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ORIGINAL RESEARCH

Integrated Analysis of CD1A Immune Infiltration and Competing Endogenous RNA Networks in COAD

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Background: The CD1A gene, a key component of the human immune system and part of the CD1 family, plays a crucial role in presenting lipid antigens to T cells. Abnormal CD1A expression is associated with various immune-related diseases and tumors. However, the biological function of CD1A in COAD is unclear.

Methods: Multiple databases were systematically employed to conduct an analysis of CD1A expression in pan-cancer and COAD, along with its clinical-pathological features. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses of CD1A were performed using the 'clusterProfiler' package. The Protein-protein interaction (PPI) analysis of CD1A was used the STRING database. Additionally, TIMER and ssGSEA tools were used to explore the relationship between CD1A expression in COAD and immune cell infiltration. The study also investigated the association between CD1A expression and N6-methyladenosine (m6A) modification genes in the TCGA COAD cohort and constructed a CD1A-centric competing endogenous RNA (ceRNA) regulatory network.

Results: CD1A displays varying expression levels in various tumors, including COAD, and is closely linked to clinical-pathological characteristics. GO analysis suggests that CD1A plays a role in important processes like antigen processing and presentation, leukocyte-mediated immunity, and lymphocyte-mediated immunity. KEGG analysis identifies CD1A's involvement in key pathways such as the Chemokine signaling pathway and Cytokine-cytokine receptor interaction. PPI analysis highlights CD1A's interactions with CD207, CD1C, CD1E, FOXP3, and ITGB2. ssGSEA analysis indicates a significant relationship between CD1A expression and the infiltration of various immune cells in COAD. Significant associations were found between CD1A and m6A modification genes in COAD. Furthermore, a CD1A-centered ceRNA regulatory network has been constructed.

Conclusion: CD1A emerges as a potential biomarker for the diagnosis and treatment of COAD, showing a strong association with tumor immune infiltration, m6A modification, and the ceRNA network.

Keywords: CD1A, COAD, immune infiltration, m6A, ceRNA

Introduction

Colon adenocarcinoma (COAD) is globally recognized as one of the prevalent malignant tumors of the digestive system, with a steadily increasing incidence across various world regions.[1] Research has established a close correlation between the onset of COAD and genetic factors, including family history and hereditary tumor syndromes, as well as lifestyle modifications such as diet and physical activity.[2] Particularly in advanced stages, COAD exhibits high malignancy, often resulting in poor prognoses without prompt treatment, thereby presenting a significant challenge to global public health. Despite advancements in early diagnosis and treatment, the five-year survival rate for advanced-stage colon

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adenocarcinoma patients remains suboptimal.[3] The pathogenesis of colon adenocarcinoma involves a complex interplay of multiple molecular aspects, including dysregulation of the cell cycle, alterations in signaling pathways like Wnt/β-catenin and PI3K/Akt, and interactions within the tumor microenvironment.[4–7] These intricate mechanisms present significant obstacles to developing effective treatments for colon adenocarcinoma. Therefore, exploring the molecular mechanisms of colon adenocarcinoma and identifying new biomarkers and therapeutic targets are crucial for improving patient outcomes and treatment effectiveness.

CD1A is a crucial element of the human immune system, responsible for transporting nonpeptide antigens such as lipids and glycolipids to T cells.[8,9] As a member of the major histocompatibility complex (MHC) class Ib, CD1A plays a significant role in the activation and recognition of various T cell subpopulations, particularly in specific anatomical locations.[10] For example, the CD1A gene is highly expressed in human Langerhans cells, which act as antigen-presenting cells in the skin and regulate the skin's immune response.[11] Furthermore, CD1A molecules are integral in the immune response to microbial infections by capturing lipids and glycolipids and presenting them to T cells and NKT cells.[12] Studies have demonstrated that CD1A-restricted T cells are crucial in both innate and adaptive immune responses, especially in anti-tumor immune reactions, showcasing potent, paracrine, and adjuvant-like properties.[13] However, the biological functions of CD1A in COAD are unclear.

In the field of oncogene therapy, there is a growing interest in tumor immunotherapy, N6-methyladenosine (m6A) modification and competitive endogenous RNA (ceRNA) regulatory networks. These biological processes play crucial roles in tumorigenesis, progression, and immune escape mechanisms.[14,15] In particular, m6A modification, an important modality of epigenetic regulation, regulates gene expression by affecting RNA stability and translational efficiency, which in turn affects the biological behavior of tumors. Meanwhile, the ceRNA network regulates multiple signaling pathways through competitive binding of microRNAs (miRNAs), which plays a key role in regulating tumor cell proliferation, invasion, and immune response. Recent studies have specifically pointed out that the CD1A gene plays a crucial role in regulating the tumor immune microenvironment.[16] The expression level of CD1A, an antigen-presenting molecule, directly influences the interaction between tumor cells and the immune system. Specifically, CD1A plays an important role in promoting specific T cell recognition and activation, which is essential for the effectiveness of tumor immunotherapy. However, studies on the role of CD1A in tumor immunotherapy, m6A modification, and ceRNA network and its regulatory mechanisms are still limited. Therefore, it is crucial to deeply investigate the function of CD1A in COAD, especially its role and regulatory mechanism in m6A modification and ceRNA network.

In this study, we comprehensively utilized the Cancer Genome Atlas as well as other public databases, such as GENT2 and TIMER, to perform an in-depth analysis of the expression levels of the CD1A gene in multiple cancer types. Multidimensional analysis methods, including quantitative gene expression analysis, differential expression analysis, and functional enrichment analysis, were used to assess CD1A expression and its biological functions in COAD. In addition, we evaluated the correlation between CD1A expression and immune infiltration, m6A modifier genes, and constructed a CD1A-centered ceRNA regulatory network. These comprehensive analyses revealed the critical role of CD1A in the pathogenesis of COAD and indicated that CD1A is a potential biomarker for the diagnosis and treatment of COAD.

Materials and Methods

GENT2 Analysis

The Gene Expression database of Normal and Tumor tissues 2 (GENT2, http://gent2.appex.kr/gent2) is a publicly accessible database providing gene expression information for analyzing specific gene expression levels in various cancers.[17] It employs Analysis of Variance (ANOVA) to compare gene expression differences between normal tissues and a range of cancer types. CD1A expression in multiple cancers was analyzed using the GENT2 database, and the screening criteria for differentially expressed genes set at $|\log2FC| > 1$ and adjusted P-value < 0.05.

TIMER Analysis

The Tumor Immune Estimation Resource (TIMER, http://timer.comp-genomics.org/timer) database serves as an online bioinformatics tool specifically designed for analyzing immune cell infiltration within the tumor microenvironment.[18]

Leveraging an extensive array of tumor sample data from The Cancer Genome Atlas, this database offers a unique platform for investigating the intricate interactions between tumors and the immune system. In this study, the TIMER tool was employed to evaluate the expression level of the CD1A gene across various tumors and to explore the correlation between CD1A expression levels and immune cell infiltration. We paid special attention to immune cell types including B cells, neutrophils, CD4+ T cells, macrophages, CD8+ T cells, and dendritic cells, as well as tumor purity. Additionally, the correlation between CD1A and diverse immune cell gene markers was analyzed. The somatic copy number alteration (SCNA) module within TIMER was utilized to investigate the relationship between CD1A genetic copy number variation (CNV) and the abundance of tumor-infiltrating cells, thereby enriching the understanding of CD1A's role in tumor immunity.

