Book Chapter

T-Cell-Associated RNAs in Colon Cancer

Yang Xi[1], Wu Wei[2], Pan Yuefen[3], Zhou Qing[4], Xu Jiamin[5] and Han Shuwen[6]*

[1]Department of Oncology, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, China
[2]Department of Gastroenterology, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, China
[3]Department of Oncology, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, People's R China
[4]Department of Critical Care Medicine, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, China
[5]Graduate School of Nursing, Huzhou University, China
[6]Department of Oncology, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, China

***Corresponding Author:** Han Shuwen, Department of Oncology, Huzhou Cent Hosp, Affiliated Cent Hops HuZhou University, Huzhou, Zhejiang Province, China, Email: shuwenhan985@163.com; Tel: +8605722023301

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Highlights

1. *ELK3* may be an immune-related gene in colon cancer.
2. After analysis of $CD4^+$ T cell-related ceRNA networks, the chr22-38_28785274-29006793.1—miR-106a-5p—*DDHD1* axis was used for further study.
3. After analysis of $CD8^+$ T cell-related ceRNA networks, the chr22-38_28785274-29006793.1—miR-4319—*GRHL1* axis was used for further study.
4. The $CD4^+$ T cell-related genes *ADAD1* and *DLG3* were associated with colon cancer prognosis.
5. A total of 175 chemical-target pairs in $CD4^+$ T cells and 9 in $CD8^+$ T cells were obtained.

Abstract

Background: Immune escape is one of the immunological mechanisms which lead to tumorigenesis and T cells play an important role in this process.
Objective: *T*o investigate the immune-related genes of the tumor infiltrating $CD4^+$ and $CD8^+$ T cells in colon cancer.

Methods: ESTIMATE was used to calculate the stromal and immune score of tumor samples, which were downloaded from The Cancer Genome Atlas (TCGA)-Colon Cancer (COAD). The differentially expressed genes (DEGs) in samples with high- *vs*. low- stromal and immune scores were screened, followed by functional enrichment of the overlapping DEGs. The DEGs related to the $CD4^+$ and the $CD8^+$ T cells were then screened. Moreover, the miRNA-mRNA and the lncRNA-miRNA pairs were predicted in order to construct the competing endogenous RNA (ceRNA) network. Furthermore, the chemical-gene interactions were predicted for the genes in the ceRNA network. The Kaplan-Meier survival curves were also plotted.

Results: A total of 83 stromal-related DEGs (5 up-regulated and 78 down-regulated) and 1270 immune-related DEGs (807 up-regulated and 293 down-regulated) were screened. The 79 overlapping DEGs were enriched in 39 **biological process terms.** Furthermore, a total of 79 $CD4^+$ T cell-related genes and eight $CD8^+$ T cell-related genes were screened, such as *ELK3*. Additionally, *ADAD1* and *DLG3*, which were related to $CD4^+$ T cells, were significantly associated with the prognosis of colon cancer patients. Moreover, the chr22-38_28785274-29006793.1-miR-106a-5p-*DDHD1* and chr22-38_28785274-29006793.1-miR-4319-*GRHL1* axis obtained from $CD4^+$ and $CD8^+$ T cell-related ceRNAs were considered for further study.

Conclusion: *ELK3* may be an immune-related gene in colon cancer. **The chr22-38_28785274-29006793.1-miR-106a-5p-*DDHD1* and chr22-38_28785274-29006793.1-miR-4319-*GRHL1* axis may be related to the** $CD4^+$ and the $CD8^+$ T cell infiltration in colon cancer.

Keywords

Colon Cancer; Immune; Competing Endogenous RNAs; $CD4^+$ T cells; $CD8^+$ T cells

Introduction

Colon cancer has remained one of the cancers with the highest mortality and incidence worldwide, especially in Asia [1]. Despite considerable advances in surgical and adjuvant therapy for colon cancer, the recurrence rates for stages Ⅰ-Ⅲ and stage Ⅳ patients were 30% and 65%, respectively [2]. With problems such as resistance, relapse, and metastasis occurring after traditional radiotherapy, chemotherapy and new targeted drug treatments, people have begun to recognize that tumors depict a systemic disease and not just mutations in oncogenes and inactivation of the tumor suppressor genes [3,4]. Tumors escape the immune surveillance mechanisms, which heralded the advent of tumor immunotherapy [5,6]. Immune evasion by tumors is one of the most important characteristics of a tumor formation [7]. It mainly occurs through the modification of tumor cells and changes in the tumor microenvironment. As such, understanding the mechanism of tumor immune escape give new strategies for immunotherapy [8]. The T cell-mediated immune response of anti-tumor is the basis of cancer immunotherapy and has been found to be correlated with favorable disease outcomes [9,10].

