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Iron Death Regulatory Network: Expression Characterization and Prognostic Correlation Study of ARL6IP1 in Colorectal Cancer

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Abstract: This study leveraged publicly available databases, including The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO), to investigate the expression patterns of ARL6IP1 in colorectal cancer (CRC) and its prognostic relevance. The results demonstrated that reduced ARL6IP1 expression is strongly associated with poorer overall survival (OS), disease-specific survival (DSS), and progression-free survival (PFS) in CRC patients, establishing ARL6IP1 as an independent prognostic marker for CRC. Further analyses using Gene Set Enrichment Analysis (GSEA) and Protein-Protein Interaction (PPI) network investigations indicated that low ARL6IP1 expression is enriched in cancer-related signaling pathways, suggesting its involvement in CRC pathogenesis through the ferroptosis mechanism. Additionally, the study uncovered a correlation between ARL6IP1 expression and immune cell infiltration within the tumor microenvironment (TME), particularly immunosuppressive cell populations such as regulatory T cells (Tregs) and M2-type macrophages. Diminished ARL6IP1 levels may promote the development of an immunosuppressive TME, thereby aiding tumor immune evasion. Collectively, these findings highlight ARL6IP1 as a critical ferroptosis-related gene that may significantly influence CRC progression and immune escape, offering a potential target for prognostic evaluation and therapeutic intervention in CRC. This study aims to investigate the expression patterns of ARL6IP1 in colorectal cancer (CRC) and its prognostic significance, as well as its correlation with immune cell infiltration in the tumor microenvironment. By leveraging publicly available databases, we sought to determine whether ARL6IP1 could serve as a potential prognostic biomarker and therapeutic target for CRC.

Keywords: Colorectal Cancer (CRC); ARL6IP1; Ferroptosis; Tumor Immune Microenvironment (TME); prognostic Biomarkers

1. Introduction

In recent years, the global incidence of colorectal cancer (CRC) has shown a consistent upward trend, positioning it as one of the leading contributors to cancer-associated deaths worldwide. CRC is a highly heterogeneous and aggressive malignancy, characterized by varied clinical presentations, rapid progression, and frequent late-stage diagnosis, which often leads to suboptimal treatment outcomes. Despite notable advancements in the diagnosis and treatment of CRC in modern medicine, the prognosis for many patients, particularly those with advanced disease, remains unfavorable. Consequently, the quest for novel molecular markers and therapeutic targets to enhance the survival and quality of life of CRC patients has become a central focus of current research efforts [1, 2].

Ferroptosis, a form of iron-dependent regulated cell death characterized by lipid peroxidation and the accu-

mulation of Reactive Oxygen Species (ROS), has attracted considerable interest in the field of oncology, given its pivotal role in modulating tumorigenesis, disease progression, and chemoresistance.Numerous studies have indicated that alterations in the expression of ferroptosis-related genes can significantly influence the prognosis of various cancers. In the context of CRC, elucidating the potential roles of ferroptosis-related genes and their mechanisms in modulating the tumor microenvironment is vital for the development of new diagnostic and therapeutic approaches [3, 4].

ADP-ribosylation factor-like 6 interacting protein 1 (ARL6IP1) is a gene associated with ferroptosis and has been implicated in various cellular metabolic processes, particularly in regulating anti-oxidative stress and lipid metabolism. Although the role of ARL6IP1 has been explored to some extent in other cancers, its expression profile, functional mechanisms, and impact on patient prognosis in CRC remain unclear. Additionally, there is limited research on whether ARL6IP1 can influence CRC progression by modulating the tumor immune microenvironment (TIME) and gut microbiota [5, 6].

This investigation is meticulously formulated to conduct a comprehensive dissection of ARL6IP1 expression in colorectal cancer (CRC) and to scrutinize its correlation with patient prognosis by leveraging publicly available databases such as The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO). Moreover, the study endeavors to clarify the nexus between ARL6IP1 expression and the infiltration levels of various immune cells in the tumor microenvironment, with the objective of uncovering the potential regulatory role of this gene within the tumor niche. It is anticipated that the findings of this research will provide a novel theoretical foundation for the early diagnosis and personalized treatment of CRC, thereby advancing therapeutic strategies for this malignancy [7, 8]. In light of the above, the present study was designed to comprehensively analyze the expression profile of ARL6IP1 in CRC and its association with patient prognosis and immune cell infiltration. We hypothesized that ARL6IP1 might play a crucial role in CRC progression and immune regulation, and could potentially serve as a prognostic biomarker and therapeutic target.

2. Materials and Methods

2.1. Data Source

The data utilized in this study were primarily sourced from publicly available gene expression and clinical information related to colon cancer patients. Specifically, these data were retrieved from well-known public databases, including The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO). To enhance the reliability of the analysis, multiple datasets were employed for validation purposes, encompassing RNA sequencing data and detailed clinical follow-up information. These comprehensive datasets provide a robust foundation for evaluating the role of ARL6IP1 gene expression in colon cancer and its influence on patient prognosis.

2.2. TCGA, GEO and HPA Databases

The TCGA Database

The Cancer Genome Atlas (TCGA) database is a comprehensive public data platform that hosts genomic data from a wide range of cancer types across the globe. As the primary repository of the TCGA program in the United States, it provides researchers with access to gene expression profiles, clinicopathologic data, and survival information for 33 distinct types of cancer. This extensive dataset serves as a crucial resource for elucidating the molecular mechanisms underlying cancer development and progression. In the context of this study, we utilized the colon cancer dataset from the TCGA database to obtain RNA sequencing data and corresponding clinical information. Our aim was to evaluate the correlation between the expression levels of the ARL6IP1 gene and the prognosis of colon cancer patients.

The GEO Database

The Gene Expression Omnibus (GEO) database, maintained by the National Center for Biotechnology Information (NCBI), is one of the largest repositories of gene chip and high-throughput gene expression data worldwide. It encompasses a vast array of gene expression datasets derived from various tissues and disease types, offering a rich resource for the analysis of gene expression patterns across different biological contexts. In this study, we leveraged the GEO database to obtain additional datasets related to colon cancer. These datasets were instrumental

in validating the expression patterns and clinical significance of the ARL6IP1 gene across multiple patient cohorts, thereby enhancing the robustness of our findings.

The HPA Database

The Human Protein Atlas (HPA) database is an online resource that provides detailed information on protein expression. It is maintained by the Human Protein Atlas project and offers a comprehensive collection of tissue and cellular protein expression data. The database covers the distribution of proteins in 44 normal tissues and 20 common cancer types, making it an invaluable tool for researchers investigating protein expression patterns in health and disease. In this study, we leveraged immunohistochemistry data from the Human Protein Atlas (HPA) database to scrutinize the expression of the ARL6IP1 protein in both cancerous and normal tissue samples. This analysis provided critical insights into the potential biological functions of ARL6IP1 in colon cancer, thereby supporting our overall research objectives.

2.3. Clinical Statistical Analysis of Prognosis, Model Development and Assessment

In this study, clinical and gene expression data for colon cancer patients were primarily sourced from the TCGA database. The prognostic significance of ARL6IP1 gene expression was assessed via comprehensive clinical statistical analyses, with a focus on overall survival (OS) and progression-free survival (PFS). These analyses were performed using Cox regression and Kaplan-Meier survival methods. Specifically, univariate and multivariate Cox regression analyses were employed to examine the correlation between ARL6IP1 expression levels and colon cancer prognosis. Additionally, the Wilcoxon signed-rank test was used to evaluate the association between ARL6IP1 expression and various clinicopathological features, with logistic regression serving as a supplementary validation tool.

An ARL6IP1-based risk score model was developed and validated by stratifying patients into high- and low-risk groups. Kaplan-Meier survival curves were used to compare the prognostic differences between these risk groups. The results of the Cox regression model were then integrated with independent prognostic factors identified through multivariate analyses. These combined data were subsequently used to predict the probability of patient survival at 1-, 3-, and 5-year intervals. The model's accuracy was rigorously evaluated using calibration curves. The results indicated that the model's predictions closely matched the actual observed outcomes across different time points, with the 45-degree calibration line confirming the robustness and reliability of the model's prognostic capabilities.

2.4. Comprehensive Protein-Protein Interaction Analysis

The STRING Web platform (https://string-db.org/) is an online tool suitable for protein interaction (PPI) data analysis. The platform provides widely integrated PPI data covering multiple biological species and protein functional relationships. In this study, after importing the expression data of the ARL6IP1 gene into the STRING platform, we retrieved the information of proteins interacting with ARL6IP1 from the PPI network. To ensure the high confidence of the data, we set the confidence threshold of the interactions to be greater than 0.7. The results of the STRING platform showed a potential interaction network of ARL6IP1 in colon cancer, which provides an important basis for further exploration of its role in iron death regulation and tumor microenvironment.

2.5. Enrichment Analysis

In this study, differentially expressed genes (DEGs) associated with the ARL6IP1 gene were analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses via the R package clusterProfiler (version 3.6.3). The GO enrichment analyses encompassed three primary categories: cellular component (CC), molecular function (MF), and biological process (BP), aiming to elucidate the potential cellular activities and biological functions involving ARL6IP1. To ensure the significance and reliability of the enrichment results, screening criteria were established, requiring a gene number greater than 15, a p-value less than 0.01, and an adjusted p-value (false discovery rate, FDR) less than 0.05.