TCGA Analysis

The Cancer Genome Atlas (TCGA, https://www.cancer.gov/ccg/research/genome-sequencing/tcga) is a valuable resource for cancer multi-omics research, containing sample information from thousands of cancer patients across different cancer types.[19] Used the 'RTCGA' package in R to download COAD mRNA sequencing data from TCGA database, which included 478 tumor samples and 41 normal samples.[20] We analyzed the expression level of CD1A in colon adenocarcinoma using TCGA COAD data and explored its correlation with clinicopathological features. Additionally, we analyzed the correlation between CD1A expression and m6A modification-related genes and assessed the differences in the expression of these genes in samples with high versus low CD1A expression. The m6A modification-related genes analyzed in this study include METTL3, YTHDC1, YTHDC2, METTL14, RBM15, RBM15B, IGF2BP1, IGF2BP2, IGF2BP3, VIRMA, WTAP, YTHDF1, YTHDF2, YTHDF3, ZC3H13, HNRNPA2B1, HNRNPC, RBMX, FTO, and ALKBH5.

GEO Analysis

The Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo), administered by the National Center for Biotechnology Information (NCBI), is a public repository that archives high-throughput gene expression data and other functional genomics experimental outcomes.[21] GEO database is instrumental in various fields, including basic biological research, elucidation of disease mechanisms, novel drug development, and biomarker discovery. To investigate the differences in CD1A gene expression between colon adenocarcinoma and normal colon tissues, COAD mRNA sequencing data from datasets GSE21510, GSE22598, and GSE37364 were obtained from the GEO database. The analysis of differential gene expression was performed using the 'limma' package in R, with criteria set at $|\log2FC| > 1$ and an adjusted P-value < 0.05.[22]

Cell Lines and Cell Culture

COAD cell lines (SW480 and HCT116) and the human normal colorectal mucosal cell line (HFC) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) enriched with high glucose (Thermo Fisher Scientific, Inc.). All cells were cultured under conditions of 37°C and 5% CO_2.

RNA Extraction and qRT-PCR

The experimental method refers to previous studies.[23] Initially, total RNA was extracted from cell samples utilizing Trizol reagent (Invitrogen, Carlsbad, CA, USA), adhering to the manufacturer's standard operating procedures. This was followed by reverse transcription of the extracted RNA into cDNA using the Prime Script RT reagent kit (Takara, Dalian, China). Post-reverse transcription, the expression of specific genes was quantified through quantitative real-time polymerase chain reaction (qRT-PCR) employing the SYBR Prime Script RT PCR kit (Takara, Dalian, China). GAPDH served as the internal reference gene for normalization in the qRT-PCR assays. The expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for CD1A and GAPDH in this study were: CD1A forward primer CGCACCATTCGGTCATTTGAGG, reverse primer TCCTGAGACCTTTCCAGAGTGC; GAPDH forward primer GTCTCCTCTGACTTCAACAGCG, reverse primer ACCACCCTGTTGCTGTAGCCAA.

LinkedOmics Analysis

LinkedOmics, an open-source database, consolidates extensive cancer omics data derived from the TCGA database.[24] This database is dedicated to the comprehensive analysis of multidimensional cancer omics data, encompassing data types such as gene expression, proteomics, and methylation. Moreover, LinkedOmics offers a robust suite of statistical analysis and visualization tools, aiding researchers in investigating and contrasting biomarkers and therapeutic targets both within specific cancer types and across diverse cancers. In this study, we utilized the LinkedOmics database to investigate genes co-expressed with CD1A in COAD, with the results visualized through volcano plots and heatmaps.

GO Functional Annotation and KEGG Pathway Enrichment Analysis

The Gene Ontology (GO) functional annotation system categorizes genes and their products based on biological processes, cellular components, and molecular functions. This standardized approach aids researchers in describing gene and protein functions, facilitating cross-species comparisons and information sharing. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis is a statistical method used to determine if a specific gene set is overrepresented in the KEGG pathway database. This analysis helps identify activated or suppressed biological pathways under various conditions, such as disease states or experimental treatments. KEGG enrichment analysis allows researchers to gain a deeper understanding of the functions of specific gene groups in complex biological processes and disease mechanisms. To elucidate the biological functions of CD1A in COAD, GO functional annotation and KEGG pathway enrichment analysis were conducted on CD1A's co-expressed genes using the 'clusterProfiler' package in R.[25]

Immune Infiltration Analysis

The single-sample Gene Set Enrichment Analysis (ssGSEA) package is a prominent bioinformatics tool used for analyzing gene expression data at the single-sample level, focusing on assessing immune cell infiltration.[26] By measuring the enrichment of specific gene sets, ssGSEA allows for a detailed examination of immune cell presence and levels in various biological samples, proving to be valuable in immunological studies and cancer research. In this study, ssGSEA was utilized to assess the changes in the relative proportions of 24 types of immune infiltrating cells linked to high or low CD1A expression in COAD.

PPI Analysis

The STRING database is a comprehensive bioinformatics resource dedicated to providing protein-protein interaction (PPI) information.[27] By combining experimentally validated data and computational predictions, the database aims to build and define networks of protein interactions. With its wide range of PPI data from different organisms, STRING has become a crucial tool for investigating protein interactions. In this study, a PPI network analysis of CD1A was conducted using the STRING database.

Prediction of miRNA

MiRwalk is an online platform focused on exploring the interactions between microRNA (miRNA) and their target genes.[28] Researchers can utilize its algorithm to discover genes that could be influenced by specific miRNAs or to identify miRNAs that might regulate particular genes. Predictions for miRNAs potentially regulating CD1A were made using miRwalk. Furthermore, an analysis was conducted to examine the correlation between these target miRNAs and CD1A, aiming to identify miRNAs that are more consistent with the ceRNA hypothesis.

Prediction of lncRNA and Construction of CeRNA Network

The miRNet database is a valuable online resource for miRNA research, offering detailed information on miRNAs and their interactions with genes, lncRNAs, and other non-coding RNAs.[29] This facilitates the analysis of miRNA function and network construction, making it a crucial tool for studying the roles of miRNAs in biological processes and diseases. Similarly, the starBase database is a comprehensive public resource dedicated to analyzing interactions among non-coding RNAs, mRNAs, and proteins.[30] By providing validated and predicted RNA-RNA and RNA-protein interactions,

starBase is essential for understanding how non-coding RNAs regulate gene expression and contribute to disease mechanisms. Both miRNet and starBase were used to predict lncRNA targets of specific miRNAs and analyze their expression correlations, identifying lncRNAs that support the ceRNA hypothesis. A ceRNA network for CD1A in COAD was constructed by combining miRNA-mRNA and miRNA-lncRNA interaction data.

Statistical Analysis

Unpaired samples were analyzed using unpaired t-tests, while paired samples were subjected to paired t-tests. Differences across multiple data groups were assessed through one-way Analysis of Variance. A significance level of $P < 0.05$ was considered in all statistical tests.

Results

Pan-Cancer Analysis of CD1A

Analysis utilizing the GENT2 database revealed that CD1A expression levels were significantly higher in tissues of Bone cancer, Colon cancer, Liver cancer, Lung cancer, Ovary cancer, Pharynx cancer, Placenta cancer, and Thyroid cancer compared to corresponding normal tissues. In contrast, CD1A expression was significantly lower in Eye cancer, Kidney cancer, Blood cancer, Adrenal_Gland cancer, Prostate cancer, Adipose cancer, Stomach cancer, Lymph_Node cancer, Brain cancer, Head_and_Neck cancer, Skin cancer, and Teeth cancer tissues than in normal tissues (Figure 1A). Furthermore, analyses from the TIMER database indicated significant upregulation of CD1A expression in BRCA, CESC, CHOL, COAD, ESCA, KIRC, KIRP, LIHC, LUAD, THCA, and UCEC compared to normal tissues (Figure 1B).

