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Complement Components C3 and C7 as Potential Therapeutic and Prognostic Targets for Adrenocortical Carcinoma

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ABSTRACT

INTRODUCTION

In cancer biology, the complement system has received increasing attention over the last decade. Complement 3 (C3) and complement 7 (C7) play important and complex roles in cancer; however, whether they act as a friend or foe remains debated. The roles of C3 and C7 in adrenocortical carcinoma (ACC) are unclear. In this study, we investigated the relevance of C3 and C7 in ACC using in silico analyses.

METHODS

Bioinformatic analyses were performed using STRING, GeneMANIA, UALCAN, BEST, cBioPortal, Metascape, TRRUST, GEPIA, LinkedOmics, and GDSC database.

RESULTS

The expression of C3 and C7 was strongly downregulated in patients with ACC. Furthermore, their expression levels affected the prognosis of patients with ACC. C3, C7, and their altered neighboring genes (ANGs) were predicted to mainly regulate complement activation, acute inflammatory reactions, hormone secretion, angiogenesis, purine nucleotide metabolism, and translation. SIRT1, STAT3, miR-151, miR-379, ATM, and CDK2 were identified as key regulatory targets of C3, C7, and their ANGs. Furthermore, anti-PD1/CTLA-4 immunotherapy was predicted to strongly upregulate the expression of C3 and C7, thereby improving the prognosis of patients with ACC. Pilaralisib and amuvatinib were predicted to strongly inhibit the growth of SW13 cells.

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CONCLUSION

This preliminarily study indicates that C3 and C7 are potential therapeutic and prognostic targets for ACC, which should provide a novel strategy for the treatment of ACC.

KEYWORDS

Adrenocortical carcinoma; Bioinformatics; Complement system; Prognostic; Therapeutic

INTRODUCTION

Adrenocortical carcinoma (ACC) is a rare but highly malignant tumor, with high mortality [1,2]. With no obvious early symptoms, most patients with ACC present with local invasion of the cancer at the time of their first visit to a doctor. As the incidence of ACC is low, being 0.77-2.0/100,000, [3] few patients with ACC are available for study. The prognosis for ACC is poor, with a 5-years survival rate of 16%-40% [4]. Currently, surgery is the only option to radically cure ACC, but is associated with a high risk (50%-85%) of recurrence [5]. In addition, effective therapeutic and prognostic biomarkers for ACC are lacking.

The complement system is an important component of innate immunity and plays an important role in the occurrence and development of malignant tumors. In addition to exerting antitumor effects, such as complement-dependent cytotoxicity and phagocytosis induced by therapeutic monoclonal antibodies, the complement system may promote immunosuppression and tumor growth and invasiveness, in particular, through anaphylatoxins that target both leukocytes and cancer cells [6]. Increasing evidence indicates that complement activation in the tumor microenvironment may play a role in tumor promotion by inducing local T-cell immunosuppression and chronic inflammation, thereby, promoting immune escape, growth, and distant metastasis of tumor [7,8]. However, the role of complement in cancer remains inconclusive. Complement 3 (C3) is an important transition point for the activation of these three pathways. It plays a key role in the activation of the complement system. C3 is an inflammatory factor that releases a variety of vasoactive substances upon activation, thereby, causing inflammation [9]. Tumor-associated inflammation is a feature of all cancers that can confer both tumor-promoting and suppressive functions [10]. Complement 7 (C7), the final product of the complement cascade [11] is one of the main rate-limiting factors in the formation of membrane attack complex [12]. C7 is also considered a potential tumor suppressor and may be used as a prognostic biomarker in some cancers [13,14]. Thus far, the roles of C3 and C7 in ACC remain unknown and were investigated in this study. We have identified C3 and C7 as important therapeutic and prognostic targets for ACC.

MATERIALS AND METHODS

GEPIA

Gene expression profiling interactive analysis (GEPIA; http://gepia.cancer-pku.cn/index.html) is an intuitive network application tool for assessing biological relationships between gene expression and prognostic information in patients with cancer. We used GEPIA to analyze the relationship between gene expression, pathological stage of tumor, and prognosis. The screening criteria were: (1) Genes: C3 and C7; (2) dataset: ACC; and (3) number of patients; 77; the threshold setting was a P-value cutoff ≤0.01. The Student's t-test was used to analyze the differences in the expression of C3 and C7 in ACC. Kaplan-Meier curves were used to analyze the prognosis of patients with ACC [15].

BEST

BEST (https://rookieutopia.com/app_direct/BEST/) provides a curated database and innovative analytical pipelines to explore cancer biomarkers at a high resolution. Protein expression, survival value, immune cell infiltration, candidate agents, and immunotherapy targeting C3 and C7 in ACC were analyzed using BEST. The "Cell infiltration," "Survival analysis," "Immunotherapy," and "Candidate agents" modules of the BEST database were used to analyze the gene expression omnibus (GEO) and the cancer genome atlas (TCGA) gene expression data, The screening criteria were set as: (1) Genes: C3 and C7; (2) dataset: ACC (10 dataset and 508 patients) [15].

UALCAN

The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN; http://ualcan.path.uab.edu/analysis.html) facilitates gene expression and survival analyses of tumor subgroups. We used UALCAN to analyze C3 and C7 expression in the ACC. The "Expression Analysis" module of the UALCAN database was used to analyze TCGA gene expression data. The screening criteria were set as: (1) Genes: C3 and C7; (2) dataset: ACC; (3) number of patients with ACC: 77 (9 in stage 1, 37 in stage 2, 16 in stage 3, and 15 in stage 4); the threshold setting was a P-value cutoff = 0.05. The Student's t-test was used for comparative analysis [15].

cBioPortal

cBioPortal (http://cbioportal.org) is an online database used for mutation analysis of tumor genes. We used cBioPortal to analyze gene alterations in C3, C7, and the top 50 altered neighboring genes (ANGs). A total of 76 ACC samples were analyzed, and mRNA expression z-scores, relative to all samples (log RNA Seq V2 RSEM), were obtained using a z-score threshold of ±2.0 [15].

STRING and GeneMANIA

STRING (https://string-db.org/cgi/input.pl) and GeneMANIA (http://www.genemania.org) are online databases used for analyzing gene-protein and protein-protein interactions. We used STRING to build a low-confidence level (0.150) PPI network and the screening criteria for species defined as humans. GeneMANIA was used to explore the functions of C3, C7, and the top 50 ANGs [15].

Metascape

Metascape (https://metascape.org) is an online database used to analyze the functions and signaling pathways of genes and proteins. We used it to analyze the function and signaling pathways of C3, C7, and the top 50 ANGs [15].

TRRUST

Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST; https://www.grnpedia.org/trrust/) is an online database used to analyze the regulatory targets of gene transcription. We used TRRUST to identify the key transcriptional regulators of C3, C7 and the top 50 ANGs [15].