Tumor immune escape is related to the decline in T cells' ability to respond, which is mainly manifested by immune tolerance to the $CD4^+$ T cells and inhibition of activation of the $CD8^+$ T cells [11]. $CD4^+$ T helper cells can assist in the activation of naive $CD8^+$ T cells and also help to eliminate the major histocompatibility antigen class Ⅱ (MHC-Ⅱ)-negative tumor cells. However, tumor cells can induce specific immune tolerance in $CD4^+$ T cells [12]. During the antitumor immune response, $CD8^+$ T cells play a major role in directly killing tumor cells by recognizing tumor antigens. However, the local tumor microenvironment contains a large number of cytokines, which can individually or synergistically affect the activation of

cytotoxic T lymphocytes (CTL) and the sensitivity of tumor cells to the CTL activity [13]. Through the interaction of various immune elements, cancer cels may enter into a dormant state or foster tumor immune evasion, which may directly promote tumor development and progression. Therefore, we analyzed the molecular mechanisms associated with $CD4^+$ and $CD8^+$ T cells in colon cancer to explore the possibility of colon cancer immunotherapy.

In order to provide a basis for the investigation of colon cancer induced T cell-mediated immune escape, miRNA target prediction and competing endogenous RNA (ceRNA) network construction were performed. Furthermore, prognostic targets were evaluated and drug screening was performed to screen prognostic indicators and treatment plans for colon cancer immunotherapy, which may provide guidance for clinical decision-making. In our study, datasets of colon cancer samples were downloaded from The Cancer Genome Atlas (TCGA) public database. Moreover, we performed enrichment analysis on the differentially co-expressed genes (DEGs) which were correlated with stroma and immune scores. Furthermore, the $CD4^+$ and $CD8^+$ T cell-related DEGs were obtained and analysis of the protein-protein interaction (PPI) was further performed. Additionally, the ceRNA networks of $CD4^+$ and $CD8^+$ T cells were analyzed and the small chemical molecules which were related to the DEGs in the ceRNA network were predicted. Finally, a survival analysis of the $CD4^+$ and $CD8^+$ T cell-related DEGs was performed.

Materials and Methods

Data Source

The dataset GDC TCGA Colon Cancer (COAD) (version 07-19-2019) was downloaded from **TCGA (**https://xenabrowser.net/**)**, which included RNAseq FPKM data and corresponding clinical phenotype data. The dataset included 448 colon cancer samples. The data were analyzed according to the workflow illustrated in Figure 1.

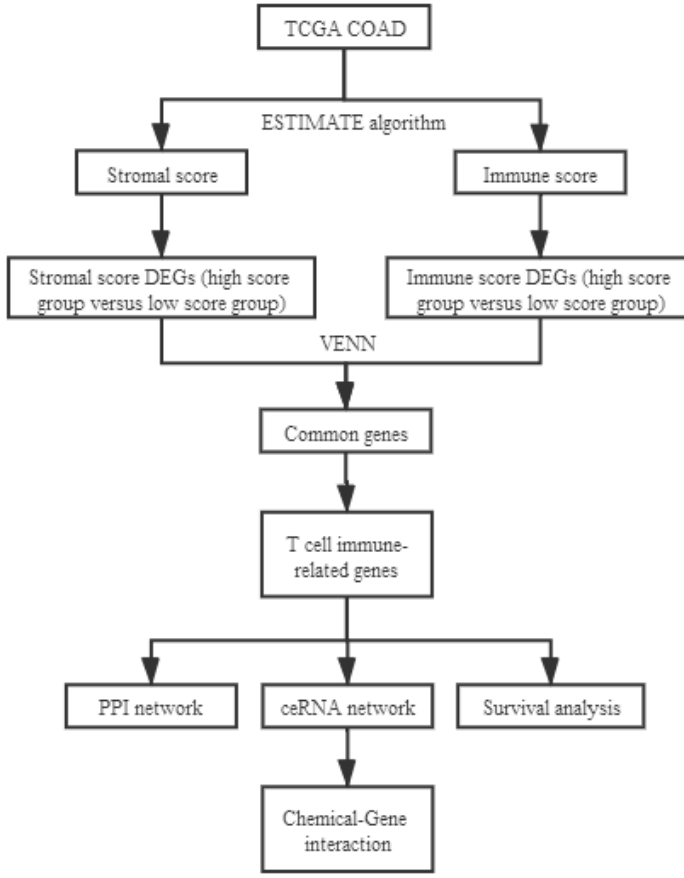

Figure 1: The workflow of sample processing used in this study.