Expression of CD1A in COAD

Analysis of sequencing data from TCGA COAD indicated a significant increase in CD1A expression levels in COAD tissues compared to normal tissues. This observation was supported by both unpaired and paired sample datasets (Figure 1C and D). Additionally, consistent results were found in three independent GEO database datasets (GSE21510, GSE22598, and GSE37364), highlighting the elevated CD1A expression in cancer tissues (Figure 1E–G). The qRT-PCR results of cellular experiments further confirmed the significant upregulation of CD1A in COAD relative to human normal colorectal mucosal cells (Figure 1H).

Relationship Between CD1A Expression and Clinical Features

By utilizing clinical data from COAD patients in TCGA, an analysis was performed to investigate the relationship between CD1A expression and specific clinical characteristics. The findings demonstrated a significant correlation between higher CD1A expression and gender ($P = 0.044$), pathological T stage ($P = 0.025$), pathological N stage ($P = 0.004$), pathological M stage ($P = 0.039$), and overall pathological stage ($P = 0.007$) (Table 1).

GO Functional Annotation and KEGG Pathway Enrichment Analysis of CD1A

Using the LinkedOmics database, an analysis was performed on genes co-expressed with CD1A in COAD samples to investigate its biological functions. As depicted in Figure 2A, a total of 5153 genes were found to be positively correlated with CD1A, while 2236 genes exhibited significant negative correlation (FDR < 0.05). Heatmaps of the top 50 genes with the strongest positive and negative correlations with CD1A are shown in Figure 2B and C, respectively.

To elucidate the biological functions of CD1A in COAD, the 'clusterProfiler' package in R was utilized for GO functional annotation and KEGG pathway enrichment analysis of CD1A's co-expressed genes. This analysis identified 23 biological processes, 3 cellular components, 7 molecular functions, and 4 KEGG pathways significantly associated with CD1A. Bubble charts, presented in Figure 2D and E, illustrate the outcomes of the GO and KEGG analyses. The GO analysis indicated CD1A's primary involvement in processes such as antigen processing and presentation, leukocyte mediated immunity, and lymphocyte mediated immunity, while KEGG analysis revealed its association with pathways including the Chemokine signaling pathway and Cytokine-cytokine receptor interaction.

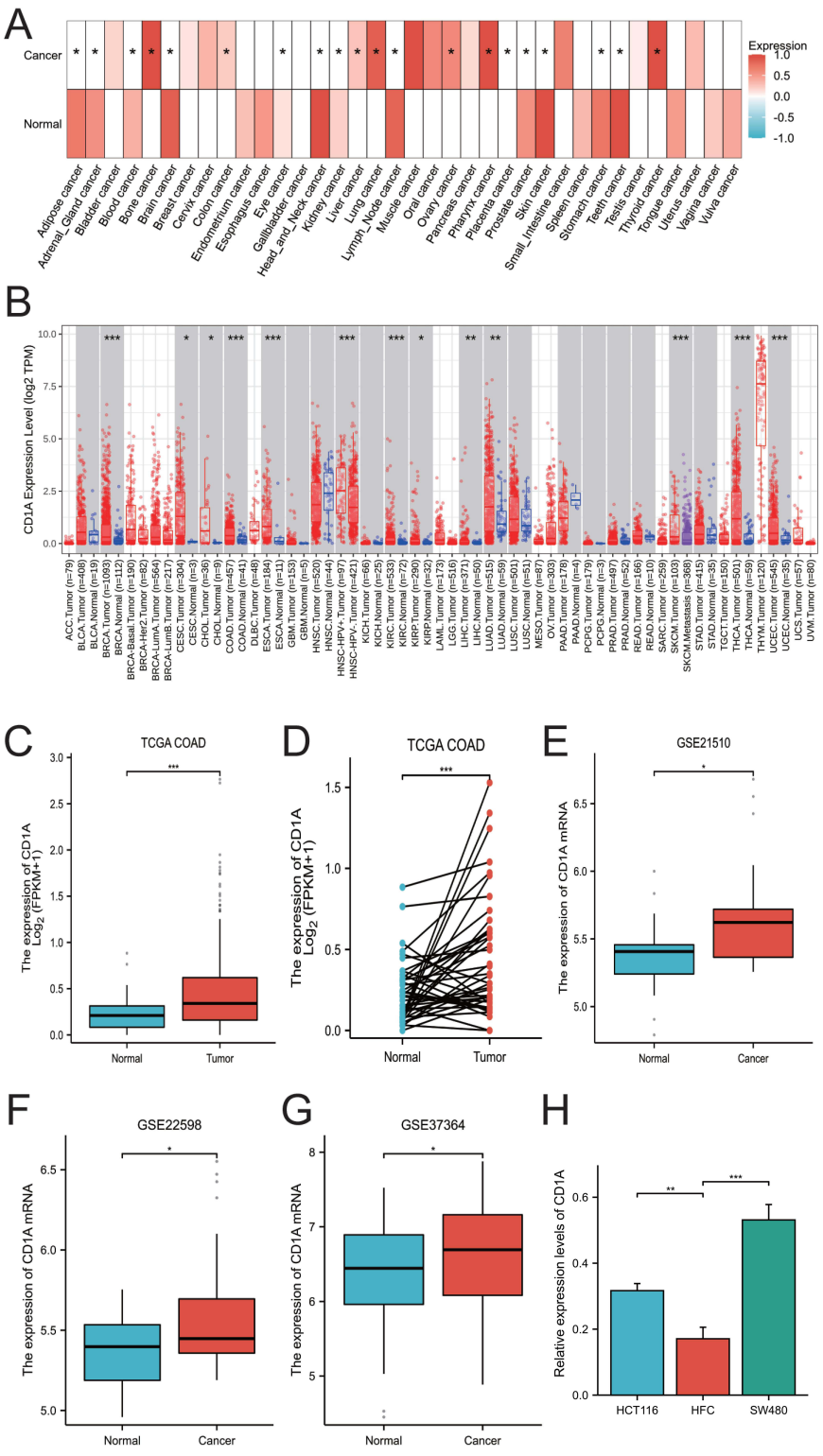

Figure 1 Expression of CD1A in COAD and Across Various Cancer Types. (**A**) Differential expression of CD1A in multiple tumor types as indicated by the GENT2 database. (**B**) Evaluation of CD1A expression levels across different tumor types using the TIMER database. (**C**) Comparative analysis of CD1A expression in COAD versus normal tissues in the TCGA database. (**D**) Analysis of CD1A expression in COAD compared to paired normal tissues using the TCGA database. (**E**) Examination of CD1A expression differences between COAD and normal tissues using the GSE21510 dataset. (**F**) Assessment of CD1A expression in COAD versus normal tissues using the GSE22598 dataset. (**G**) Investigation of CD1A expression in COAD relative to normal tissues with the GSE37364 dataset. (**H**) Analysis of CD1A expression in COAD cell lines (SW480 and HCT116) and normal human colon epithelial cell line (HCoEpiC).

Notes: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Abbreviations: COAD, Colon adenocarcinoma.