LinkedOmics

LinkedOmics (http://www.linkedomics.org/) is a public online platform for analyzing correlations between differentially expressed genes related to tumor target genes and for predicting miRNA and kinase targets. LinkedOmics was used to identify the kinase targets, miRNA targets, and differentially expressed genes related to C3 and C7 [15].

GDSC Analysis

The GDSC (http://www.cancerRxgene.org) is a specialized public database for obtaining information on potential anticancer drugs [15]. We used this database to identify the targeted drugs for C3 and C7 and to predict their anti-ACC activity.

RESULTS

C3 and C7 Expression, Prognosis, and Genetic Alterations in ACC

C3 and C7 were found to be significantly downregulated in patients with ACC (P <0.05; Figure 1A - Figure 1H). Moreover, the expression of C3 and C7 was significantly downregulated in patients with stage 4 ACC compared with that in patients with stage 2 ACC (P <0.05). The survival time was longer in patients with ACC who expressed high levels of C3 and C7 (P <0.05) (Figure 1I - Figure 1R). C3 and C7 expression levels were altered by 1.3% and 9%, respectively, in patients with ACC (Figure 1S and Figure 1T).

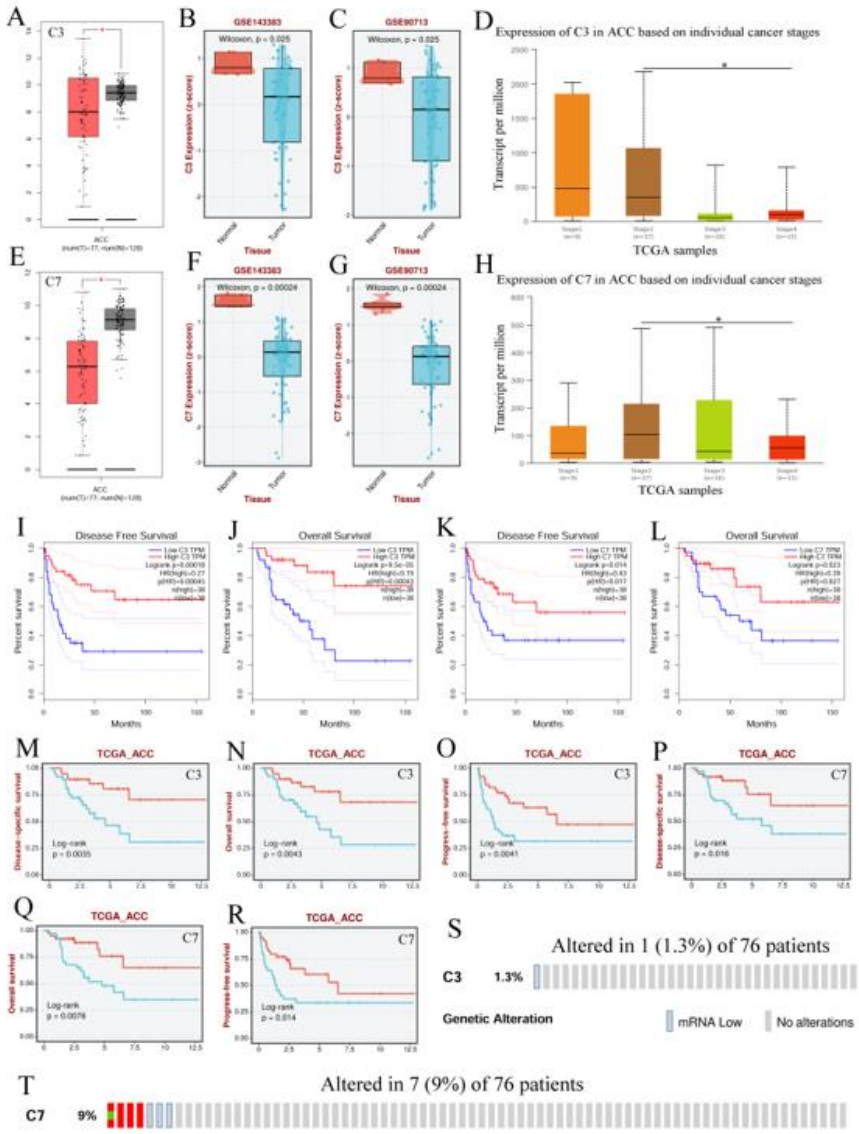

Figure 1: The transcription levels, prognostic value, and genetic alteration of C3 and C7 in ACC. **(A-D)** Boxplot showing transcription level of C3 in patients with ACC (GEPIA, BEST, and UALCAN); **(E and H)** Boxplot showing transcription level of C7 in patients with ACC (GEPIA, BEST, and UALCAN); (I) The disease-free survival curve of C3 in patients with ACC (GEPIA); **(J and N)** The overall survival curve of C3 in patients with ACC (GEPIA and BEST); **(K)** The disease-free survival curve of C7 in patients with ACC (GEPIA); **(L and Q)** The overall survival curve of C7 in patients with ACC (GEPIA); **(M)** The disease-specific survival curve of C3 in patients with ACC (BEST); **(O)** The progress-free survival curve of C3 in patients with ACC (BEST); **(P)** The disease-specific survival curve of C7 in patients with ACC (BEST); **(R)** The progress-free survival curve of C7 in patients with ACC (BEST); **(S)** Genetic alteration of C3 in patients with ACC (cBioPortal); **(T)** Genetic alteration of C7 in patients with ACC (cBioPortal); *P <0.05.

Interaction Network of C3, C7, and their ANGs in ACC

The alteration frequencies for C3 and C7 ANGs were ≥100.00% and ≥42.86%, respectively, in the 50 most frequently altered neighboring genes (ANGs) in patients with ACC (Table 1 and Table 2). ACTG1P25 (100.00%), ADIPOR1 (100.00%), and ADORA1 (100.00%) were the most frequent ANGs of C3 in patients with ACC (Table 1). The most frequent ANGs of C7 in patients with ACC were MROH2B (71.43%), C6 (57.14%), and CARD6 (57.14%) (Table 2). Thirty-eight nodes and 132 edges were found in the PPI networks (Figure 2A). A complex interaction network was discovered between C3 and its ANGs based on physical interactions, predicted co-expression, shared protein domains, colocalization, and genetic interactions (Figure 2B). Moreover, we obtained 37 nodes and 118 edges in the PPI networks (Figure 2C), and an intricate network of interactions existed between C7 and its ANGs, including co-expression, pathway, colocalization, physical interactions, and shared protein domains (Figure 2D).