Additionally, gene set enrichment analysis (GSEA) was performed to explore pathway differences between high and low ARL6IP1 expression groups. Functional pathways with significant enrichment were identified using significance thresholds of $p < 0.05$ and $FDR < 0.25$. The results revealed that gene sets significantly associated

with ARL6IP1 expression were enriched in several cancer-related metabolic, immunomodulatory, and ferroptosis pathways. The normalized enrichment score (NES) and adjusted p-value were used to evaluate the statistical significance of the enrichment outcomes.

Finally, the results of the GO, KEGG, and GSEA analyses were visualized using the ClusterProfiler tool, providing a clear depiction of the potential biological functions and key pathways involved in ARL6IP1 in colon cancer, thereby facilitating a more comprehensive understanding of its role in the disease.

2.6. Analysis of Immune Cell Infiltration

In this investigation, the role of the ARL6IP1 gene in the immune microenvironment of colon cancer was elucidated through the application of single-sample Gene Set Enrichment Analysis (ssGSEA). This method, implemented via the R package GSVA, enabled the assessment of immune cell infiltration based on gene expression profiles. By employing correlation analysis, we compared the infiltration of immune cells between groups with high and low ARL6IP1 expression. The results demonstrated a significant correlation between ARL6IP1 expression levels and the infiltration of specific immune cell types, including regulatory T cells (Tregs) and M2 macrophages, which are pivotal in immune regulation and tumor progression. Further validation was achieved through correlation tests, with significance levels indicated by symbolic markers corresponding to different p-value thresholds. The analyses revealed that ARL6IP1 expression is strongly associated with the infiltration of immune cells that contribute to an immunosuppressive microenvironment in colon cancer. For instance, elevated ARL6IP1 expression was found to correlate with increased infiltration of certain immune cells, potentially facilitating immune evasion and tumor progression.

2.7. Genetic Correlation Analysis

In the present investigation, the GEPIA platform was utilized to scrutinize the expression correlation between the ARL6IP1 gene and other immune-related genes in colon cancer, with the objective of elucidating its potential immune-regulatory functions.The GEPIA platform, which encompasses extensive gene expression data from both cancerous and normal tissues, is well-suited for conducting comprehensive gene correlation analyses. Additionally, we validated the correlation between ARL6IP1 and various immune markers using data from the TIMER platform, thereby corroborating its functional association within the tumor immune microenvironment.

3. Results

3.1. Elevated Expression of ARL6IP1 Gene in Tumors Compared to Normal Samples

In this study, we compared the expression levels of ARL6IP1 in colon cancer (COAD) tissues with those in normal tissues using data from the TCGA and GTEx databases. Our analysis demonstrated that ARL6IP1 expression was significantly higher in multiple cancer types, including colon cancer, compared to normal tissues ($p < 0.001$) (**Figure 1A**). Specifically, within colon cancer samples, ARL6IP1 mRNA expression was significantly elevated in cancerous tissues compared to adjacent normal tissues ($p < 0.001$) (**Figure 1B**). This finding was further validated by pairwise analysis, which revealed a significant difference in ARL6IP1 expression between cancerous and adjacent normal tissues from colon cancer patients ($p < 0.001$) (**Figure 1D**).

Additionally, we examined the protein expression distribution of ARL6IP1 using immunohistochemical (IHC) staining images obtained from the Human Protein Atlas (HPA) database. The results showed that ARL6IP1 staining intensity was higher in normal colon tissues, while its expression was markedly reduced in colon cancer tissues (**Figure 2A,B**). Furthermore, immunofluorescent labeling was used to analyze ARL6IP1 expression in normal colon cells and cancer cells. The findings indicated that ARL6IP1 exhibited stronger fluorescent signals in cancer cells (**Figure 2C,D**), suggesting higher expression levels in these cells.

To assess the diagnostic potential of ARL6IP1, a receiver operating characteristic (ROC) curve was constructed. The area under the curve (AUC) for ARL6IP1 was 0.966 (95% CI: 0.955–0.977), indicative of exceptional diagnostic accuracy (**Figure 1E**). Furthermore, protein expression analysis utilizing the CPTAC dataset corroborated these findings, revealing that ARL6IP1 protein expression was markedly elevated in colon cancer tissues relative to normal tissues (**Figure 1F**). Collectively, these results imply that ARL6IP1 may function as a robust diagnostic biomarker for colon cancer.

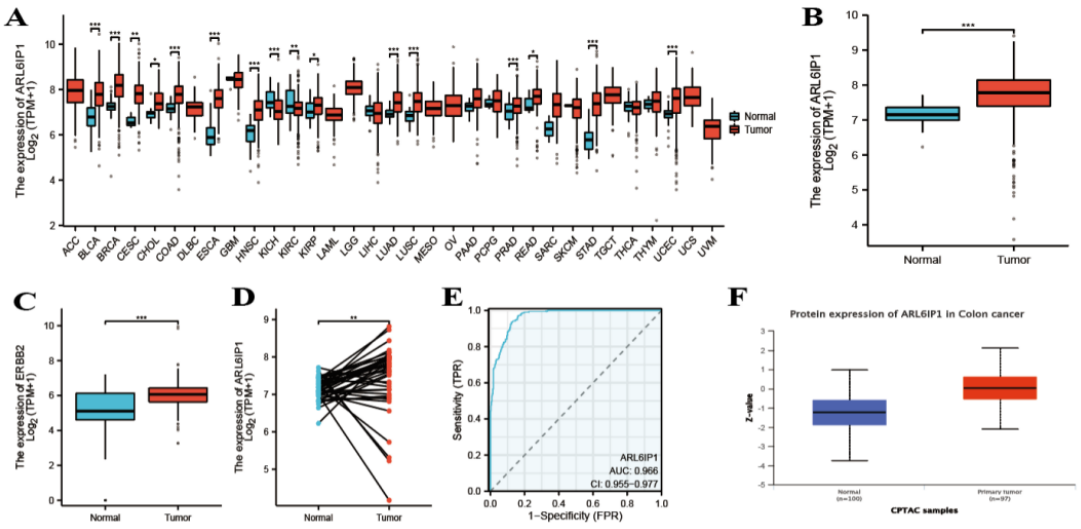


Figure 1. Expression of ARL6lP1 in Colon Cancer and Other Maignancies, and its Clinical Significance (**A**) Profle ofARL6lP1 expression in various human cancerand corresponding normal tisues, showing a significant upregulation of ARL6lP1 in colon cancer (COAD) and several other malignancies. (ns: nostatistical significance, * $p < 0.05$, ** $p < 0.01$. ** $p < 0.001$). (**B**) Signifcant iferences in ARL6lP1 expression between colon cancer (COAD) tumortissues and normal issues ($p < 0.001$). (**C**) ERBB2 gene expression in tumor and normal tissues of colon cancer, shown as a comparative marker. (**D**) Paired analysis ofARL6lP1 expression in fumor and adjacent normal tissues in colon cancer, revealing signicant upregulation in tumor tissues ($p < 0.001$). (**E**) Receiver operating characteristic (ROc) curve showing the diagnostic value ofARL6lP1 in colon cancer, with an AUc of 0.966 and Cl of 0.955–0.977, indicating a high diagnostic potential for ARL6lP. (**F**) Protein expression levels of ARL6lP1 in colon cancer tissues compared to normal tissues in the CPTAC dataset, showing significantly higher expression in tumor tissues.