Analysis of Stromal and Immune Scores

In the mRNA expression profile analysis, the gene expression value was calculated by mapping probe (obtained from the microarray dataset and the annotation files of the chip platform) to gene symbols. The average value was deemed as the level of mRNA expression when multiple probes matched to one symbol. For the analysis of stromal and immune cell infiltration in tumor tissues, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data (ESTIMATE, version 1.0.13)

[14] in the R package was used for tumor purity predictions. Thus, the stromal and immune scores of each tumor sample were obtained.

Analysis of the differentially Expressed Genes

Based on the median value of the stromal score, the tumor samples were divided into a high stromal score group and low stromal score group. Likewise, the tumor samples were divided into a high-immune score group and low-immune score group according to the median value of the immune score.

The typical Bayesian test in the limma package (Version 3.10.3) [15] was used to analyze the differentially expressed mRNAs (dif-mRNAs) between the two stromal score and two immune score groups, respectively. The dif-mRNAs with a fold change value of more than 0.263 and a p-value of less than 0.05 were selected as dif-mRNAs.

In order to screen the potential regulatory genes associated with both stromal and immune cell content, the overlapping DEGs were selected and represented by a VENN diagram.

Enrichment Analysis

Utilizing the Clusterprofiler tool [16] in R package (Version 3.2.11,), the Kyoto Encyclopedia of Genes and Genomes (KEGG) [17] pathway enrichment and the Gene Ontology [18] Biological Process (BP) enrichment were performed for the co-expressed DEGs. The significantly enriched terms with a p-value of less than 0.05 and involving no less than two DEGs were selected.

Screening $CD4^+$ and $CD8^+$ T cell-related DEGs

Based on the DEGs data of the RNA-seq expression profiles, the abundance of immune cell infiltration in the tumor samples was estimated by using the Cibersort algorithm [19]. According to the Cibersort algorithm, the infiltration of six types of immune

cells ($CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils, dendritic cells, and lymphocytes) in tumor tissues was detected.

The Pearson correlation coefficient between the expression value of the DEGs and the abundance of the infiltrated $CD4^+$ and $CD8^+$ T cells was calculated. The immune-related DEGs with an absolute value of r more than 0.15 were selected.

Construction of the PPI Network

The interactions between proteins encoded by immune-related DEGs in $CD4^+$ T cells were retrieved from the STRING [20] (version 11.0) database with a PPI score setting of 0.15 (low confidence) and the species were human. Based on the retrieved PPIs, the $CD4^+$ T cell-related PPI network was visualized using Cytoscape [21] (version 3.2.0). The $CD8^+$ T cell-related PPI network was constructed using the same method.

Construction of the ceRNA Network

miRWalk 3.0 [22] was used to predict target-miRNA regulatory relationships. The miRNAs correlated with DEGs related to the $CD4^+$ T cells were predicted and the species was selected as human. The regulatory relationships with a related score greater than 0.95 which appeared in both the TargetScan and miRDB database were selected. The miRNAs correlated with DEGs related to the $CD8^+$ T cells were predicted using the same method.

The lncRNA-miRNA relationships which were related to the $CD4^+$ T cells were predicted using the DIANA-LncBase v.2 [23] database. The lncRNA-miRNA regulatory relationships with a score of 1 were selected. The lncRNA-miRNA and target-miRNA data were integrated to construct the ceRNA network of the lncRNA-miRNA-target relationships related to the $CD4^+$ T cells. The $CD8^+$ T cell-related ceRNA network was also constructed using the same method.

Construction of the Small Chemical Molecule-Target Network

By utilizing the Comparative Toxicogenomics Database (CTD) [24] we searched for the colon cancer-related genes and small chemical molecules. Moreover, the overlapping genes which were associated with colon cancer and also belonged to the $CD4^+$ T cell-related ceRNA network were used to screen the chemical-target pairs. The $CD4^+$ T cell-related small chemical molecule-target network was constructed using the Cytoscape. The $CD8^+$ T cell-related small chemical molecule-target network was also constructed using the same method.

Survival Analysis

The clinical phenotype data related to prognosis in TCGA dataset were collected, including the Overall survival (OS) status. The $CD4^+$ T cell-related genes were divided into high/low expression groups based on the median gene expression value and the same grouping was performed for $CD8^+$ T cell-related genes. A log-rank statistical test was conducted and the genes with a p-value of less than 0.05 were considered to be significantly correlated with prognosis. Furthermore, the curves of Kaplan-Meier (K-M) survival were plotted.