Gene	Altered Group	Unaltered Group	p-Value
ACTG1P25	1 (100.00%)	0 (0.00%)	0.0132
ADIPOR1	1 (100.00%)	0 (0.00%)	0.0132
ADORA1	1 (100.00%)	0 (0.00%)	0.0132
AHI1-DT	1 (100.00%)	0 (0.00%)	0.0132
AKIRIN2	1 (100.00%)	0 (0.00%)	0.0132
ANKRD6	1 (100.00%)	0 (0.00%)	0.0132
ARL8A	1 (100.00%)	0 (0.00%)	0.0132
ASCL5	1 (100.00%)	0 (0.00%)	0.0132
ATP2B4	1 (100.00%)	0 (0.00%)	0.0132
ATP6V1G3	1 (100.00%)	0 (0.00%)	0.0132
ATP8A2	1 (100.00%)	0 (0.00%)	0.0132
BACH2	1 (100.00%)	0 (0.00%)	0.0132
BCKDHB	1 (100.00%)	0 (0.00%)	0.0132
BRINP3	1 (100.00%)	0 (0.00%)	0.0132
BRINP3-DT	1 (100.00%)	0 (0.00%)	0.0132
BTG2	1 (100.00%)	0 (0.00%)	0.0132
BTG2-DT	1 (100.00%)	0 (0.00%)	0.0132
C1ORF53	1 (100.00%)	0 (0.00%)	0.0132
C6ORF163	1 (100.00%)	0 (0.00%)	0.0132
CACNA1S	1 (100.00%)	0 (0.00%)	0.0132
CALHM6	1 (100.00%)	0 (0.00%)	0.0132
CASC6	1 (100.00%)	0 (0.00%)	0.0132
CASP8AP2	1 (100.00%)	0 (0.00%)	0.0132
CCDC190	1 (100.00%)	0 (0.00%)	0.0132
CCN2	1 (100.00%)	0 (0.00%)	0.0132
CCNC	1 (100.00%)	0 (0.00%)	0.0132
CFH	1 (100.00%)	0 (0.00%)	0.0132
CFHR1	1 (100.00%)	0 (0.00%)	0.0132
CFHR2	1 (100.00%)	0 (0.00%)	0.0132
CFHR3	1 (100.00%)	0 (0.00%)	0.0132
CFHR4	1 (100.00%)	0 (0.00%)	0.0132
CFHR5	1 (100.00%)	0 (0.00%)	0.0132
CGA	1 (100.00%)	0 (0.00%)	0.0132
CHI3L1	1 (100.00%)	0 (0.00%)	0.0132
CHIT1	1 (100.00%)	0 (0.00%)	0.0132
CLCN1	1 (100.00%)	0 (0.00%)	0.0132
CLVS2	1 (100.00%)	0 (0.00%)	0.0132
CNR1	1 (100.00%)	0 (0.00%)	0.0132
COL10A1	1 (100.00%)	0 (0.00%)	0.0132
COQ3	1 (100.00%)	0 (0.00%)	0.0132
CRB1	1 (100.00%)	0 (0.00%)	0.0132
CREB3L1	1 (100.00%)	0 (0.00%)	0.0132

CSRP1	1 (100.00%)	0 (0.00%)	0.0132
CTAGE9	1 (100.00%)	0 (0.00%)	0.0132
CYB5R1	1 (100.00%)	0 (0.00%)	0.0132
CYB5R4	1 (100.00%)	0 (0.00%)	0.0132
CYCSP52	1 (100.00%)	0 (0.00%)	0.0132
DCBLD1	1 (100.00%)	0 (0.00%)	0.0132
DDX59	1 (100.00%)	0 (0.00%)	0.0132
DDX59-AS1	1 (100.00%)	0 (0.00%)	0.0132

Table 1: The top 50 ANG of C3 in ACC (cBioPortal).

Gene	Altered Group	Unaltered Group	p-Value
MROH2B	5 (71.43%)	1 (1.45%)	6.66E-06
C6	4 (57.14%)	0 (0.00%)	2.73E-05
CARD6	4 (57.14%)	0 (0.00%)	2.73E-05
MRPS30	4 (57.14%)	0 (0.00%)	2.73E-05
MRPS30-DT	4 (57.14%)	0 (0.00%)	2.73E-05
NIPBL	4 (57.14%)	0 (0.00%)	2.73E-05
NNT	4 (57.14%)	0 (0.00%)	2.73E-05
OXCT1	4 (57.14%)	0 (0.00%)	2.73E-05
PAIP1	4 (57.14%)	0 (0.00%)	2.73E-05
PLCXD3	4 (57.14%)	0 (0.00%)	2.73E-05
RPL37	4 (57.14%)	0 (0.00%)	2.73E-05
SNORD72	4 (57.14%)	0 (0.00%)	2.73E-05
APOB	4 (57.14%)	1 (1.45%)	1.32E-04
EMB	4 (57.14%)	1 (1.45%)	1.32E-04
FRAS1	4 (57.14%)	1 (1.45%)	1.32E-04
FYB1	4 (57.14%)	1 (1.45%)	1.32E-04
HCN1	4 (57.14%)	1 (1.45%)	1.32E-04
PRKAA1	4 (57.14%)	1 (1.45%)	1.32E-04
PTGER4	4 (57.14%)	1 (1.45%)	1.32E-04
SLC1A3	4 (57.14%)	1 (1.45%)	1.32E-04
TTC33	4 (57.14%)	1 (1.45%)	1.32E-04
OR9K2	4 (57.14%)	2 (2.90%)	3.82E-04
ANXA2R-AS1	3 (42.86%)	0 (0.00%)	4.98E-04
ANXA2R-OT1	3 (42.86%)	0 (0.00%)	4.98E-04
C5ORF34	3 (42.86%)	0 (0.00%)	4.98E-04
CCDC152	3 (42.86%)	0 (0.00%)	4.98E-04
CCL28	3 (42.86%)	0 (0.00%)	4.98E-04
ERCC6	3 (42.86%)	0 (0.00%)	4.98E-04
FBXO4	3 (42.86%)	0 (0.00%)	4.98E-04
FER1L6	3 (42.86%)	0 (0.00%)	4.98E-04
GHR	3 (42.86%)	0 (0.00%)	4.98E-04
HMGCS1	3 (42.86%)	0 (0.00%)	4.98E-04
HSD17B6	3 (42.86%)	0 (0.00%)	4.98E-04
IL7R	3 (42.86%)	0 (0.00%)	4.98E-04
LINC02996	3 (42.86%)	0 (0.00%)	4.98E-04
LMBRD2	3 (42.86%)	0 (0.00%)	4.98E-04
MEGF8	3 (42.86%)	0 (0.00%)	4.98E-04
MEIS2	3 (42.86%)	0 (0.00%)	4.98E-04
MGA	3 (42.86%)	0 (0.00%)	4.98E-04
MSH2	3 (42.86%)	0 (0.00%)	4.98E-04
NADK2	3 (42.86%)	0 (0.00%)	4.98E-04
NIM1K	3 (42.86%)	0 (0.00%)	4.98E-04
NIPBL-DT	3 (42.86%)	0 (0.00%)	4.98E-04
NNT-AS1	3 (42.86%)	0 (0.00%)	4.98E-04
OXCT1-AS1	3 (42.86%)	0 (0.00%)	4.98E-04
PPM1F	3 (42.86%)	0 (0.00%)	4.98E-04
PRAMEF18	3 (42.86%)	0 (0.00%)	4.98E-04
PSG6	3 (42.86%)	0 (0.00%)	4.98E-04
RANBP3L	3 (42.86%)	0 (0.00%)	4.98E-04
RIMOC1	3 (42.86%)	0 (0.00%)	4.98E-04

Table 2: The top 50 ANG of C7 in ACC (cBioPortal).