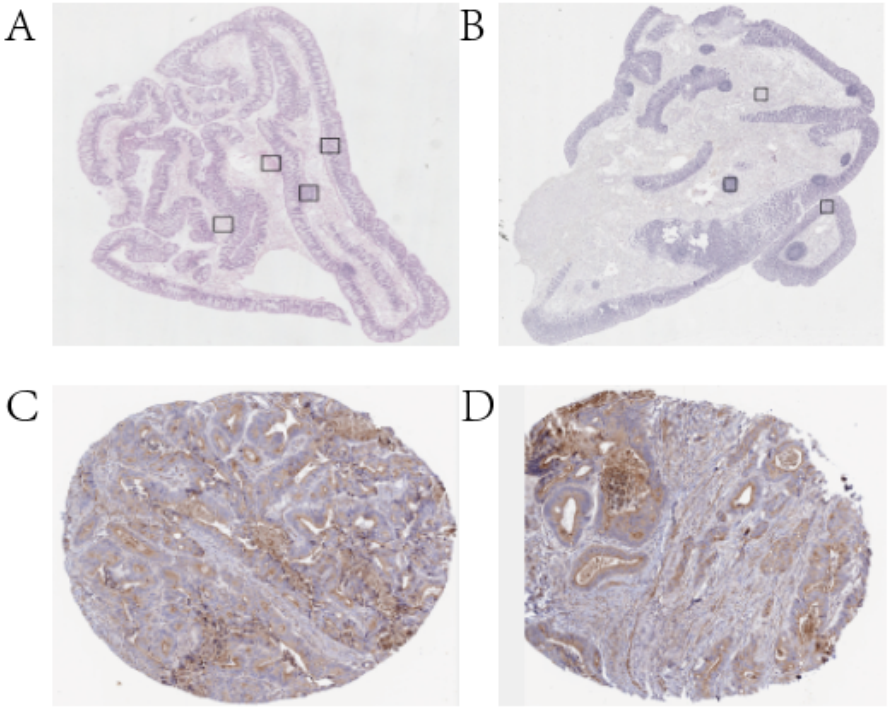


Figure 2. *Cont.*

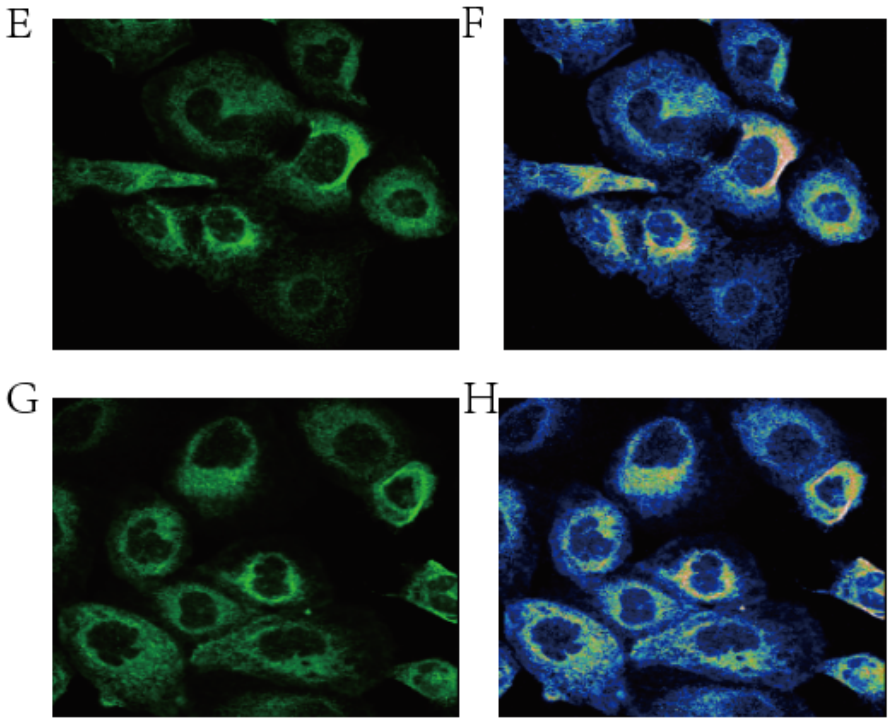


Figure 2. Expression of ARL6lPl in normal and tumor tissues ofthe colon. (**A**,**B**): Hematoxylin and eosin (HE) staining (**A**) and immunohistochemical (IHC) staining (**B**) Of ARL6IPin normal colon tissues, ARL6lP1 shows distinct expression patterns in normal tissues. (**C**,**D**): HE staining (**C**) and lHC staining (**D**) of ARL6IP1 in colon cancer tissues. Significant diferences inARL6lP1 expression are observed between tumor and normal tissues. (**E**,**F**): 1mmunofluorescence staining of ARL6IP1 in normal colon cells, showing, localization primarily in thecytoplasm (**E**: green channel; **F**: merged channel). (**G**,**H**): 1mmunofluorescence staining of ARL6IP1 in colon cancer cells, showing enhanced ARL6IP1fluorescence intensity compared to normal cells (**G**: green channel; **H**: merged channel).

3.2. Correlation of ARL6IP1 Expression with Clinical Parameters

In this study, the expression levels of ARL6IP1 in tumor samples were normalized through Z-score transformation, and subsequently, the samples were divided into low and high expression cohorts based on ARL6IP1 expression levels. To elucidate the correlation between ARL6IP1 expression and various clinical parameters, Kruskal-Wallis and Wilcoxon signed-rank tests were utilized. The results indicated that elevated ARL6IP1 expression was significantly associated with advanced T-stage, N-stage, M-stage, and pathological stage. Additionally, significant differences were observed in primary treatment outcomes (PD) and survival events (OS) ($p < 0.05$, **Figure 3A–I**). Consistent findings were obtained through Fisher's exact test or chi-square test (**Table 1**).

Univariate analysis further revealed that ARL6IP1 expression levels exhibited significant variation across different clinical parameters, particularly in pathological stage (odds ratio [OR] = 2.947, 95% CI: 1.942–4.471, $p < 0.001$), treatment outcome (OR = 0.111, 95% CI: 0.057–0.214, $p < 0.001$), and age (OR = 1.610, 95% CI: 1.052–2.463, $p = 0.028$) (**Table 2**). Conversely, no statistically significant associations were detected for gender (OR = 1.101, 95% CI: 0.746–1.625, $p = 0.627$) and histologic type (OR = 1.269, 95% CI: 0.753–2.139, $p = 0.371$) (**Table 2**). Collectively, these findings suggest that ARL6IP1 expression is closely correlated with several advanced pathological features in colon cancer patients, indicating its potential role in disease progression.

Table 1. Association of ARL6lP1 expression with clinicopathological characteristicsin patients with colorectal cancer.

Characteristics	Total(N)	HR(95% CI) Univariate Analysis	P Value Univariate Analysis	HR(95% CI) Multivariate Analysis	P Value Multivariate Analysis
Pathologic T stage	476				
T1&T2	94	Reference		Reference	
T3&T4	382	3.072 (1.423–6.631)	0.004	31931582.7035 (0.000 - Inf)	0.997
Pathologic N stage	477				
N0	283	Reference		Reference	
N1	108	1.681 (1.019–2.771)	0.042	0.080 (0.025–0.256)	<0.001
N2	86	4.051 (2.593–6.329)	<0.001	0.397 (0.144–1.092)	0.074
Pathologic M stage	414				
M0	348	Reference		Reference	
M1	66	4.193 (2.683–6.554)	<0.001	1.256 (0.465–3.388)	0.653
Pathologic stage	466				
Stage I&Stage II	267	Reference		Reference	
Stage III&Stage IV	199	2.947 (1.942–4.471)	<0.001	11.134 (3.527–35.151)	<0.001
Primary therapy outcome	250				
PD&SD&PR	42	Reference		Reference	
CR	208	0.111 (0.057–0.214)	<0.001	0.114 (0.039–0.336)	<0.001
Gender	477				
Female	226	Reference			
Male	251	1.101 (0.746–1.625)	0.627		
Histological type	472				
Adenocarcinoma	402	Reference			
Mucinous adenocarcinoma	70	1.269 (0.753–2.139)	0.371		
Race	306				
Asian&Black or African American	74	Reference			
White	232	0.865 (0.486–1.540)	0.623		
Age	477				
<=65	194	Reference		Reference	
>65	283	1.610 (1.052–2.463)	0.028	0.867 (0.345–2.180)	0.762
Residual tumor	373				
R0	345	Reference		Reference	
R1&R2	28	4.364 (2.401–7.930)	<0.001	0.781 (0.279–2.183)	0.637

Table 2. Logistic regression analysis of ARL6IP1 expression.

Characteristics	Total (N)	OR (95% CI)	P Value
Pathologic T stage (T3&T4 vs. T1&T2)	477	0.813 (0.517–1.278)	0.370
Pathologic N stage (N1&N2 vs. N0)	478	0.757 (0.525–1.092)	0.136
Pathologic M stage (M1 vs. M0)	415	0.801 (0.472–1.357)	0.409
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	467	0.748 (0.518–1.080)	0.121
Primary therapy outcome (CR vs. SD&PD&PR)	250	1.020 (0.525–1.982)	0.952
Gender (Male vs. Female)	478	0.764 (0.533–1.095)	0.143
Histological type (Mucinous adenocarcinoma vs. Adenocarcinoma)	473	0.879 (0.529–1.461)	0.618
Residual tumor (R2&R1 vs. R0)	374	0.823 (0.378–1.791)	0.623

3.3. Prognostic Relevance of ARL6IP1 Expression in Colon Cancer

Data analysis utilizing the TCGA database (**Figure 3**) revealed that diminished expression of ARL6IP1 is significantly correlated with adverse prognosis in patients with colon cancer. Kaplan-Meier survival curves indicated that patients with low ARL6IP1 expression had inferior survival outcomes, encompassing overall survival (OS), disease-specific survival (DSS), and progression-free survival (PFS). Specifically, DSS exhibited a hazard ratio (HR) of 0.50, with a 95% confidence interval (CI) spanning from 0.39 to 0.97 and a p-value of 0.039. Progression-free survival (PFS) also demonstrated a trend toward significance, with an HR of 0.74 and a 95% CI of 0.52–1.04 (p = 0.084).

Univariate Cox regression analysis further demonstrated that low ARL6IP1 expression was significantly associated with several clinical parameters, including pathological T-stage, N-stage, M-stage, and treatment outcome. Multivariate analysis corroborated the independent prognostic significance of ARL6IP1 expression (**Figure 4**).

To augment the clinical utility of these findings, a nomogram was constructed to integrate ARL6IP1 expression with other clinical features to predict the 1- and 3-year survival probabilities for colon cancer patients. Calibration curves indicated that the predicted values were highly consistent with the actual survival rates, thereby validating the accuracy of the nomogram.

