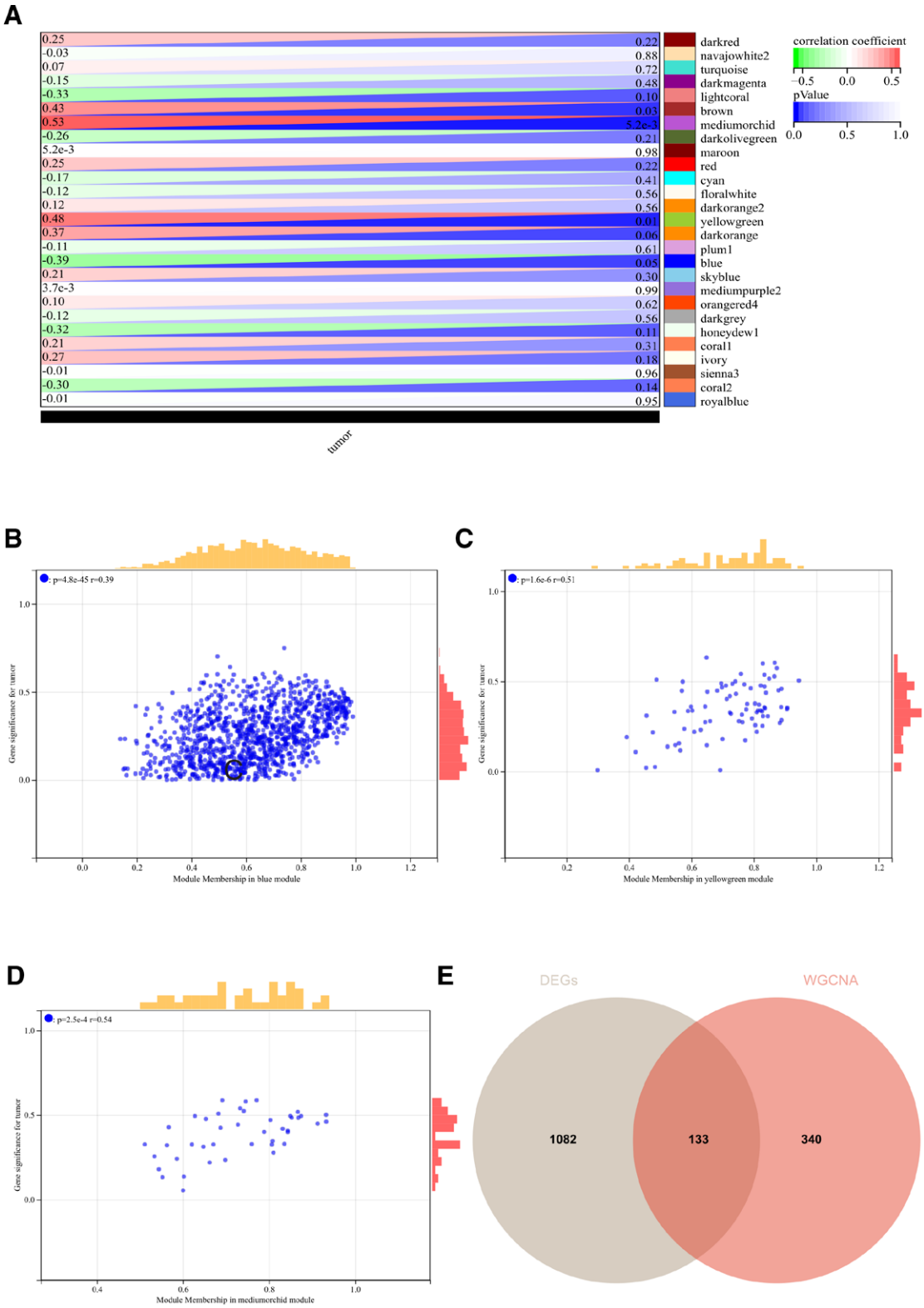

Figure 5. WGCNA. (A) Heatmaps of module-trait correlations. (B–D) Scatterplots of correlations between module eigengenes (MEs) and gene significance (GS) with clinical traits were generated. (E) A Venn diagram was generated to show the intersection of WGCNA and DEGs, which was used for the creation and analysis of protein-protein interaction networks. DEG = differentially expressed genes, WGCNA = weighted gene co-expression network analysis.