Table 1 Correlation Analysis of CD1A mRNA Expression with Clinicopathological Features in the TCGA COAD Cohort

Characteristics	Low expression of CD1A	High expression of CD1A	P value
n	239	239	
Age, n (%)			0.576
> 65	145 (30.3%)	139 (29.1%)	
<= 65	94 (19.7%)	100 (20.9%)	
Gender, n (%)			0.044
Female	102 (21.3%)	124 (25.9%)	
Male	137 (28.7%)	115 (24.1%)	
Pathologic T stage, n (%)			0.025
T1	5 (1%)	6 (1.3%)	
T2	30 (6.3%)	53 (11.1%)	
T3	168 (35.2%)	155 (32.5%)	
T4	36 (7.5%)	24 (5%)	
Pathologic N stage, n (%)			0.004
N0	141 (29.5%)	143 (29.9%)	
N1	43 (9%)	65 (13.6%)	
N2	55 (11.5%)	31 (6.5%)	
Pathologic M stage, n (%)			0.039
M0	158 (38.1%)	191 (46%)	
M1	39 (9.4%)	27 (6.5%)	
Pathologic stage, n (%)			0.007
Stage I	28 (6%)	53 (11.3%)	
Stage II	103 (22.1%)	84 (18%)	
Stage III	63 (13.5%)	70 (15%)	
Stage IV	39 (8.4%)	27 (5.8%)	
Histological type, n (%)			0.426
Adenocarcinoma	205 (43.3%)	198 (41.9%)	
Mucinous adenocarcinoma	32 (6.8%)	38 (8%)	

PPI Analysis of CD1A

The PPI network of CD1A was examined utilizing the STRING database, uncovering strong interactions with key proteins such as CD207, CD1C, CD1E, FOXP3, and ITGB2 (Figure 2F). Importantly, all of these genes are associated with regulating the immune response to tumors.[31–35] These findings suggest that CD1A could potentially have a significant impact on the development and advancement of colon adenocarcinoma.

Correlation of CD1A Expression with Immune Characteristics

Utilizing the TIMER database, an examination was conducted on the association between CD1A expression and levels of immune infiltration in COAD. Results indicated a significant positive correlation between CD1A expression and infiltration levels of B Cells ($P = 0.001$), CD8+ T Cells ($P = 0.003$), CD4+ T Cells ($P < 0.001$), Macrophages ($P < 0.001$), Neutrophils ($P < 0.001$), and Dendritic Cells ($P < 0.001$), and a significant negative correlation with Purity ($P < 0.001$) (Figure 3A). These findings imply a crucial role for CD1A in the immune infiltration within COAD. Additionally, the study found that CD1A CNV significantly impacted the infiltration levels of CD8+ T Cells, B Cells, and Dendritic Cells (Figure 3B).

Utilizing the TIMER tool, an analysis was conducted on the correlation between CD1A and various immune cell biomarkers in COAD (Table 2). The expression of CD1A demonstrated significant correlations with B cell markers CD70 and CD19 in COAD ($P < 0.05$). Further examination of different T cell types, including CD8+ T cells, Tfh cells, Th1 cells, Th2 cells, Th17 cells, Treg, and exhausted T cells, revealed significant associations between CD1A expression and T cell markers CD8A, CD8B, CD25, CD278, CD194, CD198, IL23R, CD196, FOXP3, CD73, CD127, PD-1, CTLA4, and LAG3 ($P < 0.05$), implying a potential role for CD1A in T cell-mediated immune responses in COAD. Moreover, CD1A expression was significantly correlated with markers of M1 Macrophage (IRF5), M2 Macrophage

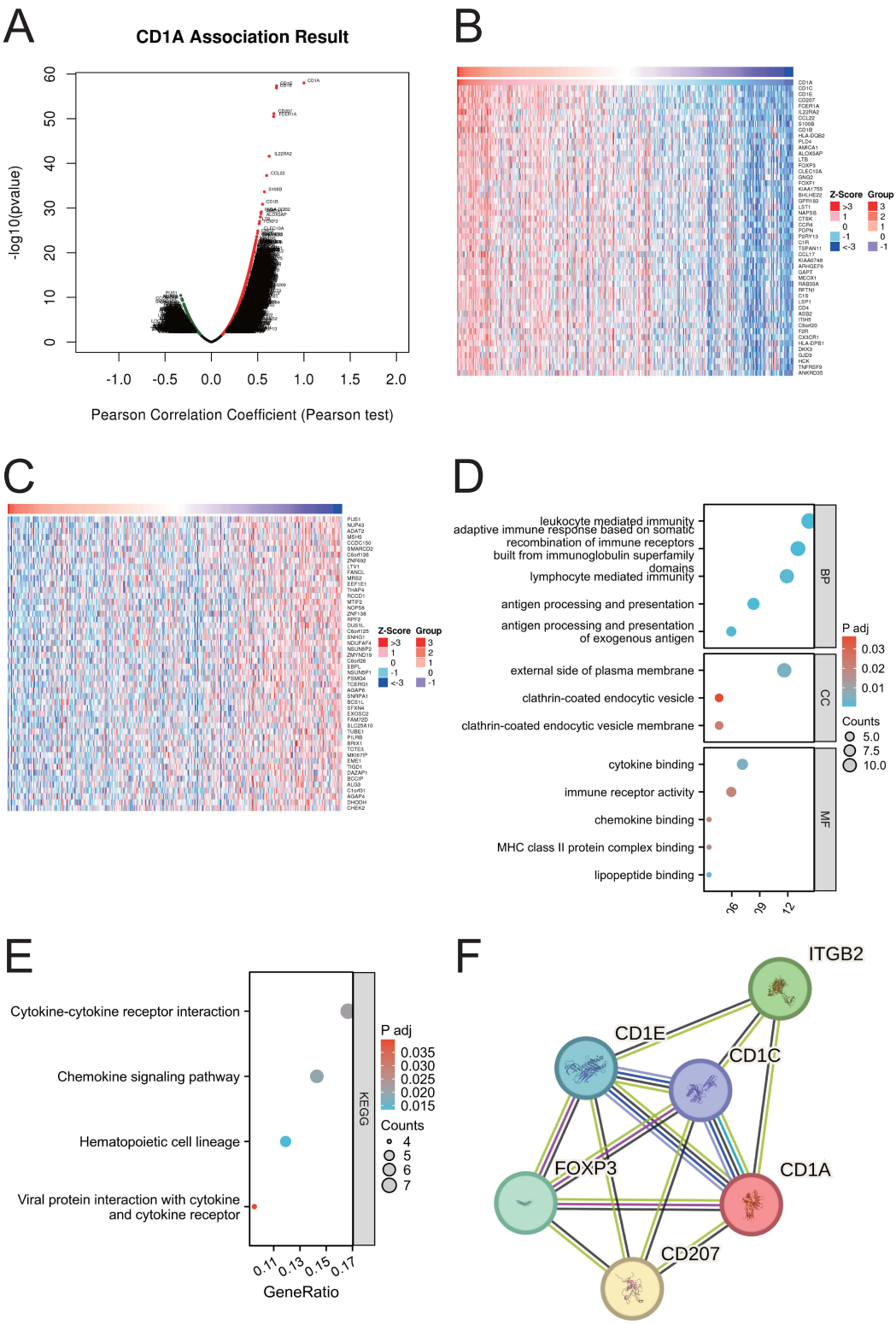

Figure 2 Analysis of CD1A's biological functions in COAD. (**A**) Identification of genes significantly associated with CD1A in COAD. (**B**) Heatmap illustrating the top 50 genes positively correlated with CD1A in COAD. (**C**) Heatmap depicting the top 50 genes negatively correlated with CD1A in COAD. (**D**) GO functional annotation analysis for genes co-expressed with CD1A. (**E**) KEGG pathway enrichment analysis for genes co-expressed with CD1A. (**F**) Protein-protein interaction network involving CD1A.