Collectively, these results suggest that low ARL6IP1 expression may function as a potential marker of poor prognosis in colon cancer patients, with significant clinical predictive value.

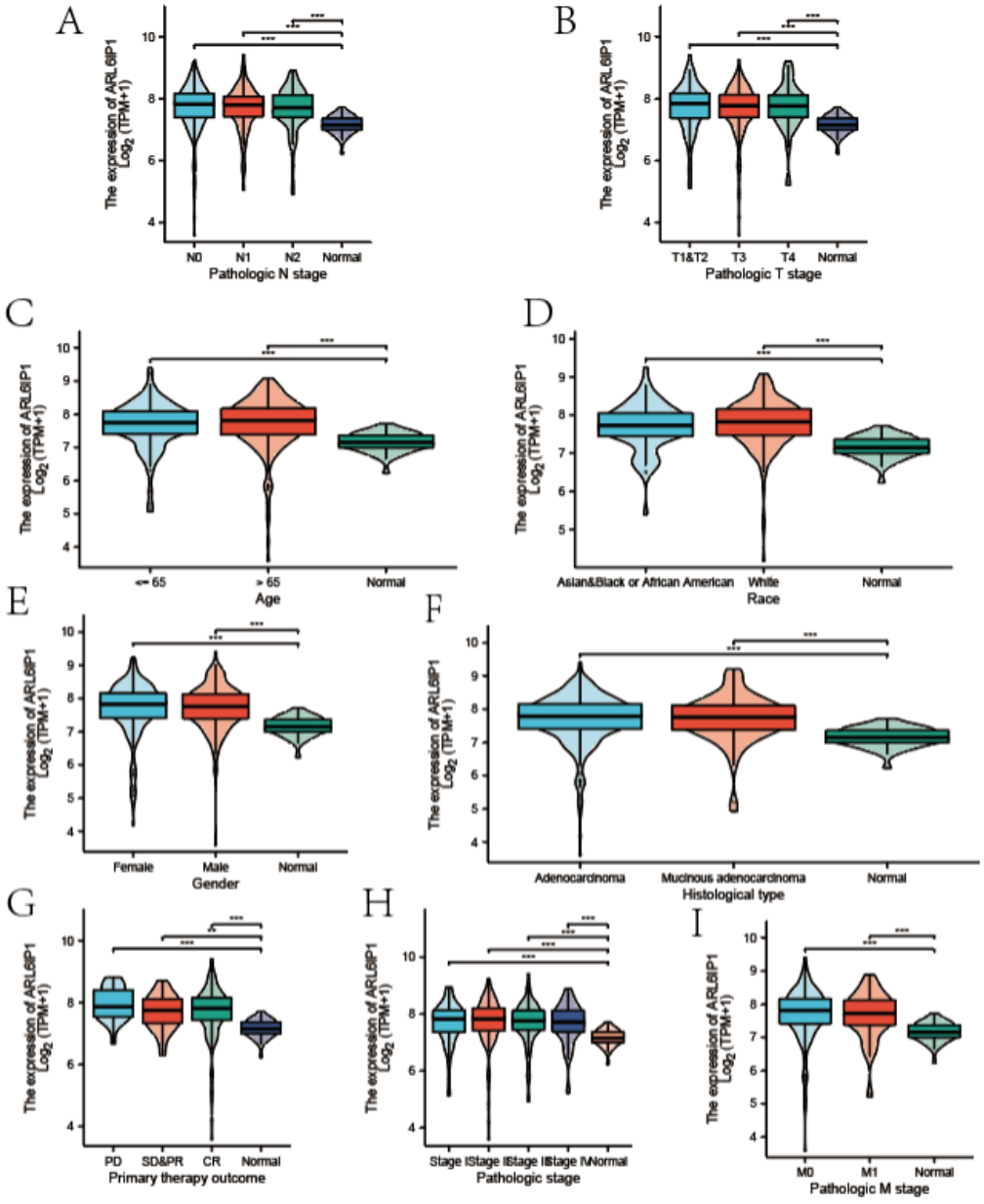


Figure 3. Analysis of ARL6IP1 expression levels in colorectal cancer tissues stratified by clinical and pathological features. (**A**,**B**): Violimn plots comparing ARL6IP1 expression across diferent pathologic N stages (**A**) and 'T stages (**B**). Expressionlevels are higher in advanced stages (N2, T4) compared to earlier stages and normal tissues. (**C**,**D**): ARL6IP1 expression by age group (**C**) and racial background (**D**). Patients <65 years and White patients exhibit-significantly elevated expression compared to their counterparts and normal tissues. (**E**,**F**): Gender-based (**E**) and histological subtype-based (**F**) comparisons of ARL6IP1 expression. Higher expression isobserved in males and in mucinous adenocarcinoma tissues compared to normal tissues. (**G**): ARL6IP1 expression stratified by primary therapy outcomes, showing significant diferences among PD, SD/PR, andR groups relative to normal tissues. (**H**,**l**): Pathological stage (**H**) and metastasis status (M stage, l) comparisons reveal increased ARL6IP1 expression in later-stages (stage ll-IV, M1) compared to early stages and normal tissues. Statistical signifcance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

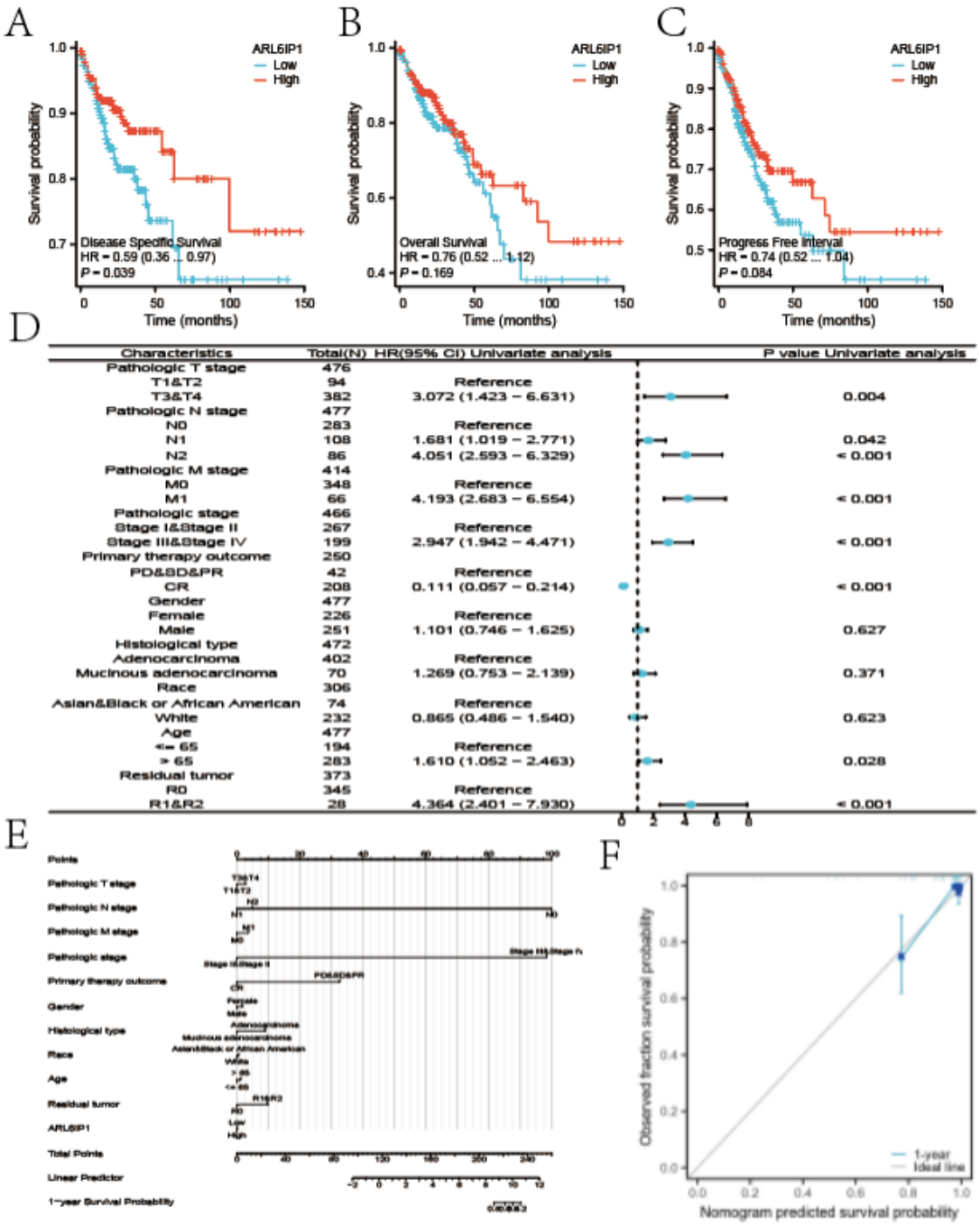
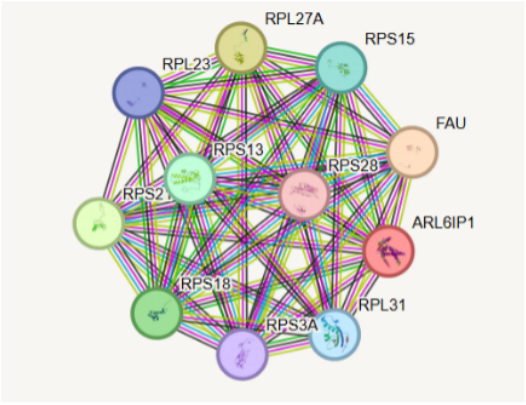


Figure 4. Prognostic analysis of ARL6IP1 expression in colorectal cancer (CRC). (**A**–**C**) Kaplan-Veier survival curves for disease-specific survival (DSS, **A**), overall survival (OS, **B**), and progression -freinterval (PFl, **C**) in CRC patients stratified by ARL6lP1 expression. Patients with high ARL6IP1 expression showedbetter survival outcomes compared to those with low expression. (**D**) Forest plot of univariate Cox regression analysis, ARL6lP1 expression is associated with favorable prognosticfeatures, including advanced pathological stages and therapyoutcomes. (**E**) Nomogram integrating ARL6IP1 expression and clinical parameters predicts 1-year survival probability. (**F**) Calibration plot confirming the accuracy of the nomogram in predicting 1-year survival probabilities.Statistical significance is indicated: <0.05, ** p < 0.01, and +** D < 0.001.