CPLX1 is specifically expressed in the brain, while CPLX2 is expressed in the brain and other secretory cells. CPLX3 and CPLX4 are also specifically expressed in the retina. CPLXs are found to inhibit spontaneous fusion and activate fast calcium-triggered fusion simultaneously. They tightly bind to SNARE complexes, preventing membrane fusion. CPLXs are also present outside the nervous system, such as in sperm, pancreatic secretory cells, and peripheral mast cells. The concentration of CPLX2 is closely related to vesicle fusion, responding to insulin redistribution to muscle cell membranes

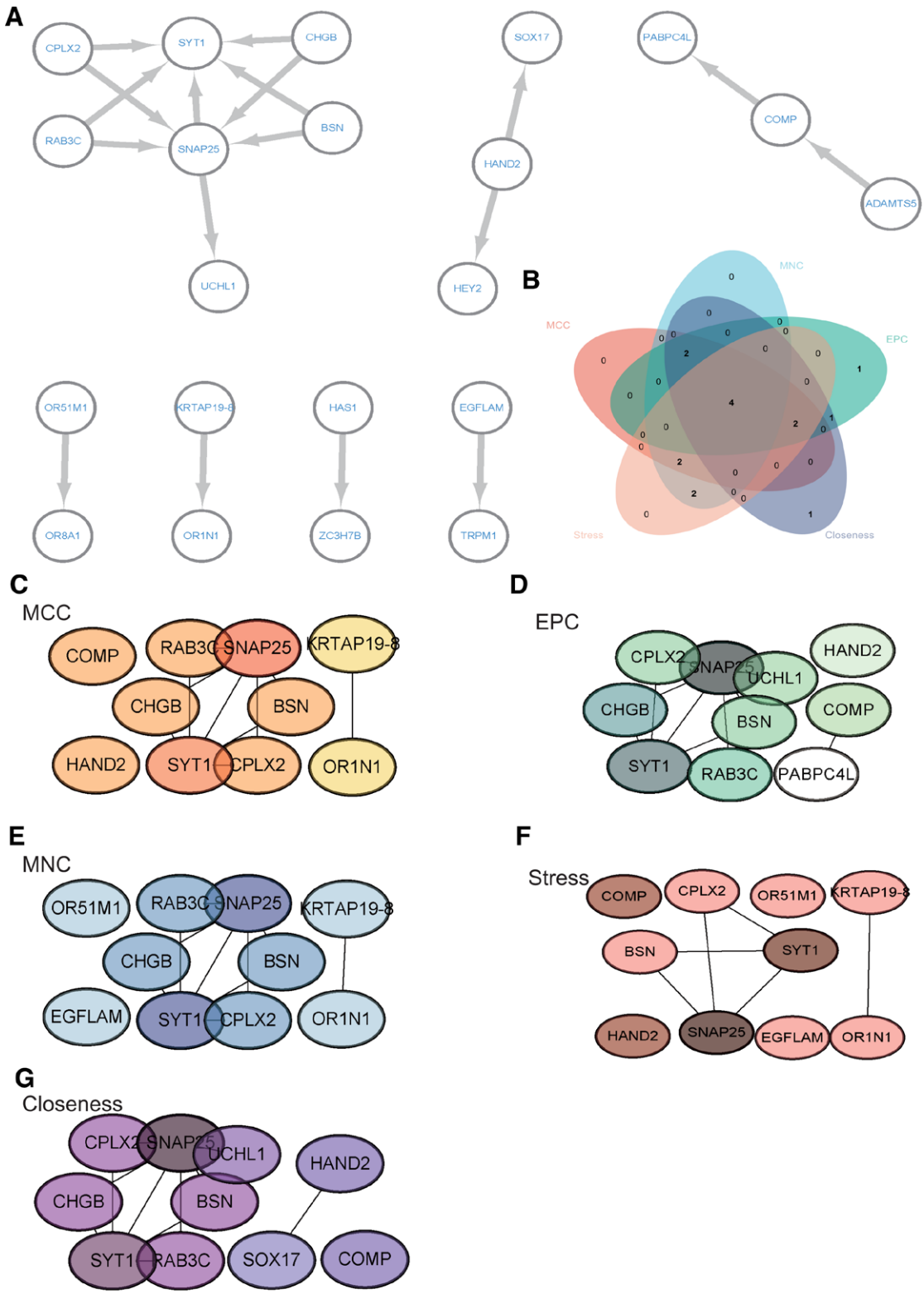

Figure 6. Construction and analysis of protein–protein interaction (PPI) network. (A) The PPI network of DEGs. (B–G) Five different algorithms were utilized to identify hub genes, and their intersection was visualized using a Venn diagram. DEG = differentially expressed genes.

and promoting GLUT4 translocation.[16] Mutations or aberrant expression of the CPLX2 gene may impact neuronal function,[17] leading to neurological disorders such as epilepsy and Parkinson's disease.

CPLX2 regulates the secretion of immunoglobulins in antibody-secreting cells[18] and is involved in cancer progression. Studies have suggested that CPLX2 regulates iron death and apoptosis through the NRF2 pathway, knocking down CPLX2 promotes sorafenib-induced cell death, suggesting CPLX2 as a potential therapeutic target for liver cancer.[19] Colorectal cancer liver metastasis is usually due to the spread of primary cancer cells to the liver through the bloodstream or lymphatic system,