Results

Differences in Gene Expression between High- vs Low-Stromal and Immune Score Colon Cancer

According to the stromal score, there were 83 DEGs (5 up-regulated and 78 down-regulated) between the high- and low-stromal score groups. Meanwhile, 1270 DEGs (including 807 up-regulated and 463 down-regulated) were screened between the high- and low- immune score groups. The volcano map of the DEGs has been shown in Figure 2. Furthermore, there were 5 up-regulated and 74 down-regulated DEGs which were screened in both the stromal and immune groups, and the number of overlapping DEGs has been shown in a VENN diagram (Figure 2C). The overlapping DEGs have been also shown in Supplementary Table 1.

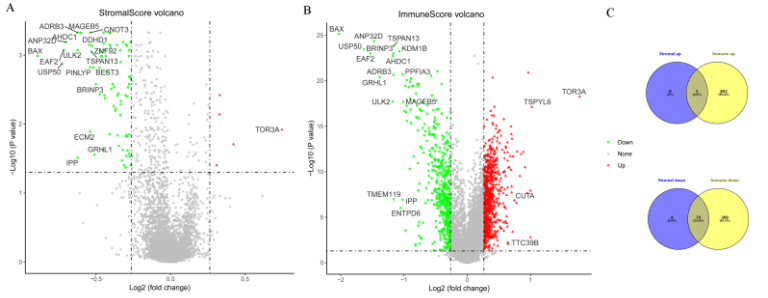

Figure 2: Gene expression profiles in colon cancer.
Volcano plot showing the expression profile of genes in high- *vs* low- stromal score (A) and in high- *vs* low- immune score (B) groups. The red dots and green dots in the volcano plot represent up-regulated genes and down-regulated genes, respectively. VEEN diagram (C) showing the overlapping differentially expressed genes between the stromal score- and immune score-related genes. The GDC TCGA Colon Cancer (COAD) dataset (version 07-19-2019) was downloaded fro**m TCGA (**https://xenabrowser.net/**)** database. For the analysis of the infiltrating stromal and immune cells in tumor tissues, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data (ESTIMATE, version 1.0.13) in the R package was used.

Enrichment Analysis of the Overlapping DEGs

In the enrichment analysis of the co-expressed DEGs, up-regulated DEGs were enriched in 22 BP, including response to copper ions and response to electrical stimuli (Figure 3A). The down-regulated DEGs were enriched in 17 BP, including immune response to tumor cells and stabilization of the membrane potential (Figure 3B). The co-expressed DEGs were not found to be enriched in any of the investigated KEGG pathways.

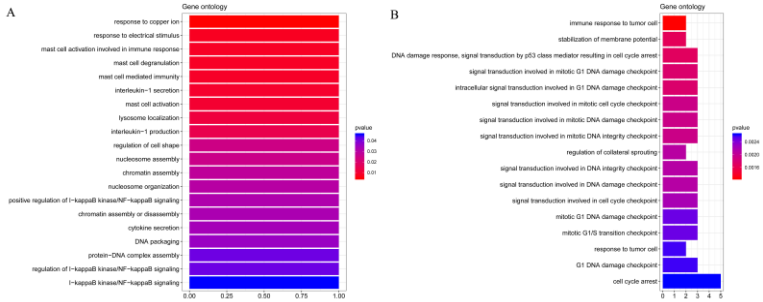

Figure 3: Enrichment analysis of the common differentially expressed genes. Gene ontology biological process term enrichment analysis of up-regulated differentially expressed genes (A) and down-regulated differentially expressed genes (B).

The CD4$^+$ and the CD8$^+$ T cell-related DEGs

Based on the Cibersort algorithm, the abundance of infiltrating immune cells (CD4$^+$ T cells, CD8$^+$ T cells, B cells, neutrophils, dendritic cells, and lymphocytes) in the tumor samples was estimated (Figure 4). Meanwhile, according to the Pearson correlation coefficient, 79 CD4$^+$ T cell-related DEGs and 8 CD8$^+$ T cell-related DEGs were screened.

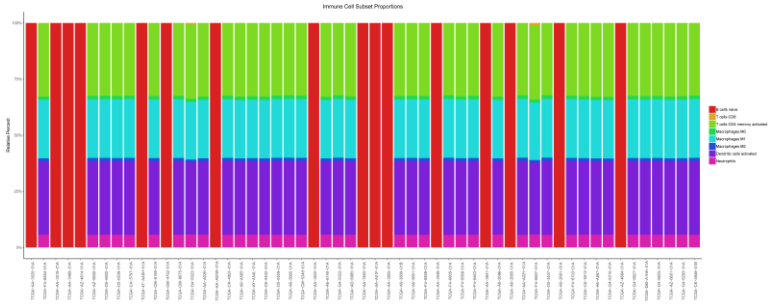

Figure 4: Infiltration of the immune cells in colon cancer.