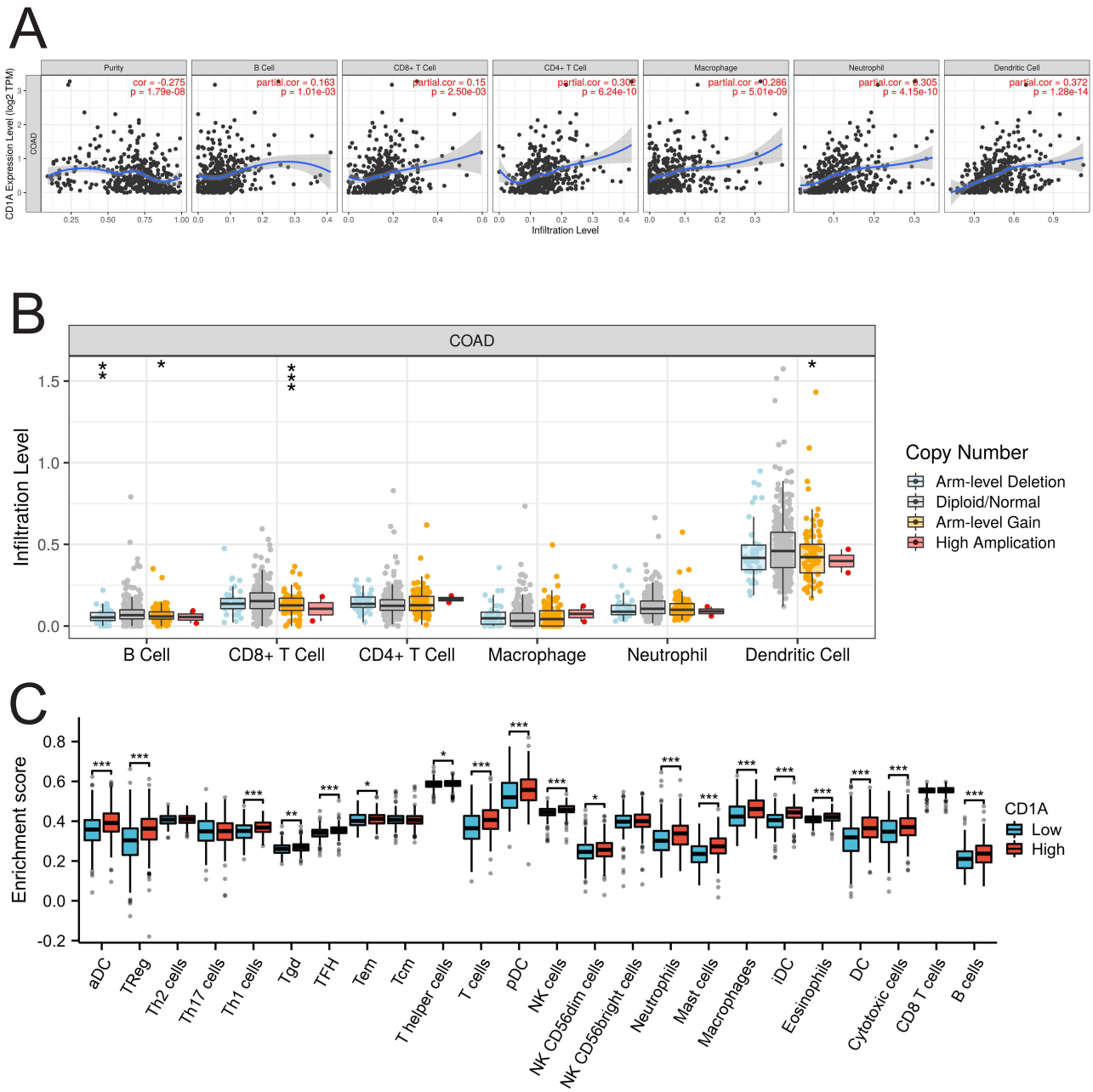

Figure 3 Correlation between CD1A Expression and Immune Cell Infiltration in COAD. (**A**) Significant correlation of CD1A expression with the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in COAD. (**B**) Impact of CD1A CNV on the infiltration levels of CD8+ T cells, B cells, and dendritic cells in COAD. (**C**) Comparative analysis of immune cell subtype proportions in groups with high versus low CD1A expression.
Notes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(CD163, CD206), Tumor-associated Macrophage (TAM) (CCL2, CD86), Monocyte (CD14, CD33), Natural killer cell (CD7), and Dendritic cell (CD1C, CD141) ($P < 0.05$). These findings indicate that CD1A may exert its function in COAD through mechanisms involving the infiltration of diverse immune cells.

We divided 478 colon adenocarcinoma tumor samples into two groups based on CD1A expression levels (high expression group and low expression group), with each group comprising 239 samples. The aim was to examine the differences in the expression of 24 types of immune cells between these groups, thereby elucidating the disparities in the tumor immune microenvironment related to CD1A expression levels in both high and low expression groups (Figure 3C). The analysis identified notable differences in the expression of various immune cell types, including aDC, Treg, Th1 cells, Tgd, TFH, Tem, T helper cells, T cells, pDC, NK cells, NK CD56dim, Neutrophils, Mast cells, Macrophages, iDC, Eosinophils, DC, Cytotoxic, and B cells

Table 2 Analysis of the Correlation Between CD1A and Immunological Markers of Immune Cells

Gene markers	Gene markers	Rho	P value
B cell	CD19	0.168	<0.001
	CD20	−0.048	0.685
	CD70	0.163	0.001
CD8+T Cell	CD8A	0.189	<0.001
	CD8B	0.133	0.008
	CD25	0.338	0.003
Tfh	CD183	0.226	0.055
	CD185	−0.025	0.835
	CD278	0.229	0.01
Th1	CD212	0.225	0.056
	CD191	0.189	0.109
	CD195	0.275	0.019
Th2	CD194	0.35	0.002
	CD198	0.343	0.003
	CD365	0.025	0.834
Th17	CD360	−0.187	0.112
	IL23R	0.139	0.005
	CD196	0.254	0.03
Treg	FOXP3	0.46	<0.001
	CD73	0.073	0.537
	CD127	−0.111	0.35
T cell exhaustion	PD-1	−0.117	0.325
	CTLA4	0.331	<0.001
	LAG3	0.157	0.002
Macrophage	CD68	0.228	<0.001
	CD11b	0.255	0.029
M1 Macrophage	NOS2	0.062	0.209
	IRF5	0.17	<0.001
M2 Macrophage	CD163	0.232	<0.001
	CD206	0.342	0.003
TAM	CCL2	0.307	<0.001
	CD86	0.329	<0.001
Monocyte	CD14	0.317	<0.001
	CD33	0.322	<0.001
Natural killer cell	CD57	−0.097	0.417
	KIR3DL1	−0.016	0.747
	CD7	0.209	<0.001
Neutrophil	CD16	0.106	0.37
	CD55	0.042	0.397
Dendritic cell	CD1C	0.698	<0.001
	CD141	0.291	0.013

between the high and low expression groups ($P < 0.05$), with a significant increase in the quantity of immune cells in the high expression group.