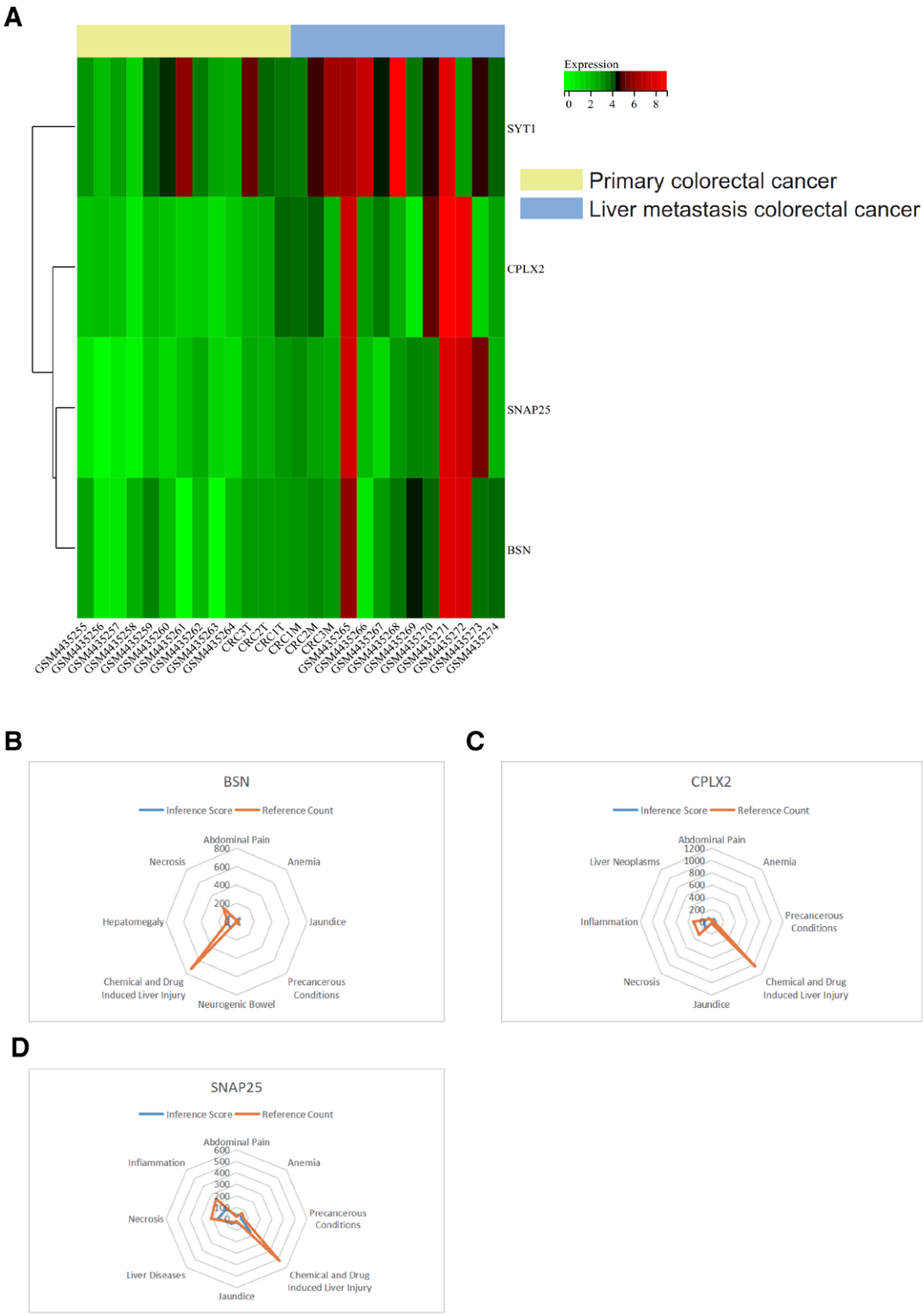

Figure 7. (A) Gene expression heatmaps. Expression heatmaps of core genes in samples. (B–D) CTD analysis. CPLX2, SNAP25, and BSN were associated with abdominal pain, jaundice, chemical and drug-induced liver injury, and necrosis. BSN = Bassoon, CPLX2 = complexin 2, CTD = Comparative Toxicogenomics Database, SNAP25 = synaptosome-associated protein 25.

forming metastatic tumors, involving multiple factors including tumor cell invasion, angiogenesis, and immune evasion. Therefore, it is speculated that the CPLX2 gene may play an important role in the recovery process of colorectal cancer liver metastasis.

SNAP25 is located on human chromosome 20, mainly present on the presynaptic membrane, and is a protein associated with synaptic vesicles, playing a crucial role in the regulation of neurotransmitter release in the nervous system.[20] During neurotransmitter release, SNAP25 interacts with SNARE proteins