PPI Network of CD4$^+$ and CD8$^+$ T cell-related DEGs

We screened 59 nodes and 77 interaction pairs in the PPI network analysis of CD4$^+$ T cell-related genes, which has been shown in Figure 5, including the ETS transcription factor

(*ELK3*). There was no PPI of DEGs related to $CD8^+$ T cells obtained under the threshold used in this study.

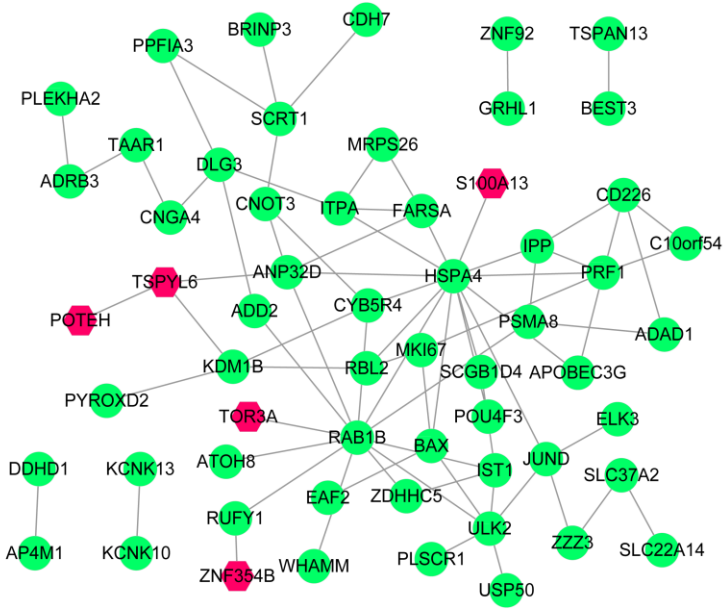

Figure 5: Protein-protein interaction networks. Protein-protein interaction network of $CD4^+$ T cell-related genes. Red hexagons represent up-regulated genes and green nodes represent down-regulated genes.

ceRNA network of $CD4^+$ and $CD8^+$ T cell-related DEGs

In the prediction of miRNA target genes, we obtained 69 miRNA-mRNA relationships (55 miRNAs and 19 target genes) in $CD4^+$ T cells (Figure 6A), while for $CD8^+$ T cells, there were only two miRNA-mRNA relationships obtained, including two miRNAs and one target gene (Figure 6B).

There were 11 lncRNAs predicted to interact with miRNAs in $CD4^+$ T cells. After integrating both this data and the data on the miRNA-target relationships, 110 ceRNA regulatory relationships were obtained for $CD4^+$ T cells, such as chr22-38_28785274-29006793.1-miR-106a-5p-DDHD1, which included 45 miRNAs and 11 mRNAs (Figure 7A).

In the prediction of lncRNAs for $CD8^+$ T cells, four lncRNAs were obtained. After integration with the miRNA-target relationships, five ceRNA regulatory relationships were obtained for $CD8^+$ T cells, such as chr22-38_28785274-29006793.1-miR-4319-GRHL1, which included one miRNA and one mRNA (Figure 7B).

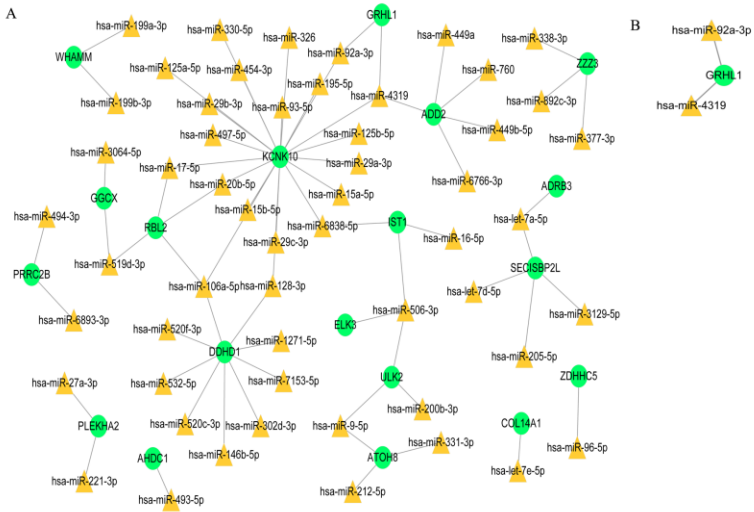

Figure 6: MiRNA-target regulatory networks.
MiRNA-target regulatory networks of $CD4^+$ **T cell-related** genes (A) and $CD8^+$ **T cell-related** genes (B). Green nodes represent down-regulated genes. Yellow triangles represent the microRNAs.