3.4. Constructing a PPI Network

In order to explore the protein interaction network involved in the development of colon cancer by ARL6IP1, we constructed an ARL6IP1-based PPI network using the STRING platform. The interaction relationship between ARL6IP1 and other important proteins, including FAU, RPL27A, RPS21, RPS18, RPS13, RPS15, RPL31, RPL23, RPS3A, and RPS28, was demonstrated in **Figure 5**. The scoring results showed that FAU had the highest association with ARL6IP1 (score 0.988), and other proteins such as RPL27A and RPS21 also showed high correlation with ARL6IP1 (scores 0.967 and 0.966, respectively). These interaction networks provide a basis for our understanding of the potential molecular mechanisms of ARL6IP1 in colon carcinogenesis and progression, suggesting that ARL6IP1 may play an important role in the regulation of tumor metabolism and cellular functions through synergistic interactions with these proteins.

A



B

Gene Symbol	Score
FAU	0.988
RPL27A	0.967
RPS21	0.966
RPS18	0.966
RPS13	0.963
RPS15	0.963
RPL31	0.961
RPL23	0.961
RPS3Q	0.96
RPS28	0.959

Figure 5. Protein-protein interaction (PPl) network of ARL6IP1 in colorectal cancer (**A**) PPI network constructed using the $'T'RlNG database, displaying interactions between ARL6lP1 and associated proteins.Key interacting partners include FAU, RPL27A, RPS21, RPS18, RPS13, RPS15, RPL31, RPL23, RPS3A, and RPS28. (**B**) Table showing the top 10 interacting proteins ranked by confidence scores, with FAU having the highest score (0.988). These interactions suggest ARL6lP1's involvement in translational and ribosomal processes, potentially impacting CRC progresior

3.5. Expression of the ARL6IP1 Gene to Whole Gene Expression Patterns

In this investigation, the expression pattern of the ARL6IP1 gene was scrutinized to elucidate its biological function in cancer. Analysis of ARL6IP1 gene expression in ccRCC samples revealed substantial alterations in gene expression profiles, characterized by a logFC exceeding 1 and a padj value below 0.05 (**Figure 6A**). Following stringent screening, the top 30 differentially expressed genes with the closest association with low ARL6IP1 expression were identified and are depicted in the heatmap (**Figure 6B**). Additionally, through Gene Ontology (GO) enrichment analysis, it was discovered that genes related to ARL6IP1 were enriched in several biological processes, including mRNA splicing, tetravalent body formation, and the regulation of lipid metabolism (**Figure 6C**).

3.6. GSEA Analysis of ARL6IP1 Gene Expression

Utilizing Gene Set Enrichment Analysis (GSEA) based on the ARL6IP1 gene expression profile in the TCGA dataset, we investigated the biological and functional pathway differences between high and low expression groups (**Table 3**). Significantly enriched pathways were identified through Normalized Enrichment Score (NES) values (**Figure 7**). The analysis revealed that the low expression group of ARL6IP1 was predominantly enriched in several key KEGG signaling pathways, such as Neuroactive Ligand Receptor Interaction, Adhesion Spot, Olfactory Transduction,

and Complement and Coagulation Cascade. Additionally, the Hedgehog signaling pathway, cancer-related pathways, and calcium signaling pathway also demonstrated notable enrichment trends.

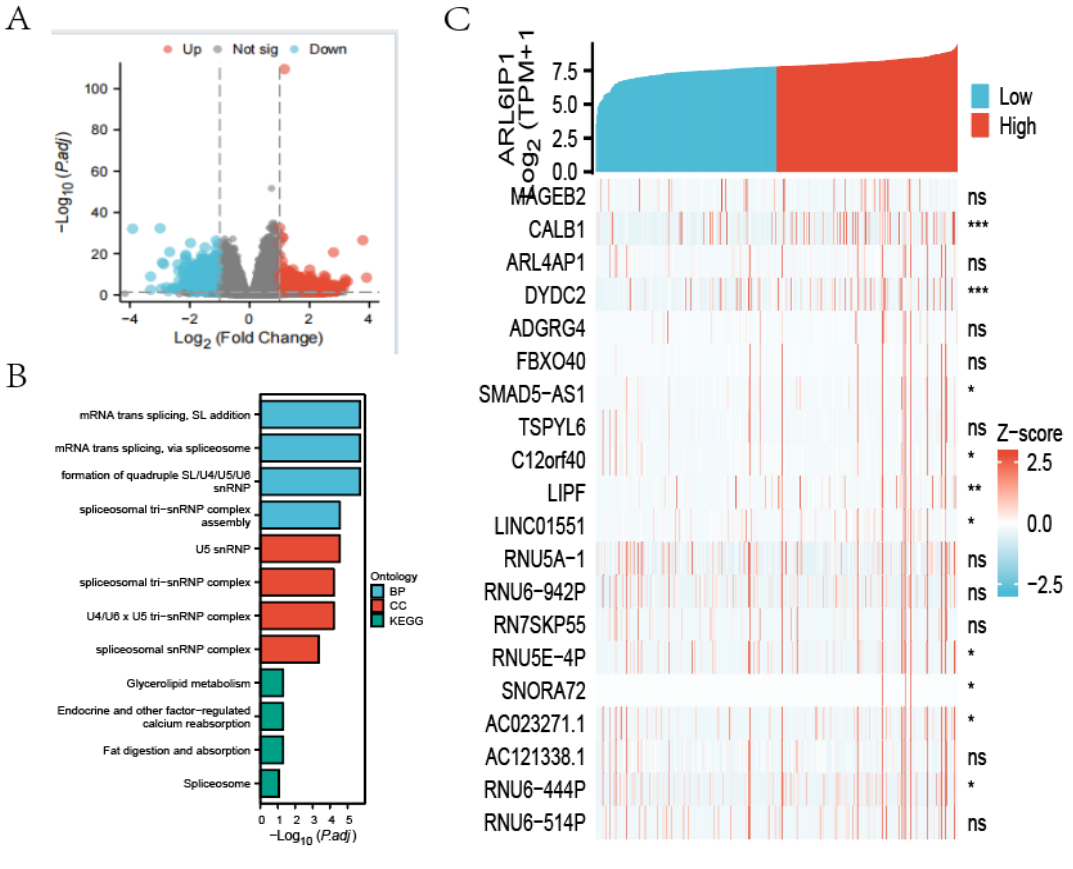


Figure 6. Differential expression analysis and enrichment of ARL6IP 1-related genes in colorectal cancer (CRC). (**A**) Volcano plot ilustrating the diferential expression of genes in CRC tissues compared to normal tissues. Red and blue dotsrepresent significantly upregulated and downregulated genes, respectively (log2FC > 1, adjusted p-value < 0.05). (**B**) GO and KEGG pathway enrichment analysis of ARL6lP1-associated differentially expressed genes (DEGs). Top pathwaysinclude mRNA splicing via spliceosome, U5 snRNP complex assembly, and glycerolipid metabolism, highlighting ARL6IP1'potential involvement in RNA processing and lipid metabolic regulation. (**C**) Heatmap ofthe top 20 DEGs stratified by ARL6IP1 expression levels (high vs. low). Z-scores indicate expression changes,with significant upregulation in CALB1 and ARL4AP1 observed in the high ARL6IP1 expresion group (*** p < 0.001).

Table 3. Results of gene ontologyenrichment analysis.