Correlation Analysis of CD1A Expression with m6A Modification Related Genes

In the investigation of COAD pathogenesis, there has been a growing interest in the role of N6-methyladenosine (m6A) modification. This study examined the relationship between CD1A expression and genes related to m6A modification. Through a comprehensive analysis of TCGA COAD data, the study revealed significant correlations between CD1A expression and 20 m6A modification-related genes. Specifically, notable associations were found with FTO ($R = 0.166$, $P < 0.001$),

HNRNPA2B1 ($R = 0.141$, $P = 0.002$), IGF2BP2 ($R = 0.133$, $P = 0.004$), METTL3 ($R = -0.126$, $P = 0.006$), RBM15 ($R = -0.171$, $P < 0.001$), and RBMX ($R = -0.111$, $P = 0.015$). Visual representations in scatter plots (Figure 4A-F) depict these correlations. Additionally, the study categorized 478 COAD tumor samples into high (239 samples) and low (239 samples) CD1A expression

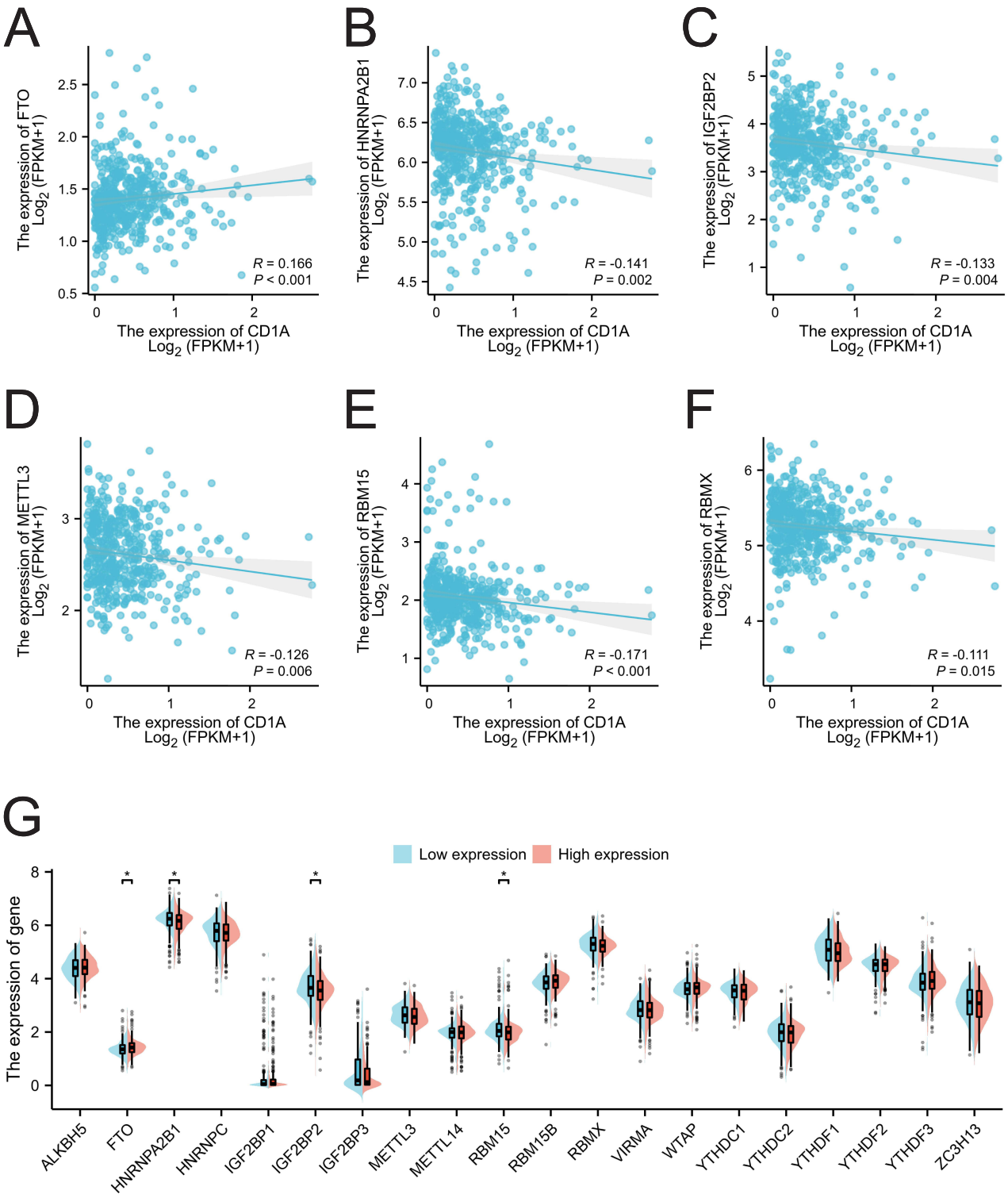

Figure 4 Correlation between CD1A expression and m6A-related genes in COAD. (**A-F**) Correlation analysis between CD1A expression and FTO, HNRNPA2B1, IGF2BP2, METTL3, RBM15 and RBMX. (**G**) Differences in the expression of m6A-related genes between CD1A high-expression and low-expression groups in COAD.
Note: *$P < 0.05$.
Abbreviations: m6A, N6-methyladenosine.

groups to analyze the expression variances of the 20 m6A-related genes (Figure 4G). Results showed significant differences in expression levels of HNRNPA2B1, IGF2BP2, RBM15, and FTO between the high and low expression groups ($P < 0.05$). These findings suggest a strong link between CD1A and m6A modification in COAD.

Construction of a CeRNA Network for CD1A

The growing body of research highlights the crucial role of ceRNA networks, which consist of lncRNA, miRNA, and mRNA, in various human cancers. Therefore, our aim was to analyze and construct the ceRNA network of CD1A to investigate its biological functions in COAD. Using the miRwalk database, we identified 12 potential target miRNAs that may interact with CD1A, such as hsa-miR-27b-5p, hsa-miR-516b-5p, hsa-miR-589-3p, hsa-miR-33b-5p, hsa-miR-548p, hsa-miR-3160-3p, hsa-miR-3185, hsa-miR-4733-3p, hsa-miR-2467-5p, hsa-miR-6511b-5p, hsa-miR-6801-5p, and hsa-miR-6811-5p. Subsequently, we performed a correlation analysis between the target miRNAs and CD1A expression levels to determine the most relevant miRNAs for the ceRNA network. Our analysis indicated that certain target miRNAs exhibited a significant negative correlation with CD1A expression, include hsa-miR-33b-5p ($R = -0.116$, $P = 0.015$), hsa-miR-516b-5p ($R = -0.263$, $P = 0.034$), and hsa-miR-589-3p ($R = -0.132$, $P = 0.006$) (Figure 5).

Utilizing miRNet and starBase databases, potential lncRNAs that could bind to hsa-miR-33b-5p, hsa-miR-516b-5p, and hsa-miR-589-3p were predicted. The results were illustrated using Venn diagrams (Figure 6A-B). Notably, hsa-miR-589-3p did not predict any specific lncRNAs. Following the ceRNA network theory, lncRNA expressions typically show an inverse relationship with miRNA expressions. To investigate this, TCGA COAD data was analyzed to assess the correlation between target lncRNAs and miRNAs. The analysis revealed significant negative correlations of miR-516b-5p with three lncRNAs (H19, LINC01106, and LINC01123) (Figure 6C-E). However, only EGOT demonstrated a negative correlation with hsa-miR-33b-5p (Figure 6F). Based on the ceRNA hypothesis, a ceRNA network involving CD1A was constructed, including EGOT-hsa-miR-33b-5p-CD1A, H19-miR-516b-5p-CD1A, LINC01106-miR-516b-5p-CD1A, and LINC01123-miR-516b-5p-CD1A (Figure 6G).