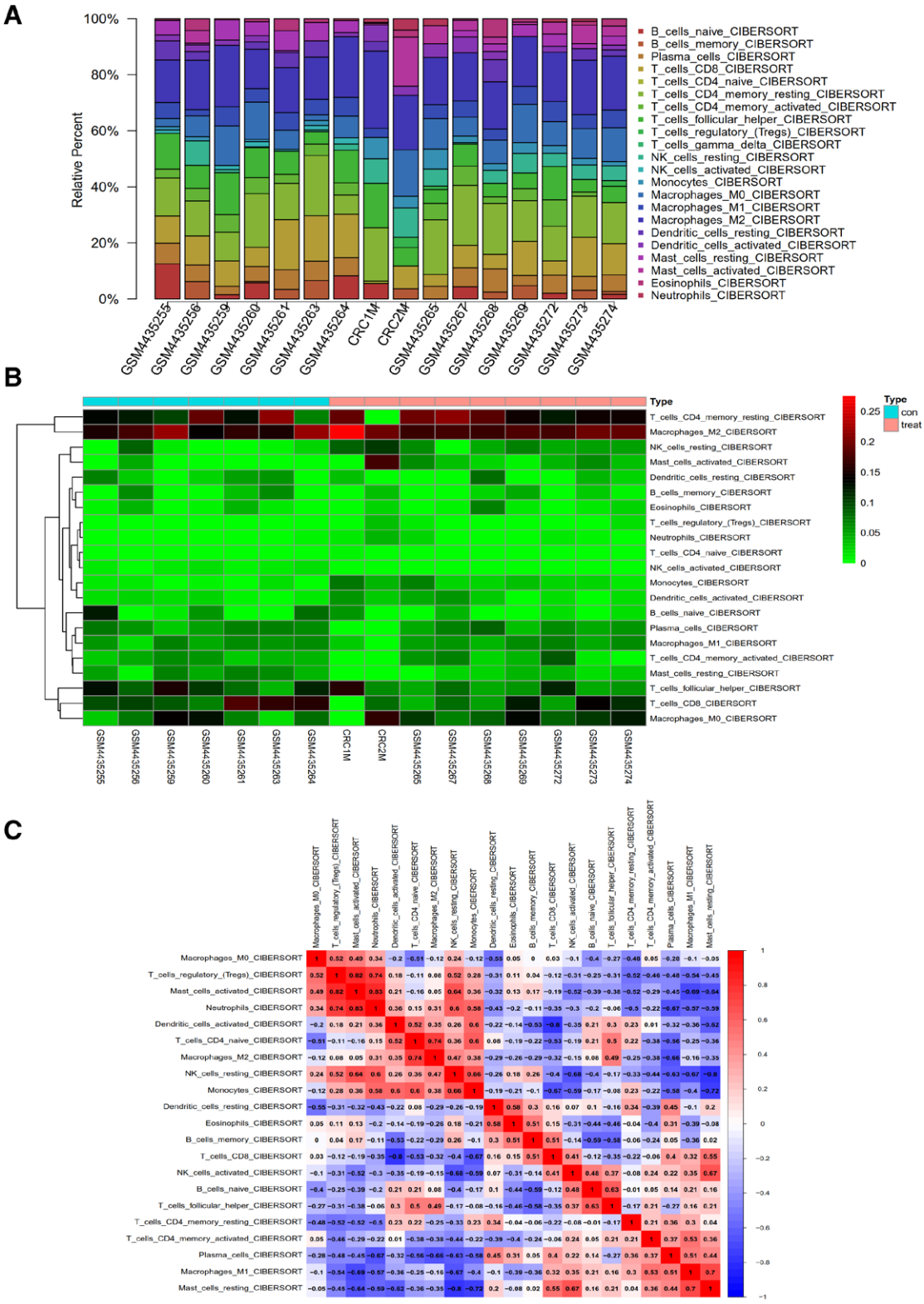

Figure 8. Immune infiltration analysis. (A)The proportion results of immune cells in the full gene expression matrix. (B) The heat map of immune cell expression in the dataset. (C) The co-expression patterns among immune cell components.

on the presynaptic membrane to promote fusion between synaptic vesicles and the presynaptic membrane, releasing neurotransmitters into the synaptic cleft. Aberrant expression or dysfunction of SNAP25 may affect neuronal signal transmission and be associated with the occurrence and development of various neurological disorders.[21] Novel mutations in SNAP25 can lead to early-onset developmental and epileptic encephalopathy.[22]

The protein encoded by the SNAP25 gene is an essential member of the synaptic vesicle fusion protein complex on the presynaptic membrane. As part of the SNARE complex, SNAP25 interacts with proteins on the presynaptic membrane

Table 1

A summary of miRNAs that regulate hub genes.

	Gene	MiRNA		
1	CPLX2	hsa-miR-1-3p	hsa-miR-206	hsa-miR-613
2	SNAP25	hsa-miR-181d-5p	hsa-miR-181b-5p	hsa-miR-181c-5p
3	BSN	hsa-miR-140-3p.1	hsa-miR-140-3p.1	

BSN = Bassoon, CPLX2 = complexin 2, SNAP25 = synaptosome-associated protein 25.

to facilitate neurotransmitter release, crucial for normal neuronal function, affecting synaptic signaling.[23] SNAP25 protects primary cortical neurons from hypoxic-ischemic injury associated with CREB signaling.[24] SNAP25 is a key gene regulating dopamine secretion in neurons. Lack of SNAP25 can lead to impaired dopamine secretion and excessive accumulation.[25] SNAP25 belongs to the SNARE complex, critical for mediating synaptic vesicle fusion and exocytosis, regulating minimal fusion mechanisms, intercellular signaling, and ion channel