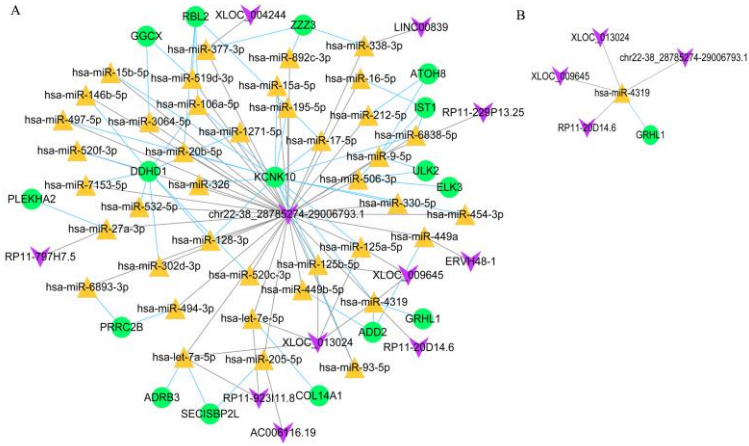

Figure 7: Competing endogenous RNAs (ceRNA) networks.
The ceRNA network of $CD4^+$ **T cell-related** genes (A) and $CD8^+$ **T cell-related** genes (B). Green nodes represent down-regulated genes. Yellow triangles represent the microRNAs. Purple inverted triangles represent the long non-coding RNAs.

Colon Cancer-Related Small Chemical Molecules Target the mRNAs in the ceRNA Network

According to the results of the prediction of small chemical molecule-target interactions in $CD4^+$ T cells, there were 175 small chemical molecule-target interactions obtained, including two lncRNAs, ten genes, and 64 types of small chemical molecules (Figure 8A). In the prediction analysis for $CD8^+$ T cells, there were nine small chemical molecule-target interactions obtained, including one gene and nine types of small chemical molecules (Figure 8B).

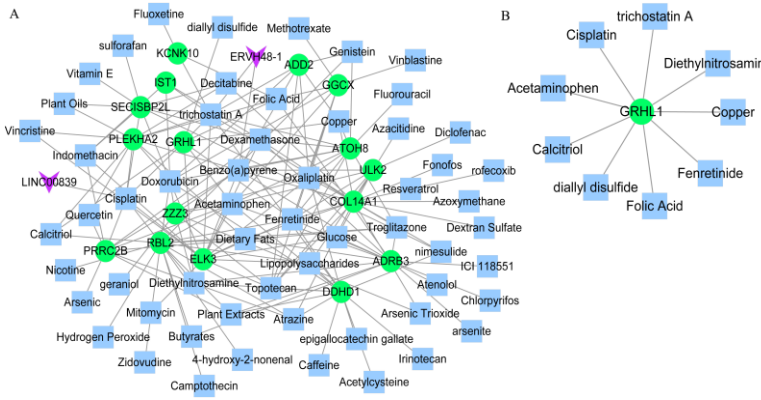

Figure 8: Chemical-gene interaction networks.
Chemical-gene interactions were predicted for the genes in the competing endogenous RNAs (ceRNA) networks by using the CTD database. The chemical-gene interaction network of genes in the $CD4^+$ **T cell-related** ceRNA network (A) and $CD8^+$ **T cell-related** ceRNA network (B). Green nodes represent down-regulated genes. Blue squares represent the small chemical molecules. Purple inverted triangles represent the long non-coding RNAs.

$CD4^+$ and $CD8^+$ T cell-related Genes associated with Colon Cancer Prognosis

After screening for DEGs associated with survival, the adenosine deaminase domain containing 1 (*ADAD1*), and the discs large MAGUK scaffold protein 3 (*DLG3*) were selected from the 77 $CD4^+$ T cell-related DEGs. However, there were no survival-related DEGs in the eight $CD8^+$ T cell-related genes. The K-M survival curve of *ADAD1* has been represented in Figure 9A and the K-M survival curve of *DLG3* has been represented in Figure 9B.