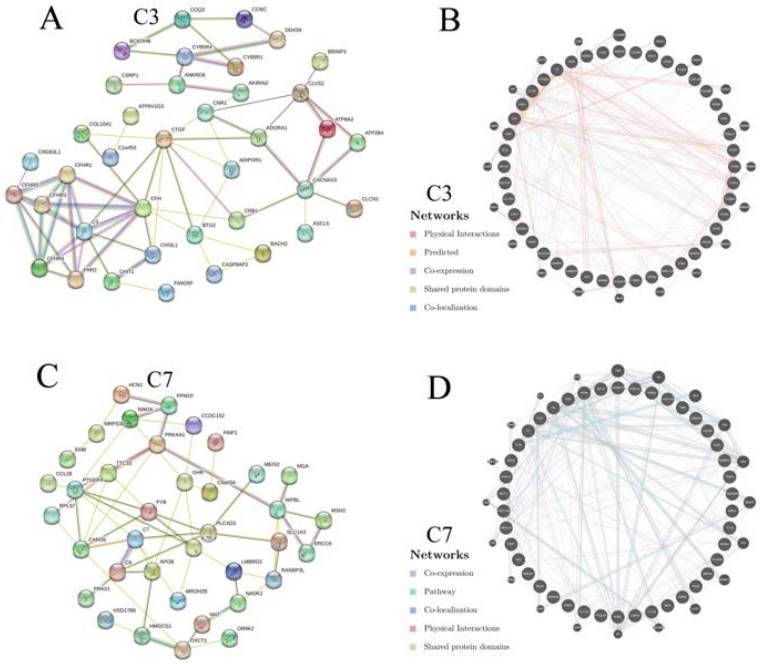

Figure 2: Interaction analyses of C3, C7, and their ANG in ACC. **(A)** PPI network of C3 and its ANG in patients with ACC (STRING); **(B)** Network analyses of C3 and its ANG in patients with ACC (GeneMANIA); **(C)** PPI network of C7 and its ANG in patients with ACC (STRING); **(D)** Network analyses of C7 and its ANG in patients with ACC (GeneMANIA).

Gene Ontology (GO) Function and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis of C3, C7, and their ANGs in ACC

As shown in Figure 3A, the molecular functions related to C3 and its ANGs were mainly associated with complement component C3b binding, monoatomic ion transmembrane transporter activity, ATP-dependent activity, and transcription coregulatory activity. Moreover, complement activation, regulation of acute inflammatory response, secretion, regulation of angiogenesis, and response to hormone were the main biological processes for C3 and its ANGs (Figure 3B). The cellular components of C3 and its ANGs included blood micro particle, transporter complex, and secretory granule lumen (Figure 3C). Complement and coagulation cascades, cAMP signaling pathway, and amino sugar and nucleotide sugar metabolism were the main KEGG pathways for C3 and its ANGs (Figure 3D). The molecular functions of C7 and its ANGs were protein C-terminus binding and protein homodimerization (Figure 3E). Furthermore, the main biological processes for C7 and its ANGs were purine nucleotide metabolism, translation, lymphocyte differentiation, and lymphocyte-mediated immunity (Figure 3F). The cellular components of C7 and its ANGs were neuronal cell body and mitochondrial matrix (Figure 3G). The KEGG pathways for C7 and its ANGs were coronavirus disease (COVID-19) and cytokine-cytokine receptor interaction (Figure 3H).

Transcription Factor, miRNA, and Kinase Targets of C3 and C7 in Patients with ACC

As shown in Table 3, SIRT1 and STAT3 were the key transcription factors involved in the network of C3 and its ANGs ($P < 0.05$). SIRT1 regulates the functions of CFH and COL10A1. CHI3L1 and CSRP1 were the main regulatory genes for STAT3. The miRNA targets of C3 and C7 were identified using LinkedOmics (Table 4). MiR-151, miR-492, miR-29A, miR-29B, and miR-29C were the miRNA targets of C3 in ACC ($P < 0.001$) (Table 4). However, the miRNA targets of C7 in ACC were miR-151, miR-379, miR-517A, and miR-517C ($P < 0.05$) (Table 4). ATM, AURKB, and AURKA were found to be the kinase targets of C3 in patients with ACC ($P < 0.001$) (Table 5).

Moreover, the kinase targets of C7 were CDK2, CHEK1, and CDK1 in patients with ACC (P <0.001) (Table 5).