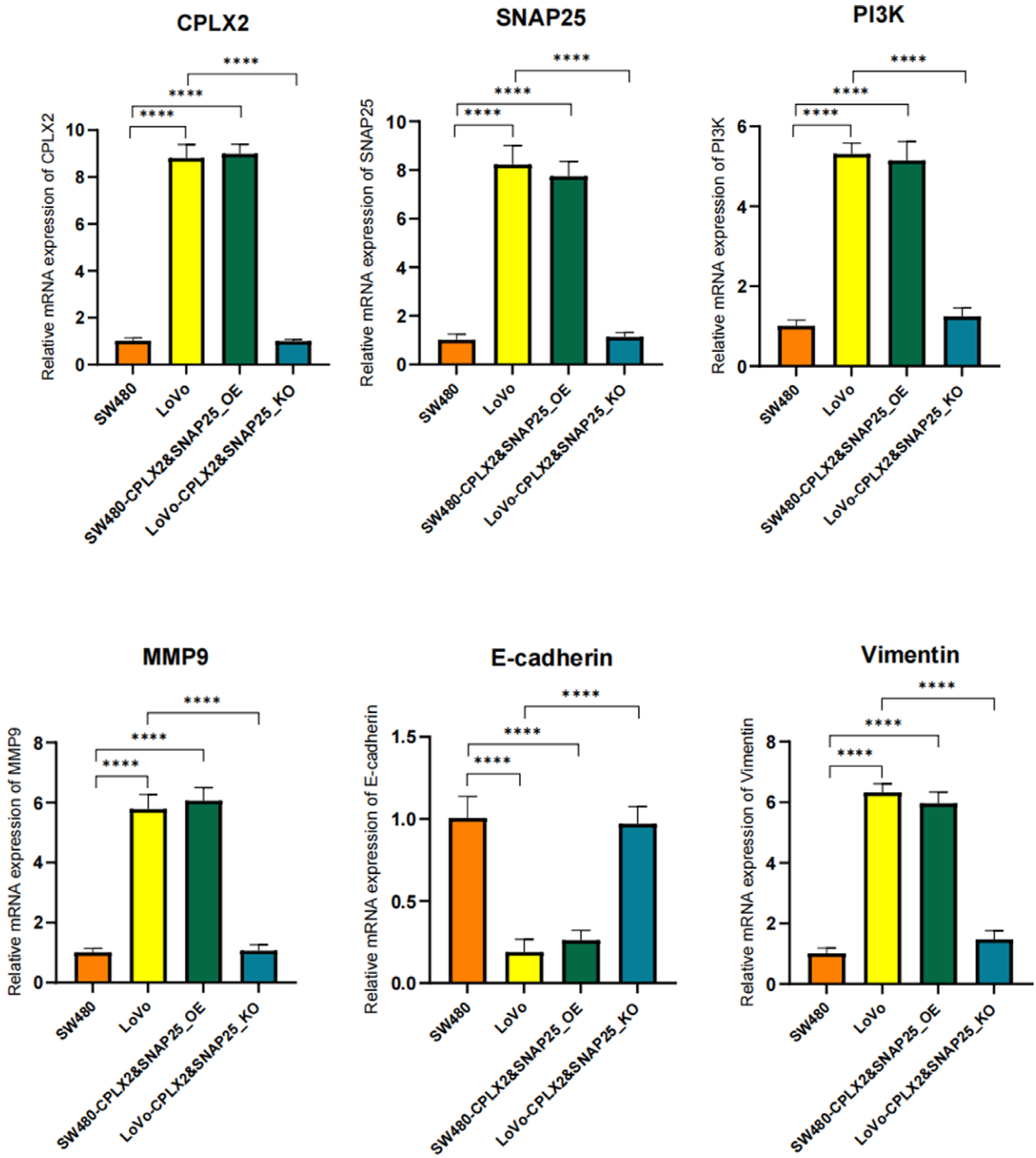

Figure 9. Through RT-qPCR analysis, the effects of overexpression or knockdown of genes CPLX2 and SNAP25 on the expression of metastasis-related genes (E-cadherin, Vimentin, MMP9) were investigated. (* indicates statistically significant differences). CPLX2 = complexin 2, MMP9 = matrix metalloproteinase 9, RT-qPCR = real-time quantitative polymerase chain reaction, SNAP25 = synaptosome-associated protein 25.