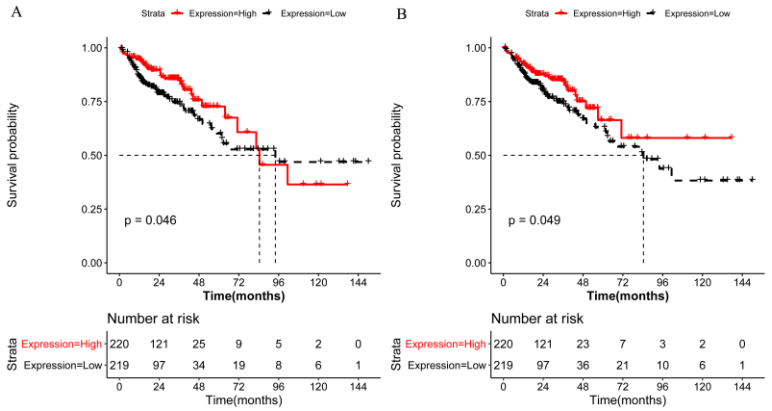

Figure 9: The Kaplan-Meier curves of overall survival in patients with colon cancer.

After screening for differentially expressed genes associated with survival, the adenosine deaminase domain containing 1 (ADAD1), and the discs large MAGUK scaffold protein 3 (DLG3) were selected from 77 $CD4^+$ **T cell-related** genes. There were no survival-related differentially expressed genes in the eight $CD8^+$ **T cell-related** genes. The Kaplan-Meier curves of overall survival showing the prognosis value of ADAD1 (A) and DLG3 (B).

Discussion

In this study, by comparing the samples in the database between groups with high- or low- stromal and immune scores, we obtained a total of 83 DEGs (5 up-regulated and 78 down-regulated) between the two stromal score groups, and 1270 DEGs (807 up-regulated and 293 down-regulated) between the two immune score groups. A total of 79 co-expressed DEGs were obtained, which were associated with stromal and immune scores. The 5 up-regulated co-expressed DEGs were enriched in 22 BP (response to copper ions and response to electrical stimuli) and the 74 down-regulated co-expressed DEGs were enriched in 17 BP (immune response to tumor cells and stabilization of membrane potential). Moreover, 79 $CD4^+$ T cell-related DEGs were screened, with 77 edges and 59 nodes in the PPI network, while only eight $CD8^+$ T cell-related DEGs were obtained. Furthermore, there were 110 ceRNA relationships in the network of $CD4^+$ T cell-related DEGs and five ceRNA

relationships in the network of $CD8^+$ T cells. Finally, in order to understand the clinical applications of our findings, as well as provide a possible method for subsequent prediction of clinical prognosis and targeted drug selection, prognostic genes were analyzed and small-molecule drugs were screened. In the analysis of small chemical molecule-gene interactions, 175 pairs were obtained for $CD4^+$ T cells and nine pairs for $CD8^+$ T cells. In the survival analysis, *ADAD1* and *DLG3* were selected for $CD4^+$ T cells, as they were the most strongly correlated with prognosis. As such, the analysis of the molecular mechanism of $CD4^+$ and $CD8^+$ T cells in colon cancer may provide novel targets for colon cancer immunotherapy.

ELK3 is a transcription factor belonging to the E26 transformation-specific (ETS) family [25]. The PI3K/Akt/mammalian target of rapamycin (mTOR) and ERK signaling pathways activated ELK3 [26]. Notably, the studies have showed that *ELK3* regulated cell migration and invasion in hepatoma cells and breast cancer [27]. In the regulation of colorectal cancer stemness, Wang et. al. demonstrated that *ELK3* was involved in. Moreover, *ELK3* was shown to be a potential target of miR-507, and the expression was restored with the abrogation of LINC00525-knockdown [28]. Many important biologic processed were regulated by the ETS protein family, such as the function of immune cell [29,30]. *ELK3* regulated the expression of heme oxygenase-1 (HO-1) as a transcriptional repressor. Moreover, inflammatory mediators tend to affect the expression of *ELK3,* which was down-regulated by bacterial endotoxins. A study by Tsoyi showed that the ETS protein family played a role in the immune response. During the inflammatory response to infection, *ELK3* and *HO-1* were important for macrophage function [31]. In our study of $CD4^+$ T cell-related DEGs, *ELK3* was found to be downregulated.