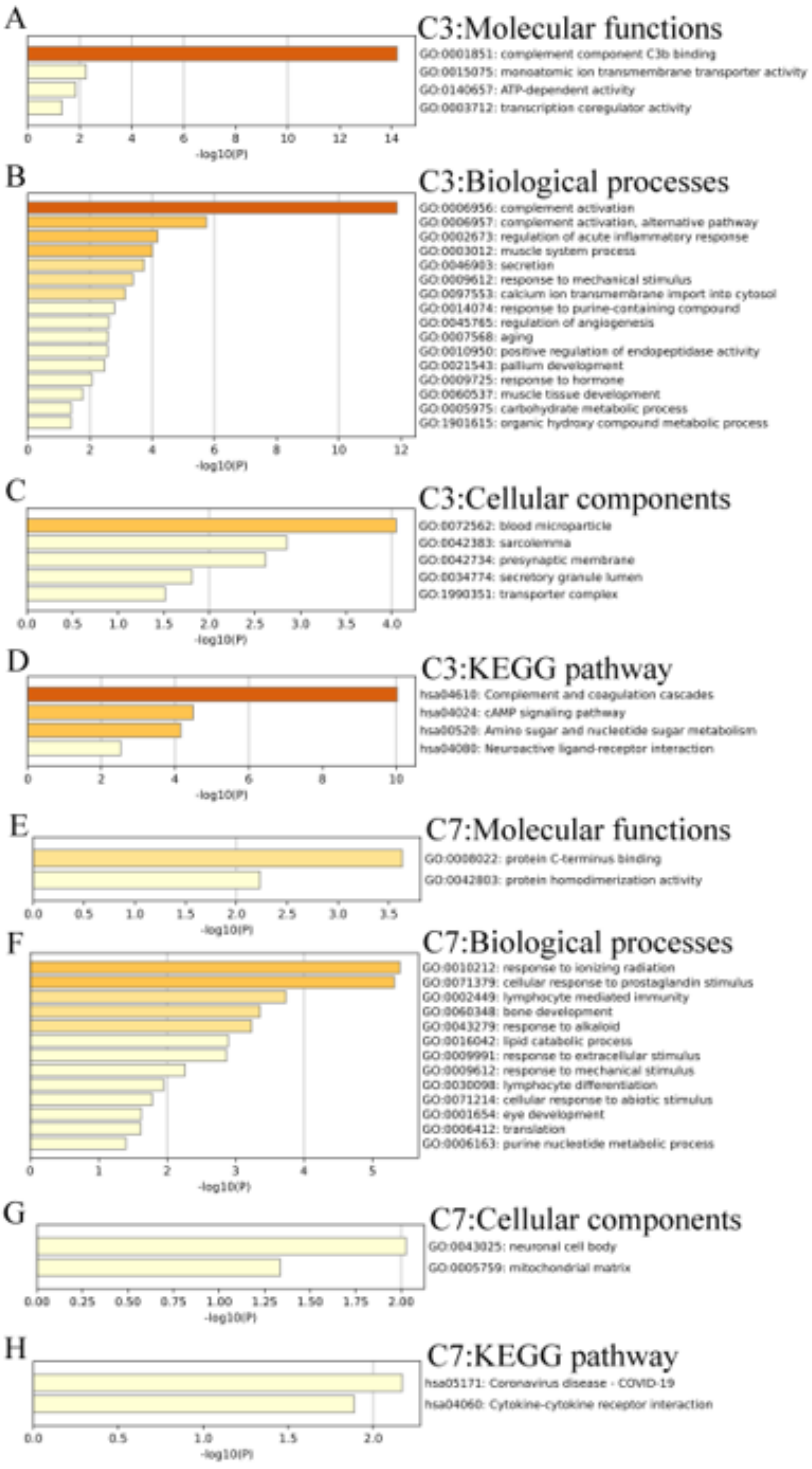

Figure 3: GO function and KEGG pathways enrichment analyses of C3, C7, and their ANG in patients with ACC (Metascape). **(A)** Molecular functions of C3 and its ANG in patients with ACC; **(B)** Biological processes of C3 and its ANG in patients with ACC; **(C)** Cellular components of C3 and its ANG in patients with ACC; **(D)** KEGG pathway analysis of C3 and its ANG in patients with ACC; **(E)** Molecular functions of C7 and its ANG in patients with ACC; **(F)** Biological processes of C7 and its ANG in patients with ACC; **(G)** Cellular components of C7 and its ANG in patients with ACC; **(H)** KEGG pathway analysis of C7 and its ANG in patients with ACC.

Key TF	Description	Regulated gene	Mode of regulation	P-value
SIRT1	Sirtuin 1	CFH, COL10A1	Repression	0.00442
STAT3	Signal transducer and activator of Transcription 3	CHI3L1, CSRP1	Activation	0.0348

Table 3: Key regulated factor of C3 and its top 50 ANG in ACC (TRRUST).

Gene	Gene set	Leading Edge Number	P-value
C3	AGTCTAG,miR-151	11	<2.2e-16
	CAGGTCC,miR-492	25	<2.2e-16
	TGGTGCT,miR-29A,miR-29B,miR-29C	130	<2.2e-16
C7	AGTCTAG,miR-151	6	0.018692
	GTCTACC,miR-379	8	0.012552
	TGCACGA,miR-517A,miR-517C	6	0.039823

Table 4: The top three miRNA target of C3 and C7 in ACC (LinkedOmics).

Gene	Gene Set	Leading Edge Number	P-value
C3	AGTCTAG,miR-151	11	<2.2e-16
	CAGGTCC,miR-492	25	<2.2e-16
	TGGTGCT,miR-29A,miR-29B,miR-29C	130	<2.2e-16
C7	AGTCTAG,miR-151	6	0.018692
	GTCTACC,miR-379	8	0.012552
	TGCACGA,miR-517A,miR-517C	6	0.039823

Table 5: The top three miRNA target of C3 and C7 in ACC (LinkedOmics).

Gene	Kinase target	Description	Leading edge number	P-value
C3	Kinase_ATM	ATM serine/threonine kinase	54	<2.2e-16
	Kinase_AURKB	Aurora kinase B	37	<2.2e-16
	Kinase_AURKA	Aurora kinase A	16	<2.2e-16
C7	Kinase_CDK2	Cyclin dependent kinase 2	104	<2.2e-16
	Kinase_CHEK1	Checkpoint kinase 1	54	<2.2e-16
	Kinase_CDK1	Cyclin dependent kinase 1	102	<2.2e-16

Table 6: The top three kinase target of C3 and C7 in ACC (LinkedOmics).

Correlation of Differentially Expressed Genes and C3 and C7 Expression in Patients with ACC

As shown in Figure 4A and Figure 4D, 6,411 and 5,818 genes were closely related to C3 and C7, respectively, in patients with ACC. These genes showed positive (3,517 and 2,943) or negative (2,894 and 2,875) correlations with C3 and C7 expression (Figure 4A and Figure 4D). In addition, 50 important genes were positively and negatively correlated with C3 and C7 expression in patients with ACC (Figure 4B and Figure 4F). Among them, the expression C3 was strongly positively associated with NUPR1 (Pearson correlation coefficient (PCC) = 0.6832, P = 4.027e-12) (Figure 4G), CST3 (PCC = 0.6818, P = 4.611e-12) (Figure 4H), and RERG (PCC = 0.6716, P = 1.247e-11) (Figure 4I).

The expression C7 was positively associated with PLCXD3 (PCC = 0.7446, P = 3.676e-15) (Figure 4J), PIK3R1 (PCC = 0.629, P = 5.358e-10) (Figure 4K), and RERG (PCC = 0.6246, P = 7.676e-10) (Figure 4L).

Correlation of Immune Cell Infiltration with C3 and C7 Expression and Anti-PD1/CTLA-4 Immunotherapy in ACC

The expression of C3 and C7 in patients with ACC was positively associated with immune cell infiltration (P<0.05; Figure 5A - Figure 5G). In addition, C3 and C7 were significantly upregulated in patients treated with anti-PD1/CTLA-4 antibodies (P <0.05; Figure 5H - Figure 5J). However, the expression of C7 in ACC patients treated with the anti-PD-L1 antibody was significantly downregulated (P <0.05; Figure 5K).