Discussion

The surface molecule encoded by the CD1A gene is crucial in the immune system, notably in the antigen presentation process to T cells.[12] Recent findings demonstrate that CD1A expression in various tumor types is intricately linked with disease progression and patient prognosis, particularly influencing the tumor microenvironment.[36,37] In pathological studies of human tumors, aberrant CD1A expression has been directly associated with tumor cell proliferation.[36] For example, in certain skin cancer types, reduced CD1A expression may facilitate tumor cell evasion from immune surveillance and further tumor advancement.[38] This phenomenon indicates CD1A's role in tumorigenesis and its potential as a novel target for tumor immunotherapy. Moreover, recent studies have highlighted that CD1A expression patterns

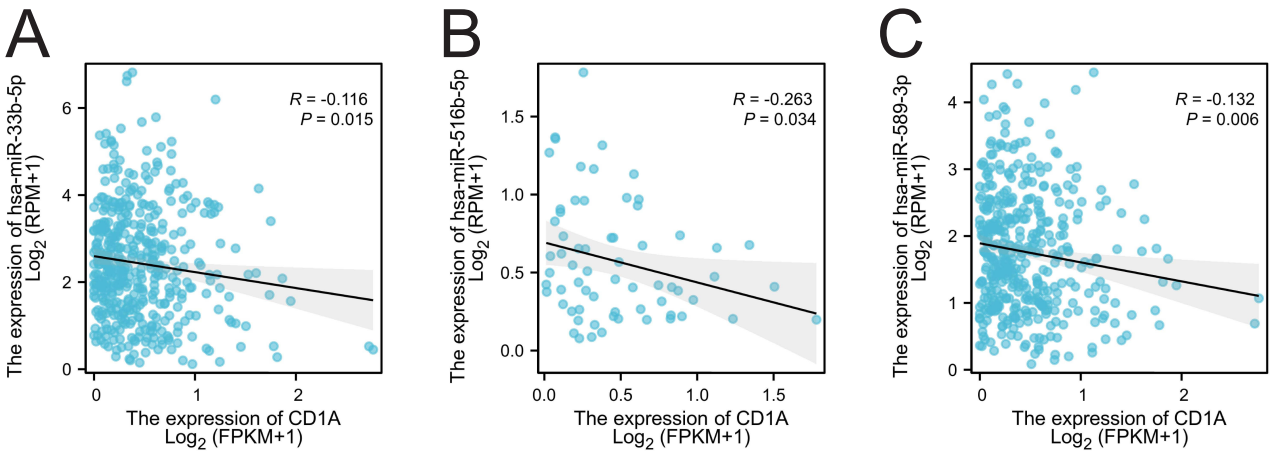

Figure 5 Correlation analysis of CD1A with target miRNAs. (**A**) Correlation analysis of CD1A expression with hsa-miR-33b-5p expression. (**B**) Correlation analysis of CD1A expression with that of hsa-miR-516b-5p. (**C**) Correlation analysis of CD1A expression with that of hsa-miR-589-3p.

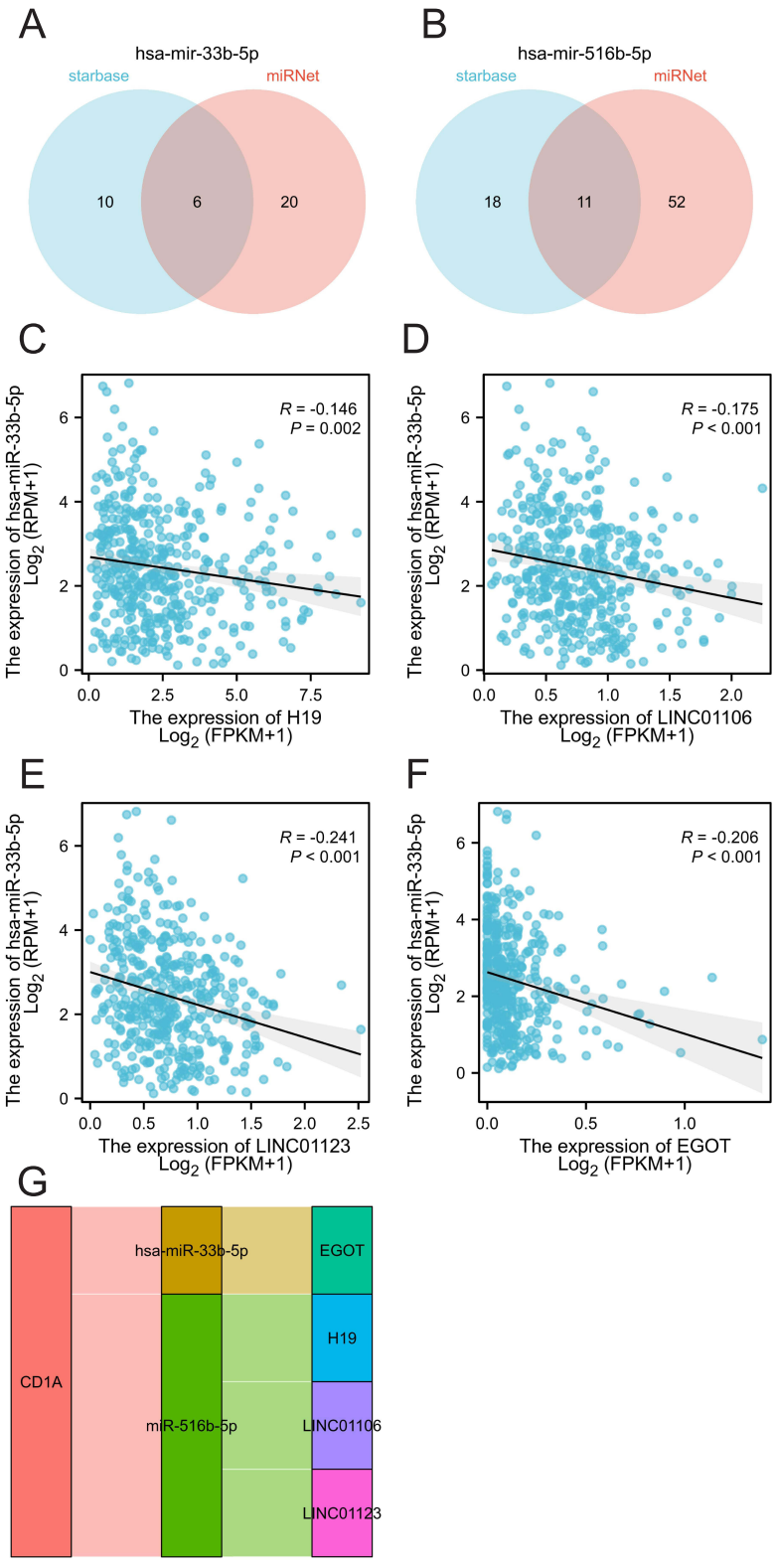

Figure 6 CeRNA network construction of CD1A in COAD. (**A**) Prediction of target lncRNA of has-miR-33b-5p using starbase and miRNet. (**B**) Prediction of target lncRNA of has-miR-33b-5p using starbase and miRNet. (**C-F**) Correlation analysis of miRNAs with targeted lncRNAs. (**G**) Sankey diagram showing the lncRNA-miRNA-mRNA regulatory network of CD1A.

differ across tumor types, potentially correlating with the tumor stage, grade, and patient prognosis.[11,38] However, the biological function of CD1A in COAD is unclear. Therefore, in this study, we comprehensively explored CD1A in COAD by experimental and bioinformatics methods.

We explored the expression pattern of CD1A in a variety of cancers through the analysis of multiple databases, including GENT2 and TCGA. Based on the analysis of the GENT2 database, CD1A was found to be highly expressed in 12 cancers. Similarly, data from the TCGA database showed that CD1A was highly expressed in 13 cancers. In particular, in COAD, we found a significant increase in CD1A expression in COAD samples compared to normal tissues ($P < 0.05$) by analyzing the GEO dataset and the TCGA COAD cohort. qRT-PCR experiments further verified that CD1A was significantly differentially expressed between COAD cells and human normal colorectal mucosal cells. These findings highlight the possible important role of CD1A in cancer pathogenesis, and CD1A has the potential to serve as a diagnostic or prognostic biomarker for COAD.