In the present study, the chr22-38_28785274-29006793.1—miR-106a-5p—*DDHD1* axis from the $CD4^+$ T-cell related ceRNA network was selected for further analysis. The phospholipase A1 (PLA1) family was classified as extracellular and intracellular proteins, which was implicated in different intracellular mechanisms. As a phosphatidic acid (PA)-pre-ferring PLA1

(PA-PLA1), intracellular *DDHD1* was extensively studied for its implication in cancer development. *DDHD1* was involved in the synthesis of lysophosphatidylinositol (LPI) [32]. LPI activity was correlated with tumor growth and aggressiveness, which was mediated by the interaction with the G protein-coupled receptor 55 (GPR-55) [33-36]. Moreover, *DDHD1* was shown to support proliferation and survival of colon cancer cell. Studies have also demonstrated that through the inhibition of the MAPK/ERK and PI3K/Akt signaling pathways, viability of colon cancer cell *in vitro* was reduced and apoptotic cell death was increased by the silencing *DDHD1* via small interference RNA [37]. These results were consistent with previous studies, which supported lysophospholipid mediators *DDHD1* promote tumor. In neoplastic cells, by interacting with GPR-55, LPI was inducing the ERK and the Akt signaling [34]. MiR-106a-5p belongs to the miR-17 family. According to the consensus seed region, there are three clusters in the miR-17 family. MiR-106a-5p is located on Xq26.2, which belongs to miR-106a-363 cluster. MiR-106a-5p was found to be highly expressed in gastric [38-42], breast [43,44], colorectal [45], and non-small cell lung cancers [46]. In squamous cell carcinomas [47], colon cancers [48], and gliomas [49], miR-106a-5p was expressed at lower levels. Studies have also shown that in colorectal cancer, by inhibiting the anti-metastatic gene transforming growth factor-b receptor 2 (*TGFBR2*), cell migration and invasion are increased by miR-106a-5 [45]. In our study, *DDHD1* was found to be a potential target of miR-106a-5p in colon cancer cells, which influence the progression in disease.

In our study, the chr22-38_28785274-29006793.1—miR-4319—*GRHL1* axis from the $CD8^+$ T-cell related ceRNA network was chosen for further analysis. Grainyhead-like 1 (*GRHL1*), belongs to the GRHL transcription factor family, which comprises *GRHL1, GRHL2,* and *GRHL3* [50]. Studies have suggested that the Grainyhead family genes have adopted a DNA-binding immunoglobulin folded homologous to the DNA-binding domain of the key tumor suppressor p53 and that they participanted in wound healing, embryonic neural tube closure, and epidermal integrity [51-53]. Recently, these transcription factors involved in different cancer, such as skin squamous cell

carcinoma, gastric cancer, breast cancer, colorectal cancer, and cervical cancer [54]. Moreover, a study has shown that *GRHL2*-knockdown in colorectal cancer cells inhibited cell proliferation by targeting ZEB1 [55]. Huang et. al. revealed that the expression of miR-4319 was inversely related with patients' survival in colorectal cancer. Moreover, overexpression of miR-4319 was shown to markedly reduce colorectal cancer cell proliferation by infecting the ankyrin repeat and the BTB domain containing 1 (*ABTB1*) and alter the cell cycle distribution [56]. Thus, we hypothesized that the chr22-38_28785274-29006793.1—miR-4319—*GRHL1* axis may be correlated with $CD8^+$ T cells and correlated with the pathogenic mechanism of colon cancer.

In conclusion, a series of bioinformatics analyses were conducted on DEGs related to $CD4^+$ and $CD8^+$ T cells in the colon cancer tissue. *ELK3* was found to be down-regulated, which may be correlated with colon cancer and $CD4^+$ T cells. Moreover, the chr22-38_28785274-29006793.1—miR-106a-5p—*DDHD1* axis from the $CD4^+$ T cell-related ceRNA network was selected for further analysis. In the analysis of the $CD8^+$ T cell-related ceRNA network, the chr22-38_28785274-29006793.1—miR-4319—*GRHL1* axis was chosen for further analysis. In the survival analysis of $CD4^+$ T cell-related DEGs, *ADAD1* and *DLG3* were selected, which had a strong correlation with prognosis. Furthermore, 175 small chemical molecule-gene interaction pairs in $CD4^+$ T cells and nine in $CD8^+$ T cells were screened. In the present study, screening of T cell-related RNAs, ceRNA network construction, and miRNA target prediction may provide the basis for relevant studies on colon cancer-induced T cell-mediated immune escape. The prediction of small chemical molecule drugs and survival differences based on differential RNA expression may provide a novel direction of clinical decision making for the treatment and prognosis evaluation of colon cancer from the perspective of immunity.

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Supplementary Material

Supplementary Material can be accessed online at https://videleaf.com/wp-content/uploads/2023/09/PACR3ED-23-08_Supplymentary-Table.doc