ID	Description	Set Size	Enrichment Score	NES	p Value	p. Adjust	q Value
KEGG_COMPLEMENT _AND_COAGULATION _CASCADES	KEGG_COMPLEMENT _AND_COAGULATION _CASCADES	66	−0.622518782	−2.556956081	1E-10	9.3E-09	6.68421E-09
KEGG_OLFACTORY _TRANSDUCTION	KEGG_OLFACTORY _TRANSDUCTION	147	0.594626319	2.507055558	1E-10	9.3E-09	6.68421E-09
KEGGFOCAL_ ADHESION	KEGG_FOCAL _ADHESION	198	−0.426726032	−2.100766874	6.22856E-10	2.89628E-08	2.08165E-08
_KEGG_NEUROACTIVE _LIGAND_RECEPTOR _INTERACTION	KEGG_NEUROACTIVE _LGAND_RECEPTOR _INTERACTION	255	−0.399650041	−2.024449619	5.26568E-10	2.89628E-08	2.08165E-08

Table 3. *Cont.*

ID	Description	Set Size	Enrichment Score	NES	p Value	p. Adjust	q Value
KEGGECM _RECEPTORINTE RACTION	KEGGECM _RECEPTORINTE RACTION	83	−0.561631714	−2.427734206	1.05692E-09	3.93174E-08	2.82587E-08
__KEGG_CALCIUM _SIGNALING_PATHWAY	__KEGG_CALCIUM _SIGNALING_PATHWAY	175	−0.422700401	−2.037136978	1.17881E-08	3.65431E-07	2.62647E-07
KEGGMELANOGENESIS	KEGGMELANOGENESIS	100	−0.482498483	−2.152450134	1.49345E-07	3.77422E-06	2.71265E-06
_KEGG_PATHWAYS _IN_CANCER	_KEGGPATHWAYS _IN_CANCER	323	−0.335328016	−1.757271434	1.62332E-07	3.77422E-06	2.71265E-06
KEGGHEDGEHOG _SIGNALINGPATHWAY	_KEGG_HEDGEHOG_ SIGNALING_PATHWAY	55	−0.575947756	−2.285818281	1.89368E-07	3.91361E-06	2.81284E-06
__KEGG_BASAL _CELL_CARCINOMA	KEGG_BASAL_ CELL_CARCINOMA	54	−0.586731854	−2.32104271	2.81016E-07	5.2269E-06	3.75674E-06
KEGG_AXON _GUIDANCE	KEGG_AXON _GUIDANCE	128	−0.431468888	−1.987890268	3.75434E-07	6.34824E-06	4.56269E-06
KEGG_DILATED_ CARDIOMYOPATHY	KEGG_DILATED _CARDIOMYOPAHY	89	−0.475136301	−2.072010695	5.22378E-06	8.09686E-05	5.81947E-05
KEGG_VASCULAR_ SMOOTHMUSCLE_ CONTRACTION	KEGG_VASCULAR _SMOOTHMUSCL ECONTRACTION	115	−0.423290174	−1.92668749	8.36644E-06	0.000119705	8.60355E-05
_KEGG_MAPK_ SIGNALING_PATHWAY	__KEGG_MAPK_ SIGNALING_PATHWAY	263	−0.326176627	−1.657624319	9.26936E-06	0.00012315	8.85119E-05
KEGG_HEMATOPOIETIC _CELL_LINEAGE	KEGG_HEMATOPOIETIC _CELL_LINEAGE	83	−0.45971068	−1.987165816	1.19341E-05	0.000147983	0.00010636
KEGG_CELL_ADHESION _MOLECULES_CAMS	KEGG_CELL_ADHESION _MOLECULES_CAMS	129	−0.40237189	−1.853177677	1.99986E-05	0.000232483	0.000167093
KEGG_CYTOKINE_ CYTOKINE_RECEPTOR _INTERACTION	KEGG_CYTOKINE_ CYTOKINE_RECEPTOR _INTERACTION	241	−0.31832118	−1.606045214	2.75142E-05	0.000301038	0.000216366
KEGG_WNT_ SIGNALING_PATHWAY	KEGG_WNT_SIGNALING _PATHWAY	149	−0.365208781	−1.718454674	4.10792E-05	0.000424485	0.000305091
KEGG_ARACHIDONIC _ACID_METABOLISM	KEGG_ARACHIDONIC_ ACID_METABOLISM	56	−0.505197136	−2.022497068	4.64585E-05	0.000454805	0.000326883
KEGG_GNRHSIGNALING _PATHWAY	KEGGGNRHSIGNALING PATHWAY	100	−0.410334607	−1.830523437	6.98827E-05	0.000649909	0.00046711
_KEGGREGULATION _OF_ACTIN_ CYTOSKELETON	___KEGGREGULATION _OFACTIN_ CYTOSKELETON	208	−0.322882832	−1.612022791	0.000119211	0.001055871	0.000758889
_KEGG_LEUKOCYTE_ TRANSENDOTHELIAL_ MIGRATION	__KEGG_LEUKOCYTE_ TRANSENDOTHELIAL_ MIGRATION	113	−0.388189528	−1.761880281	0.000145839	0.001233002	0.000886198
KEGG_PPAR_ SIGNALING_PATHWAY	KEGG_PPAR_ SIGNALING_PATHWAY	68	−0.447093242	−1.860582387	0.00024197	0.0019568	0.001406415
KEGG_ ARRHYTHMOGENIC _RIGHT_ VENTRICULAR_ CARDIOMYOPATHY _ARVC	KEGG_ ARRHYTHMOGENIC _RIGHT_ VENTRICULAR_ CARDIOMYOPATHY _ARVC	73	−0.426073541	−1.786279791	0.000334906	0.002595522	0.001865485
KEGG_ CHEMOKINESIGNALING _PATHWAY	KEGG_ CHEMOKINE_ SIGNALINGPATHWAY	186	−0.316946362	−1.548427322	0.000670546	0.004988862	0.003585657
_KEGG_GAP _JUNCTION	_KEGG_GAP _JUNCTION	89	−0.396017224	−1.726982176	0.000755547	0.005405064	0.003884794
KEGG_ HYPERTROPHICCAR DIOMYOPATHYHCM	KEGG HYPERTROPHICCAR DIOMYOPATHYHCM	82	−0.399632773	−1.716046312	0.000795924	0.005483029	0.00394083

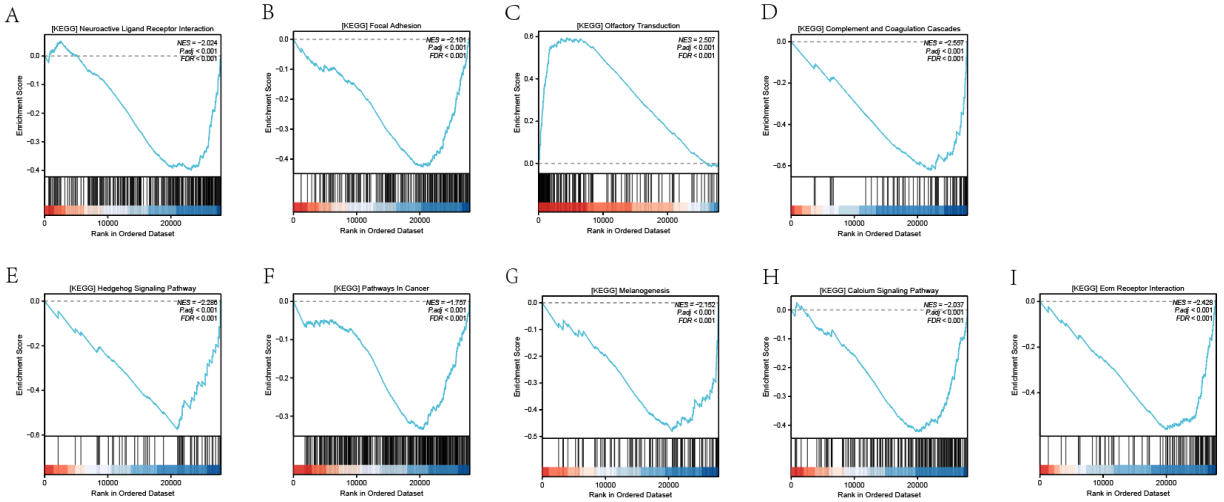


Figure 7. Gene Set Enrichment Analysis (GSEA) Of ARL6IP1 in colorectal cancer (ORO). (**A–l**) GsEA plotsilustrating enriched KEGG pathways in the ARL6P1 low-expresion group compared o the high-expresion group. ignicanty enriched pathwaysincude. (**A**) Newroacirveligand- eceptor interaction, (**B**) Focal adhesion, (**C**) 0ladtory transducion, (**D**) Complement and coapulktion casades, (**E**) Hedgehog signaing patliway (**E**) Pathwaysin cancer (**G**) Meanogenesil (**H**) Calcium signaling pathway, and (**I**) ECM receptor interaction. Normalized Fmichment Sores NEsl pxrales andFDR wlues areindicsted foreach natwey hiohighhngthe ikemetofARlfiplin aucad cancer-related and metahoicprocese.

These findings suggest that ARL6IP1 may play a crucial role in key biological processes such as cell signaling, immune response, and tumorigenesis. Specifically, the enrichment of the Hedgehog signaling pathway indicates potential involvement in tissue homeostasis and tumorigenesis. The calcium signaling pathway's enrichment highlights its role in intracellular signaling and regulation of various cellular processes. Overall, these results highlight the multifaceted biological functions of ARL6IP1 and its potential impact on colon cancer progression.