To elucidate the biological functions of CD1A, the 'clusterProfiler' package in R was employed for GO functional annotation and KEGG pathway enrichment analysis of its co-expressed genes. GO analysis showed that CD1A was involved in processes such as antigen processing and expression, leukocyte-mediated immunity and lymphocyte-mediated immunity, whereas KEGG analysis indicated CD1A was involved in signaling pathways such as the chemokine signaling pathway and cytokine-cytokine receptor interaction. Further, the PPI network analysis of CD1A revealed significant interactions with proteins such as CD207, CD1C, CD1E, FOXP3, and ITGB2, which are important in regulating the tumor immune response.[31–35] Thus, the results of these analyses not only confirm the central role of CD1A in the regulation of immune responses, but also highlight its potential as a potential target for cancer therapy.

To explore the relationship between CD1A and immune cell infiltration in COAD, we analyzed the correlation between CD1A expression and the level of infiltration of multiple immune cells using the TIMER database. The results of the analysis revealed that CD1A expression was significantly correlated with the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells, which underscores the potentially pivotal role of CD1A in regulating the tumor immune microenvironment. In particular, the correlation of CD1A with specific marker genes such as CD70, CD19, CD8A of these cells highlights its complexity and versatility in immune regulation. In addition, CNV analysis of CD1A further supported its effect on CD8+ T cells, B cells and dendritic cell infiltration, which may enhance anti-tumor immune responses by directly or indirectly affecting the function of these cells. The significant increase in CD1A high expression groups in a variety of immune cell types, such as regulatory T cells and Th1 cells, underscores the role of CD1A in the promotion of potential mechanisms in immune cell infiltration and activation. Overall, the expression level of CD1A was not only closely associated with immune cell infiltration in COAD, but also this association may act through the activation of different immunoregulatory pathways. Therefore, CD1A may serve as an important target for regulating tumor immune responses, and changes in its expression level may regulate the dynamic balance of the tumor immune microenvironment, thereby affecting the biological behavior and efficacy of cancers.

M6A is the most common form of modification on eukaryotic mRNAs and is known to play an important role in a variety of biological processes including tumor proliferation and migration.[39] Our analysis showed a significant correlation between the expression level of CD1A and m6A-related genes such as IGF2BP2, YTHDF2, HNRNPC, METTL3, VIRMA, FTO, and ALKBH5 in COAD. This finding implies that CD1A may regulate tumor cell behavior through a mediating mechanism that affects m6A modifications. In particular, m6A modifications regulate the expression of relevant genes by affecting mRNA stability and translation efficiency. Thus, the co-expression of CD1A with these m6A-modified genes may reveal a complex regulatory network, which may play a key role in the tumor biological properties of COAD. For example, increased expression of FTO and ALKBH5, which act as m6A demethylases, may alter the methylation status of mRNAs, thereby affecting tumor cell proliferation and migration. Notably, the generally elevated expression levels of these m6A-associated proteins in samples with elevated CD1A expression may indicate the presence of a specific tumor microenvironment in which m6A modifications promote or inhibit tumor progression by modulating intracellular signaling and gene expression patterns. In conclusion, our results emphasize the importance of in-depth studies of the interactions between CD1A and m6A modification-related genes and the potential regulatory role of these interactions in COAD development.

The CeRNA network regulates mRNA expression through lncRNAs that act as miRNA sponges, a mechanism that reduces the abundance of miRNAs and thus their repressive effects on downstream target genes.[40] In COAD, studies of the ceRNA regulatory network have revealed its complexity and importance. For example, Du et al found that lncRNA ELFN1-AS1 enhanced colon adenocarcinoma cell growth and migration through the miR-191-5p/SATB1 axis,[41] while Wu et al observed that lncRNA MALAT1 induced colon adenocarcinoma by regulating the miR-129-5p/HMGB1 axis.[42] Our study further explored this network, especially around the expression of CD1A. Utilizing the miRwalk database, we identified 12 potential upstream miRNAs and found that hsa-miR-33b-5p, hsa-miR-516b-5p, and hsa-miR-589-3p were significantly negatively correlated with CD1A expression ($P < 0.05$). This finding emphasizes the potential role of CD1A as a miRNA target gene in COAD and supports the ceRNA theory that lncRNAs act as sponges for miRNAs reducing their activity and decreasing the inhibition of target genes such as CD1A. In addition, by predicting the upstream lncRNAs of these miRNAs, we identified four key lncRNAs: H19, LINC01106, LINC01123, and EGOT, which play roles in several key biological processes in COAD. h19 enhances the migration and invasion of tumor cells through activation of the MAPK signaling pathway,[43] while LINC01106 promotes tumor growth and stem cell properties through a feedback mechanism affecting Gli family factors.[44] LINC01123 has been found to enhance proliferation, invasion, and chemotherapy tolerance of COAD cells,[45] while high expression of EGOT is associated with increased proliferation and metastasis of COAD.[46] Based on the ceRNA theory, we constructed a CD1A registry including EGOT-hsa-miR-33b-5p-CD1A, H19-hsa-miR-516b-5p-CD1A, LINC01106-hsa-miR-516b-5p-CD1A and LINC01123-hsa-miR-516b-5p-CD1A the CD1A ceRNA regulatory networks. These networks not only reveal the possible regulatory mechanisms of CD1A in COAD, but also highlight the need to further validate the role of these networks in actual biological functions.

The innovative aspect of our study is the first systematic exploration of the link between CD1A and COAD. However, we must acknowledge that there are some limitations to our work. First, our study relied heavily on bioinformatics analyses, and although this provided us with a great deal of valuable information, we only performed a simple one-cell validation experiment. Future studies should incorporate more in vivo and in vitro experiments to validate our findings, and these experiments will help to further consolidate our findings and gain a deeper understanding of the role of CD1A in COAD. Second, the number of databases used in this study is relatively limited, and although our preliminary results are convincing, cross-validation in more diverse datasets is essential. In-depth analyses of multiple datasets can improve the reliability of our results and provide a more solid foundation for further research. In conclusion, despite the limitations of our study, it is only the first step in our understanding of the role of CD1A in COAD. We look forward to more in-depth studies in the future to overcome these limitations and reveal more mechanistic details.

Conclusion

In summary, this study underscores the pivotal role of CD1A in COAD, as evidenced by its significant associations with tumor immune infiltration, m6A modification, and regulatory involvement within the ceRNA network. Our comprehensive analyses demonstrate that CD1A not only varies in expression across different tumors but is also intimately linked with clinical-pathological features specific to COAD. The established correlations between CD1A expression and various immune cells, along with its interaction with m6A modification genes, enhance our understanding of its potential mechanisms in tumor immunity. Importantly, the construction of a CD1A-centric ceRNA network provides insights into the molecular regulatory landscape in which CD1A operates, highlighting its potential as a valuable biomarker for diagnosing and treating COAD.

Data Sharing Statement

The datasets generated and analyzed during the current study are available in the TCGA repository (https://portal.gdc.cancer.gov/) and The Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The datasets used and analyzed in this study are available upon request from corresponding author Chunxiang Zhou.

Ethics Approval and Consent to Participate

The research was approved by the Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine (2019LWKZ009). All the procedures were followed in accordance with the Declaration of Helsinki under the Ethics approval and consent to participate heading.

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Author Contributions

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

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Disclosure

All authors declare no competing interests in this study.

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