modulation. SNAP25 is the only SNARE protein that can localize to the outer mitochondrial membrane and may participate in neuronal mitochondrial autophagy.[26] SNAP25 is involved in B cell receptor signaling pathways, cell adhesion molecule signaling pathways, chemokine signaling pathways, complement, and coagulation cascade reactions, T cell receptor signaling pathways, adipokine signaling pathways, aldosterone-regulated sodium reabsorption, glycosaminoglycan biosynthesis heparan sulfate, and insulin signaling pathways. SNAP25 mutations disrupt metabolic homeostasis, steroid hormone production, and central nervous behavior.[27]

SNAP25's expression in other cells and tissues suggests its role beyond the nervous system, possibly involving processes such as cell adhesion, migration, and differentiation.[28] Therefore, the SNAP25 gene plays a critical role in maintaining normal nervous system function and other biological processes. SNAP25 is a member of the SNARE family and a membrane-binding protein in neurons, associated with severe synaptic diseases such as schizophrenia and protein diseases such as Alzheimer's disease.[29,30] SNAP25 is a potential factor in normal vesicle fusion and lysosomal transport. Significant correlation of SNAP25 with lymphocytes (natural killer, macrophages, mast cells, and natural killer T) suggests a potential association between the tumor microenvironment and SNAP25. Abnormal expression of the SNAP25 gene and its encoded protein in tumors may be related to tumor invasiveness and metastatic ability. Studies have shown that SNAP25 is not only closely related to the clinical manifestations of prostate cancer but also involved in tumor-related signaling pathways and immune and metabolic processes, providing new targets for studying the potential mechanisms of prostate cancer.[31] SNAP25 is a microenvironment-related and immune-related gene that can predict adverse outcomes in colon cancer. Bioinformatics analysis suggests that SNAP25 is involved in tumor-related signaling pathways, immunity, and metabolism, providing new targets for studying the potential mechanisms of colorectal cancer.[32] Therefore, it is speculated that the SNAP25 gene may play an important role in the progression of colorectal cancer liver metastasis recovery.

The results of this study have potential clinical implications. Targeted therapy or biomarkers can be developed to detect colorectal cancer liver metastasis. The high expression of CPLX2 and SNAP25 genes can be used as biomarkers. By detecting the expression levels of related genes in patients, it is helpful to detect colorectal cancer liver metastasis in the early stage of the disease and improve the accuracy of diagnosis. For some patients with colorectal cancer who are difficult to determine whether there is liver metastasis by imaging or other examination methods, detection of CPLX2 and SNAP25 gene expression can assist doctors in differential diagnosis, determine whether liver lesions are colorectal cancer metastases, and avoid misdiagnosis and missed diagnosis. The highly expressed CPLX2 and SNAP25 genes can be used as potential therapeutic targets. The development of targeted drugs targeting these two genes or their related signaling pathways can act on tumor cells more accurately, inhibit the proliferation, invasion, and metastasis of tumor cells, improve the therapeutic effect, and reduce the damage to normal tissues. Understanding the expression of CPLX2 and SNAP25 genes in colorectal cancer tissues of patients is helpful to predict the sensitivity of patients to chemotherapy drugs. For patients with high expression of these 2 genes, it may be necessary to choose a more effective chemotherapy regimen or use a combination of targeted drugs against these 2 genes to improve the efficacy of chemotherapy. For patients with low or no expression, some ineffective or side effects of chemotherapy drugs can be avoided, and individualized chemotherapy regimens can be achieved.

Although rigorous bioinformatics analysis was conducted in this study, there are still some limitations, such as database bias or limitations of the algorithms used, that are discussed.

5. Conclusion

In summary, the CPLX2 and SNAP25 genes are upregulated in colorectal cancer liver metastasis, and higher expression of CPLX2 and SNAP25 genes is associated with a worse prognosis. CPLX2 and SNAP25 play crucial roles in the recovery process of colorectal cancer liver metastasis, providing important molecular targets for further research and laying the foundation for the development of new therapeutic strategies.

Author contributions

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