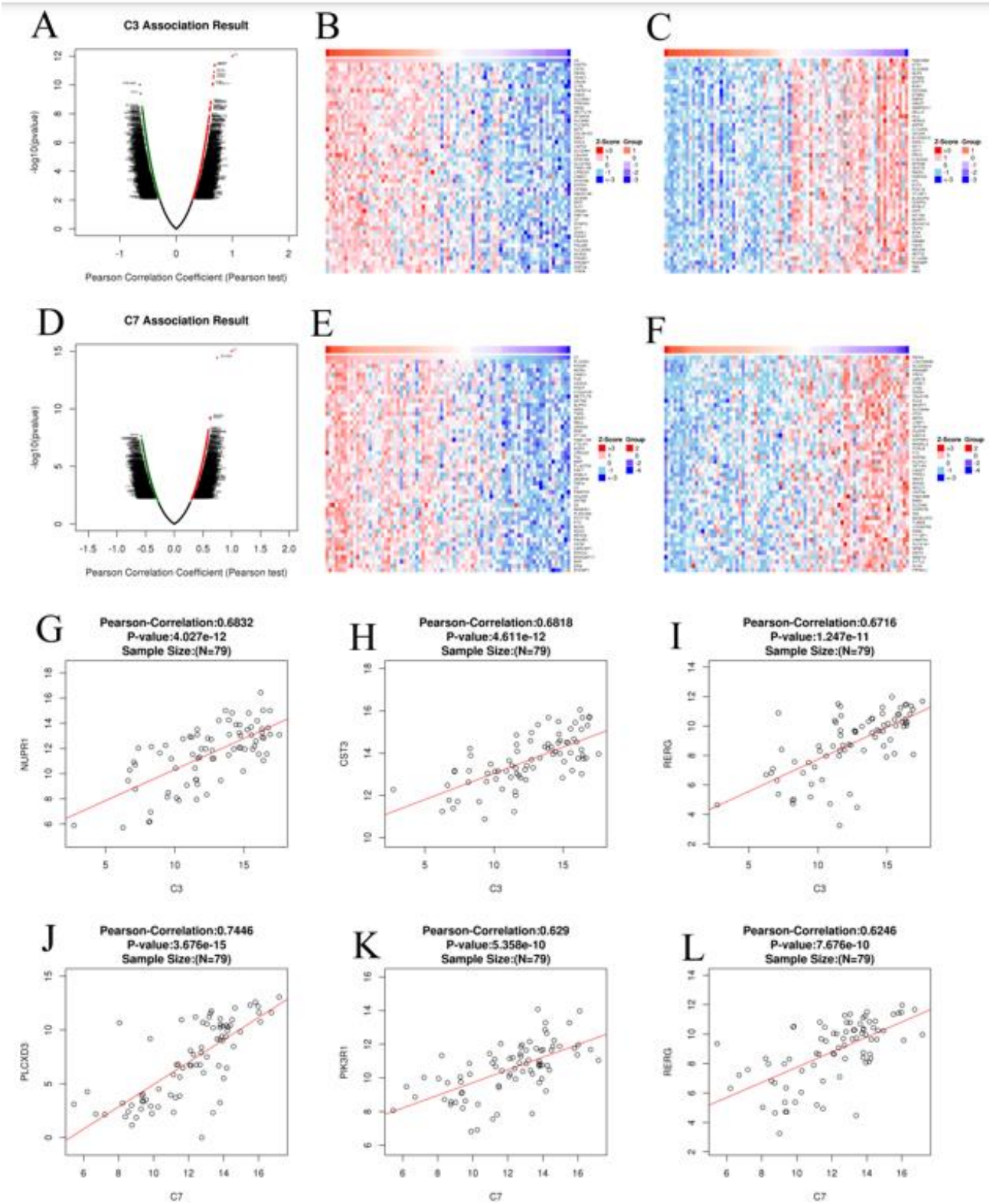

Figure 4: Genes differentially expressed in correlation with C3 and C7 expression in ACC (LinkedOmics). **(A and D)** Pearson test was used to analyze correlations between C3, C7 and genes differentially expressed in ACC, respectively; **(B, C, E, and F)** Heat maps showing genes positively and negatively correlated with C3 and C7 in ACC, respectively (TOP 50 genes); The scatter plot shows pearson correlation of C3 and C7 expression with expression of NUPR1 **(G)**, CST3 **(H)**, RERG **(I)**, PLCXD3 **(J)**, PIK3R1 **(K)**, and RERG **(L)** in ACC; Red indicates positively correlated genes and blue indicates negatively correlated genes.