3.7. Relationship between ARL6IP1 Gene Expression and Immune Cell Infiltration

In the correlation analysis between ARL6IP1 gene expression and various immune cell subtypes in clear cell renal cell carcinoma (ccRCC), it was revealed that ARL6IP1 expression levels exhibited significant correlations with the infiltration of multiple immune cell types. Notably, the associations between ARL6IP1 and T helper cells (Th cells), regulatory T cells (Tregs), Tγδ cells, mast cells, and macrophages were particularly pronounced (**Figure 8A**). Further investigation indicated that ARL6IP1 expression was markedly elevated in immune cell populations with active functions, such as dendritic cells (DCs), natural killer (NK) cells, and macrophages, while it was relatively lower in certain immune cells with low expression or in an inactivated state, such as neutrophils.

Additionally, scatter plots illustrated a positive correlation between ARL6IP1 expression and the proportion of specific immune cells (**Figure 8E**). For instance, in macrophages and T-cell subpopulations, higher ARL6IP1 expression levels were directly proportional to the extent of immune cell infiltration in tumor tissues. Collectively, these findings suggest that ARL6IP1 expression may play a crucial role in modulating the immune microenvironment of ccRCC, particularly in influencing the degree of immune cell infiltration, thereby highlighting its potential function in tumor immunoregulation.

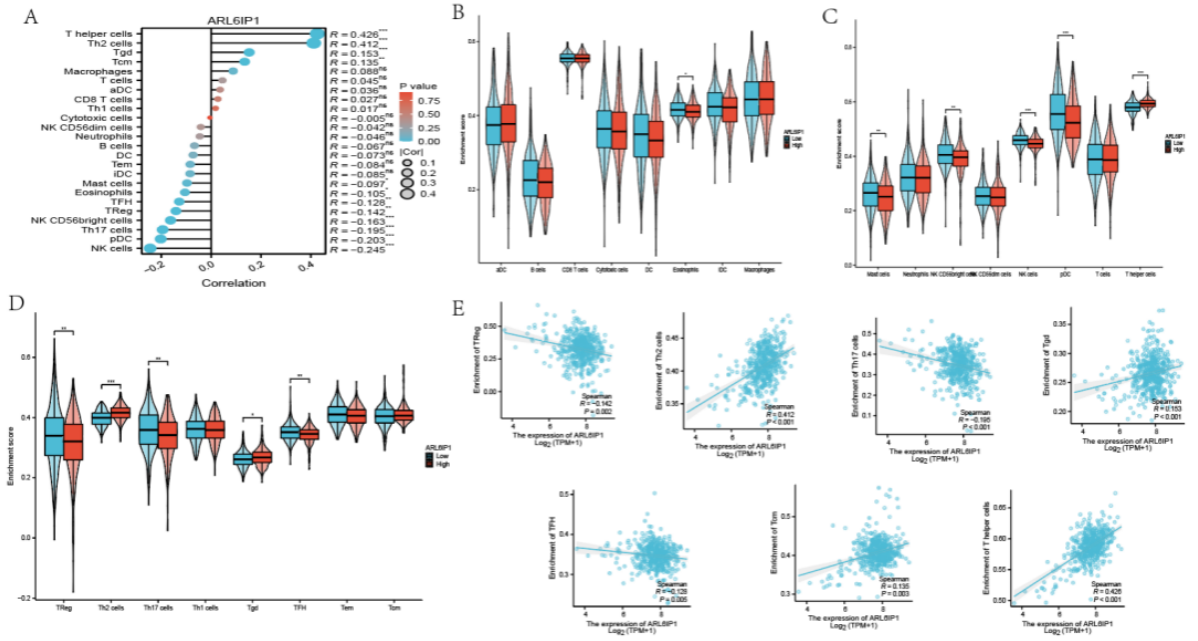


Figure 8. (**A**) Correlation of ARL6lPl expression with immune cell infltration in colorectal cancer(CRC). Correlation analysis between ARL6lP1 expression cell infltration levels in CRC, including T helper calls, macrophages, CD8+ T cells, and regulatory T cells (Tregs). Correlation coeficients (R) and p-values are shown. (**B**–**D**) Violin plots comparing immune cell infltraties scores in ARL 6IP1 high-expression and low-expression groups. Specific immune cell types, such as aDCs, macrophages, and various T cell subsets, show significant differences (* $p < 0.05$, * $p < 0.01$, ** $p < 0.001$). (**E**) Scatter plots illustrating the positive correlation between ARL6IP1 expression and infltration levels of Th2 cells, Tregs, and T helper cells. Spearman correlation coeficients (R) and p-values are annotated.

4. Discussion

In the present study, we conducted a comprehensive and meticulous investigation into the expression profile of the iron death-associated gene ARL6IP1 in colorectal cancer (CRC). This investigation was specifically designed to elucidate the associations between ARL6IP1 expression and patient prognosis, tumor immune microenvironment, and intestinal flora. Our analysis, which leveraged multiple public database resources, uncovered that ARL6IP1 expression is significantly downregulated in CRC tissues compared to normal tissues. This downregulation is closely correlated with adverse patient outcomes, including shortened overall survival (OS) and progression-free survival (PFS). Further analysis utilizing Cox regression models confirmed that low ARL6IP1 expression remains an independent risk factor for prognosis in CRC patients, even after accounting for various clinicopathologic variables. Collectively, these findings suggest that ARL6IP1 may serve as a valuable prognostic biomarker for CRC, potentially guiding the optimization of clinical treatment strategies [9, 10].

Our study provided insights into the potential mechanisms underlying the role of ARL6IP1 in colorectal cancer (CRC) progression. Specifically, ARL6IP1 has been implicated in the regulation of apoptosis in other cancer types, such as cervical cancer, where it has been shown to mediate cisplatin-induced apoptosis in CaSki cells. This suggests that ARL6IP1 may similarly influence cell survival and death pathways in CRC [11, 12]. Additionally, recent studies have highlighted the importance of iron death in cancer progression and treatment resistance. For instance, the TRPML1-ARL8B pathway has been identified as a key regulator of iron death resistance in AKT-hyperactivated cancers. Given the significant downregulation of ARL6IP1 in CRC tissues, it is plausible that this gene may also play a role in modulating iron death pathways in CRC, thereby influencing tumor progression and response to therapy [13–15].

Furthermore, the tumor immune microenvironment is a pivotal determinant in the progression of colorectal cancer (CRC), and our findings demonstrate that ARL6IP1 expression is intricately associated with the composition and function of the immune microenvironment in CRC [4, 14]. The intestinal flora, which is intricately linked

to the immune system, also appears to be influenced by ARL6IP1 expression. Recent research has shown that gut microbiota can modulate immune responses and influence tumor progression through various mechanisms, including the production of bioactive metabolites. Thus, the downregulation of ARL6IP1 in colorectal cancer (CRC) may exert a direct impact on tumor cells while also indirectly modulating the tumor microenvironment and immune response via alterations in the intestinal microbiota [12, 16].

To summarize, our investigation has established that ARL6IP1 is markedly downregulated in colorectal cancer (CRC) tissues and is correlated with unfavorable patient outcomes. The potential mechanisms underlying these associations may encompass the regulation of ferroptosis pathways, modulation of the tumor immune microenvironment, and alterations in the intestinal microbiota [17, 18]. Future research endeavors should concentrate on corroborating these findings within more extensive cohorts and elucidating the molecular mechanisms through which ARL6IP1 exerts its influence on colorectal cancer (CRC) progression and the tumor microenvironment.Additionally, the potential therapeutic implications of targeting ARL6IP1 in CRC warrant further investigation [19, 20].

To provide further insights into the role of ARL6IP1 in colorectal cancer (CRC), we employed a multifaceted analytical approach, integrating various bioinformatics tools and methodologies. Specifically, we utilized Gene Set Enrichment Analysis (GSEA) to identify gene sets that are significantly enriched in CRC tissues with low ARL6IP1 expression. This approach allows us to uncover potential biological pathways and processes associated with ARL6IP1 downregulation in CRC [21, 22]. This comprehensive analysis revealed significant enrichment in several key biological pathways, including neuroactive ligand-receptor interactions, cell adhesion molecules, and apoptosis regulation. These pathways are known to play crucial roles in various cellular processes, and their enrichment suggests that ARL6IP1 may influence the iron death process by modulating intercellular communication, redox homeostasis, and signal transduction. This, in turn, may contribute to the development of malignant tumor phenotypes [19, 23, 24].

Notably, the association of ARL6IP1 expression with complement and coagulation cascade response pathways suggests its potential involvement in tumor progression through modulation of the inflammatory microenvironment. This finding underscores the complexity of the interactions between ARL6IP1 and the tumor microenvironment, highlighting the need for further investigation into the specific mechanisms underlying these associations [2, 25]. Future studies should focus on elucidating the precise molecular interactions between ARL6IP1 and these pathways, as well as exploring the potential therapeutic implications of targeting these pathways in CRC. Additionally, experimental validation of these bioinformatics findings in in vitro and in vivo models is warranted to confirm the functional relevance of ARL6IP1 in CRC progression [20, 26].

Beyond its potential involvement in the regulation of iron-dependent cell death, ARL6IP1 appears to exert a substantial influence on the tumor immune microenvironment. Our analysis demonstrated that low expression of ARL6IP1 is positively correlated with the infiltration levels of various immunosuppressive cells, including regulatory T cells (Tregs) and M2-type macrophages [21, 27]. Conversely, ARL6IP1 expression is inversely associated with the infiltration levels of anti-tumor immune cells, such as effector CD8+ T cells and natural killer cells (NK cells) [28, 29]. This suggests that ARL6IP1 may facilitate immune evasion by tumor cells through the promotion of an immunosuppressive microenvironment. This finding is consistent with the established mechanism of iron death in regulating the immune microenvironment, which involves influencing the activity and infiltration level of immune cells through metabolites or inflammatory factors. Based on these observations, we hypothesize that ARL6IP1 may affect the immune microenvironment of CRC by regulating the process of iron death, thereby promoting immune escape of tumor cells and tumor malignancy. Future research endeavors should concentrate on elucidating the precise mechanisms through which ARL6IP1 modulates the immune microenvironment and its interaction with iron death pathways [30, 31].

Notwithstanding the comprehensive nature of our investigation, several limitations must be acknowledged. First, this study was primarily based on bioinformatics analysis of publicly available databases and lacked validation through in vitro and in vivo experiments. While bioinformatics approaches provide valuable insights, they are inherently limited by the quality and availability of data. Therefore, future studies need to further elucidate the specific mechanisms of ARL6IP1's role in iron death regulation and immune microenvironment modulation through cellular experiments and animal models. This will help to validate the findings from our bioinformatics analysis and provide a more robust understanding of the biological functions of ARL6IP1 in CRC [32, 33].

In addition, considering the remarkable heterogeneity of CRC, the roles of ARL6IP1 in different molecular sub-

types may differ. Subsequent studies could further clarify its functional roles in combination with molecular typing, thereby providing a more nuanced understanding of its contributions to CRC progression [34, 35]. Moreover, the clinical relevance of ARL6IP1 as a prognostic biomarker and potential therapeutic target should be evaluated in larger cohorts and across diverse patient populations to ensure its applicability in clinical settings. Future research should also explore the potential interactions between ARL6IP1 and other signaling pathways involved in CRC development and progression, which may reveal additional therapeutic opportunities [36, 37].

Moreover, the tumor immune microenvironment is a highly complex ecosystem encompassing diverse cell types, cytokines, and metabolic products, which not only influence tumor initiation and progression but also modulate immune cell infiltration and function. Our analysis demonstrated that low expression of ARL6IP1 is positively correlated with the infiltration levels of various immunosuppressive cells, such as regulatory T cells (Tregs) and M2-type macrophages—cells that are well-known for their contribution to an immunosuppressive microenvironment and facilitation of tumor immune evasion. In contrast, ARL6IP1 expression is inversely associated with the infiltration levels of anti-tumor immune cells, including effector CD8+ T cells and natural killer cells (NK cells). These findings suggest that ARL6IP1 may promote immune evasion by tumor cells through the establishment of an immunosuppressive microenvironment.This finding is consistent with the established mechanism of iron death in regulating the immune microenvironment, which involves influencing the activity and infiltration level of immune cells through metabolites or inflammatory factors. Based on these observations, we hypothesize that ARL6IP1 may affect the immune microenvironment of CRC by regulating the process of iron death, thereby promoting immune escape of tumor cells and tumor malignancy. Future studies should focus on elucidating the specific mechanisms through which ARL6IP1 modulates the immune microenvironment and its interplay with iron death pathways [38, 39].

In this study, we have uncovered compelling evidence that ARL6IP1, a gene associated with iron-dependent cell death, is intricately connected to multiple critical aspects of colorectal cancer (CRC). Specifically, our integrative analysis, leveraging data from various databases, has illuminated the complex relationships between ARL6IP1 expression and several key factors in CRC, including patient prognosis, the tumor immune microenvironment, and alterations in the intestinal microbiota. Our findings suggest that low expression of ARL6IP1 is correlated with a more aggressive disease course and the development of malignant phenotypes in CRC, mediated through several distinct yet interconnected mechanisms [34, 40].

Initially, ARL6IP1 seems to exert a substantial influence on the regulation of ferroptosis—a form of cell death marked by iron-dependent lipid peroxidation. This regulatory role is particularly significant in the context of colorectal cancer (CRC), given that ferroptosis has garnered increasing attention as a potential therapeutic target in cancer treatment. By modulating ferroptosis, ARL6IP1 may alter the equilibrium between cell survival and cell death within tumor tissues, consequently impacting tumor progression.For instance, studies have shown that iron death can be induced in cancer cells through the manipulation of specific metabolic pathways, leading to tumor cell death and potentially reducing tumor burden [27, 32].

Secondly, our analysis revealed that ARL6IP1 expression is closely correlated with the composition and function of the tumor immune microenvironment. The immune microenvironment is a complex and dynamic network of immune cells, cytokines, and other signaling molecules that can either promote or inhibit tumor growth. We found that low expression of ARL6IP1 is associated with an increase in the infiltration of immunosuppressive cells, such as regulatory T cells (Tregs) and M2-type macrophages, which are known to contribute to an immunosuppressive microenvironment and facilitate tumor immune evasion. Conversely, ARL6IP1 expression exhibits an inverse correlation with the infiltration of anti-tumor immune cells, such as effector CD8+ T cells and natural killer (NK) cells.This suggests that ARL6IP1 may modulate the immune microenvironment in a way that promotes immune evasion by tumor cells, thereby contributing to tumor progression [29, 35].

Moreover, ARL6IP1 appears to influence the metabolic network of the intestinal flora, which has been increasingly recognized as a critical factor in CRC development and progression. The intestinal flora, or microbiota, consists of a diverse community of microorganisms that can influence various aspects of host physiology, including immune function and metabolism. Our findings indicate that alterations in ARL6IP1 expression may affect the composition and function of the intestinal flora, potentially contributing to the development of a pro-tumorigenic environment [16, 30]. For example, certain microbial metabolites have been shown to modulate immune cell function and influence the efficacy of cancer therapies. By affecting the intestinal flora, ARL6IP1 may indirectly influence the immune

microenvironment and overall tumor biology.

The multifaceted roles of ARL6IP1 in colorectal cancer (CRC) underscore its potential as a valuable target for both mechanistic studies and clinical applications. Our findings provide a theoretical foundation for further exploration of the functional mechanisms of ARL6IP1 in CRC and its potential as a prognostic biomarker and therapeutic target. Future research should concentrate on elucidating the specific molecular pathways through which ARL6IP1 regulates ferroptosis, immune microenvironment remodeling, and intestinal flora metabolism. This will necessitate a combination of in vitro and in vivo experimental approaches to validate the findings from our bioinformatics analysis and provide a more comprehensive understanding of ARL6IP1's biological functions [2, 21, 32].

In addition, considering the remarkable heterogeneity of CRC, it is essential to explore the roles of ARL6IP1 in different molecular subtypes of the disease. This will help to identify specific patient populations that may benefit from targeted therapies based on ARL6IP1 expression. Furthermore, the clinical relevance of ARL6IP1 as a prognostic biomarker and potential therapeutic target should be evaluated in larger cohorts and across diverse patient populations to ensure its applicability in clinical settings [20, 25].

In a nutshell, our study has provided important insights into the multifaceted roles of ARL6IP1 in CRC, highlighting its potential as a key regulator of iron death, immune microenvironment remodeling, and intestinal flora metabolism. Future research endeavors should prioritize the detailed elucidation of the molecular mechanisms underpinning these roles and the exploration of the therapeutic potential of targeting ARL6IP1 in colorectal cancer (CRC). This will not only enhance our understanding of the molecular basis of CRC progression but also pave the way for the development of new precision therapies and personalized medicine approaches for this disease.

5. Conclusions

In a nutshell, the present study demonstrated that ARL6IP1 was under-expressed in colon cancer, which may promote colon cancer progression by regulating iron death and related signaling pathways.Reduced expression of ARL6IP1 was significantly associated with poor prognosis of the patients, suggesting that it may be a potential marker for survival prediction in colon cancer patients. In addition, ARL6IP1 expression correlated with the level of infiltration of a variety of immune cells, especially in immunosuppressive cells such as regulatory T cells (Tregs), M2-type macrophages, etc. ARL6IP1 may further contribute to the malignant phenotype of colon cancer by affecting the immunosuppressive features of the tumor microenvironment. Future studies can combine in vitro and in vivo experiments to further validate the function and mechanism of ARL6IP1 in colon cancer and provide an in-depth understanding of its role in colon carcinogenesis, progression and immune regulation. In summary, our study demonstrated that low expression of ARL6IP1 in colon cancer is associated with poor prognosis and may promote cancer progression by regulating iron death and related signaling pathways. The findings highlight the potential of ARL6IP1 as a prognostic marker and its role in modulating the tumor immune microenvironment. Future research should further validate these results and explore the underlying mechanisms, aiming to develop targeted therapies for CRC patients.

Author Contributions

All members of this article participated in the first draft of the article writing, G.L. responsible for most of the writing of the article. S.L. responsible for the experimental data visualization. J.G., R.Z. are responsible for the revision and integration of the whole article. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

All data were obtained from public databases.

Data Availability Statement

Data are available upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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