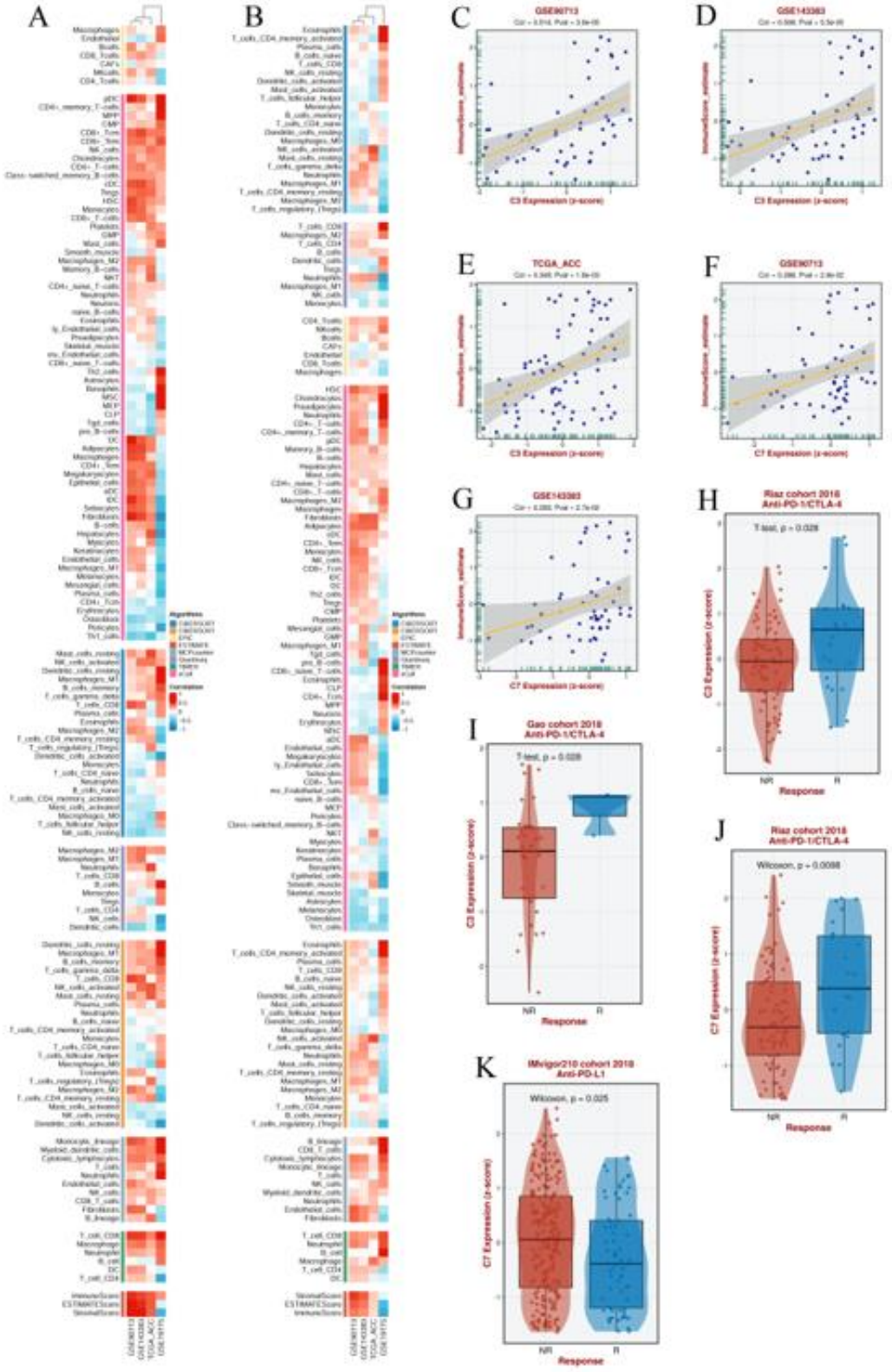

Figure 5: The correlation between C3, C7 expression and immune cell infiltration and immunotherapy in ACC (BEST). **(A and B)** Heat maps showing the correlation between C3, C7, and immune cell infiltration in ACC, respectively; **(C-E)** The correlation between C3 expression and immunescore in ACC, respectively; **(F and G)** The correlation between C7 expression and immunescore in ACC, respectively; **(H and J)** Boxplot showing the correlation between C3, C7 expression and anti-PD1/CTLA-4 immunotherapy in ACC, respectively; **(K)** Boxplot showing the correlation between C7 expression and anti-PD1 immunotherapy in ACC, respectively.

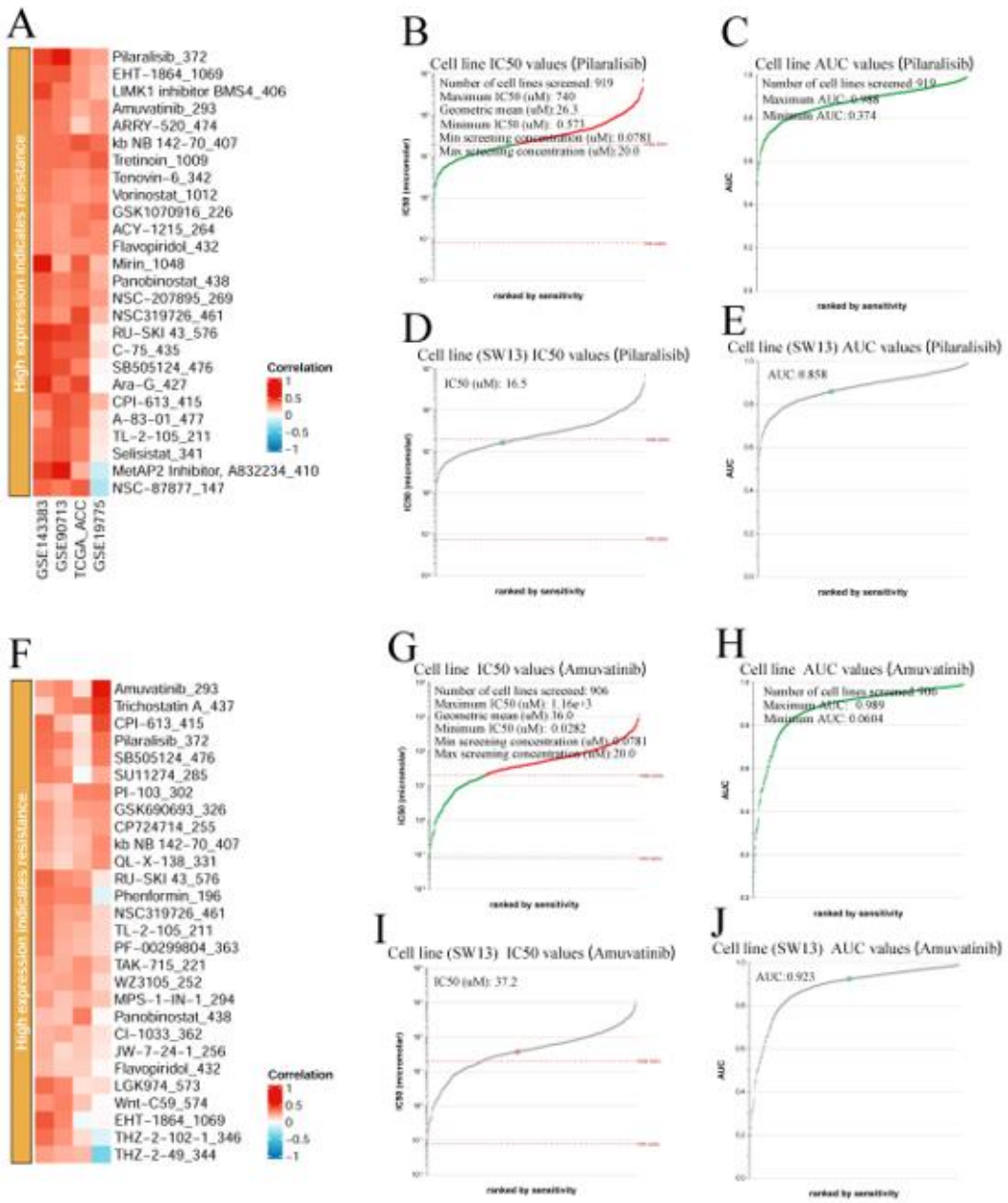

Figure 6: IC50 evaluation of pilaralisib and amuvatinib in different tissue types of cancer (BEST and Genomics of Drug Sensitivity in Cancer). **(A and F)** Heat maps showing C3 and C7 high expression indicates resistance drugs ranking, respectively; **(B)** Cell line IC50 values of pilaralisib; **(C)** Cell line AUC values of pilaralisib; **(D)** SW13 cell line IC50 values of pilaralisib; **(E)** SW13 cell line AUC values of pilaralisib. **(F and G)** Cell line IC50 values of amuvatinib; **(H)** Cell line AUC values of amuvatinib; **(I)** SW13 Cell line IC50 values of amuvatinib; **(J)** SW13 cell line AUC values of amuvatinib.

Therapeutic Drugs Targeting C3 and C7 in ACC

We used the Biomarker exploration of solid tumors (BEST) database to evaluate C3 and C7 high expression indicates resistance drugs and found that pilaralisib and amuvatinib were the first drugs (Figure 6A and 6F). Next, the Genomics of Drug Sensitivity in Cancer (GDSC) database was used to evaluate the inhibitory effects of pilaralisib and amuvatinib on ACC cell lines. Pilaralisib inhibited 919 cell lines with area under the curve (AUC) values greater than 0.374 (Figure 6C), indicating a good inhibitory effect (0.573 ≤IC50 (μ M) ≤740) (Figure 6B). Moreover, the ACC cells, SW13, were inhibited by pilaralisib (AUC = 0.858; IC50 (μM) = 16.5; Figure 6D

and 6E). However, amuvatinib inhibited 906 cell lines with AUC values greater than 0.0604 (Figure 6H), indicating a good inhibitory effect (0.0282 ≤IC50 (μM) ≤1160; Figure 6G). Furthermore, SW13 cells were considerably inhibited by amuvatinib (AUC values = 0.923; IC50 (μM) = 37.2; Figure 6I and 6J).

DISCUSSION

We observed a strong downregulation of C3 and C7 expression by 1.3% and 9% in patients with ACC, which suggests that C3 and C7 are potential targets for ACC therapy. Next, we aimed to explain the abnormal expression of C3 and C7 in ACC patients through an

analysis of genetic alterations. Our results suggest that genetic alterations may not be the main reason for the observed decrease in C3 and C7 expression. However, further investigations are required to address this issue. Finally, we evaluated the prognostic value of C3 and C7 expression in patients with ACC. We found that the survival of patients with ACC exhibiting high C3 and C7 expression was longer than of those with low expression. Thus, C3 and C7 may serve as potential prognostic markers in patients with ACC.

We further evaluated the potential interactions between C3, C7, and their ANGs. Based on their co-expression, pathway, and co-localization, C3, C7, and their ANGs formed a complex interaction network. The functions of C3, C7, and their ANGs were evaluated. The functions related to C3 and its ANGs mainly included complement activation, regulation of acute inflammatory response, secretion, regulation of angiogenesis, and response to hormones. These functions affect hormone secretion, tumor proliferation, invasion, and angiogenesis [15-17]. However, the functions related to C7 and its ANGs mainly include purine nucleotide metabolic process, translation, lymphocyte differentiation, and lymphocyte-mediated immunity. These functions have been reported to affect tumor proliferation, invasion, metastasis, and metabolism [18-20]. Taken together, C3, C7, and their ANGs may contribute to the development and progression of ACC. Therefore, the ACC may be regulated by these genes.

We examined the transcription factors, and miRNA and kinase targets of C3 and C7 in patients with ACC. Our results show that SIRT1 and STAT3 are key transcription factors for C3 and its ANGs in patients with ACC. SIRT1 is a protein deacetylase that regulates many proteins that are often functionally implicated in tumor development and progression. SIRT1 can be used as a new target for the treatment of pancreatic cancer [21]. However, its role in ACC remains unclear. STAT3 can promote angiogenesis in patients with ACC, which makes it a selective target for molecular-targeted therapy of ACC [22]. Moreover, we found that miR-151, miR-492, miR-29A, miR-29B, miR-29C, miR-379, miR-517A, and miR-517C are the targets of C3 and C7 in patients with ACC. These miRNAs are involved in the proliferation, migration, invasion, and drug resistance of tumor cells, which make them promising targets for cancer treatment [23-25]. However, they have not been reported to be associated with ACC. ATM, AURKB, AURKA, CDK2, CHEK1, and CDK1 are kinase targets of C3 and C7 in patients with ACC. ATM plays an important role in ACC tumorigenesis [26]. Overexpression of genes involved in DNA damage and those regulating cell cycle pathways, including AURKB, CHEK1, and AURKA, is correlated with poorer overall survival in patients with ACC [27]. CDK1 serves as a therapeutic target for adrenocortical carcinoma via regulation of epithelial-mesenchymal transition, G2/M phase transition, and PANoptosis [28]. Studies have shown that the expression of CDK2 mRNA is strongly upregulated in ACC and that the CDK inhibitor, flavopiridol, has a dose-dependent antiproliferative effect [29]. Apparently, these transcription factors and miRNAs, as well as the kinase targets of C3, C7, and their ANGs, may be able to treat ACC.

Next, we examined the correlation between C3 and C7 expression and the differentially expressed genes in patients with ACC. A correlation was found between C3 and C7 expression and the expression of 6,411 and 5,818 genes, respectively, including NUPR1, CST3, PLCXD3, PIK3R1, and RERG, in patients with ACC. Among these, the top three genes were positively correlated with the expression of C3 and C7. This may provide additional therapeutic targets for ACC. Infiltration of immune cells plays a pivotal role in cancer [30]. The deficiency of C3a receptor reduced monocyte infiltration induced by chronic stress [31] C5a and its receptor C5aR1 play key roles in initiating and maintaining several inflammatory reactions by recruiting and activating neutrophils and monocytes [32]. As expected, immune cell infiltration positively correlated with the expression of C3 and C7 in patients with ACC. Furthermore, we found that C3 and C7 were strongly upregulated in ACC patients treated with anti-PD1/CTLA-4 antibodies. This is beneficial for the prognosis of ACC patients. However, the expression of C7 in ACC patients treated with the anti-PD-L1 antibody was significantly downregulated. Attention should be paid to whether decreased C7 levels affect the prognosis of patients with ACC treated with PD-L1. Furthermore, pilaralisib and amuvatinib were evaluated for their inhibitory effects on ACC. Pilaralisib and amuvatinib inhibited 919 and 906 cancer cell lines, respectively. Among these, pilaralisib had a better inhibitory effect on SW13 cells than amuvatinib. In summary, pilaralisib and amuvatinib inhibited the growth of a broad spectrum of cancer cells. Studies have shown that pilaralisib and amuvatinib have good antitumor and safety properties [33,34]. However, their effect on AAC remains unclear. Pilaralisib, amuvatinib, and inhibitors of C3 and C7 or their regulatory targets may be important strategies for treatment of ACC.

CONCLUSION

In conclusion, the results of this preliminary study indicate that C3 and C7 could be potential therapeutic and prognostic targets for ACC. Moreover, the study provides insights into the mechanism underlying the pathogenesis of ACC and offers potential avenues for its treatment.

AUTHOR CONTRIBUTIONS

ZS and QX performed data analysis work and aided in writing the manuscript. YS designed the study and assisted in writing the manuscript. XJ, ZH, JZ, LL, QL, JC, and LD edited the manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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