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Research article

Identify AGAP2 as prognostic biomarker in clear cell renal cell carcinoma based on bioinformatics and IHC staining

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ABSTRACT

Background: Arf GTPase-activating proteins are aberrantly expressed in a variety of tumors, but their role in clear cell renal cell carcinoma (ccRCC) was unclear. Exploring the biological role of Arf GAP with GTP binding protein like domain, Ankyrin repeat and PH domain 2 (AGAP2) in ccRCC could improve our understanding on the aggressiveness and immune relevance of ccRCC.
Methods: The expression of AGAP2 was analyzed based on the Cancer Genome Atlas (TCGA) database and verified in ccRCC samples using immunohistochemistry. The association between AGAP2 and clinical cancer stages was explored by TCGA dataset and UALCAN. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed to analyze the biological functions of AGAP2-related genes. Moreover, the relationship between AGAP2 and immune cell infiltration was investigated with TIME and TCGA dataset.
Results: Compared to normal tissues, AGAP2 was upregulated in ccRCC tissues. Higher expression of AGAP2 was associated with clinical cancer stages, TNM stages, pathologic stages, and status. Prognostic analysis on AGAP2 showed that AGAP2 overexpression was associated with KIRC overall survival (OS) reduction ($P = 0.019$). However, higher expression of AGAP2 may improve the OS of CESC ($P = 0.002$), THYM ($P = 0.006$) and UCEC ($P = 0.049$). GO and KEGG analysis showed that AGAP2-related genes was related to T cell activation, immune activity and PD-L1 expression and PD-1 checkpoint pathway. Furthermore, our study showed that AGAP2 were significantly associated with T cells, Cytotoxic cells, Treg, Th1 cells, CD8 T cells, T helper cells. And AGAP2 expression level affected the abundance of immune cells infiltration. The infiltrating level of immune cells was different between the AGAP2 high-expression and low-expression groups.
Conclusion: The expression of AGAP2 in ccRCC was higher than that in normal kidney tissues. It was significantly associated with clinical stage, poor prognosis, and immune cell infiltration. Therefore, AGAP2 may become an important component for ccRCC patients who receive precision cancer therapy and may be a promising prognostic biomarker.

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1. Introduction

Renal cell carcinoma (RCC) is a malignancy with the eighth highest incidence in the world. Annually, there are about 79,000 new cases and 13,920 deaths from RCC in 2022 in the United States alone [1]. The incidence is increasing but varies in different regions [2]. RCC can be classified depending on different morphologic and molecular genetic features. In general, clear cell renal cell carcinoma (ccRCC) nearly makes up 80% of all RCC [3]. The gradual development of RCC is often accompanied with unobvious early clinical manifestations. About 1/3 of RCC patients present with metastasis by the time of initial diagnosis and therefore miss the opportunity for taking surgery. Although surgical treatment remains the primary treatment for RCC, surgical treatment is not a solution once and for all. Metastases still occur to 20%–30% of cases in patients who undergo surgical treatment, which had poor survival time [4]. Due to great advances in diagnosis and treatment, the prognosis of patients with RCC has been greatly improved. However, many patients, especially those at advanced stages, still have a poor prognosis. Therefore, there is an urgent need to explore prognostic biomarkers in ccRCC.

AGAP2 was preliminarily identified as Arf GAP with GTPase domain, ankyrin repeat, and PH domain 2 [5]. AGAP2 is one of AGAP family members involved in coordinated changes of membrane and actin cytoskeleton under several motor cell structures and are essential for normal physiological functions, such as development, wound healing, and phagocytosis [6–8]. Recent studies indicated that the coordination of membrane and actin cytoskeleton contributed to pathological behaviors of cancer cell invasion and metastasis. The AGAP family members also function as scaffolds and perform intrinsic activities, such as membrane bending, which may directly contribute to abnormal behaviors of cancer cells. For example, He et al. showed that AGAP2 could promote the proliferation of prostate cancer cells via interacting directly with UNC5B [9]. And Jee-Yin Ahn reported that AGAP2 bound to activated Akt to increase the invasion of glioblastoma cells and showed a protective effect on glioblastoma cell apoptosis [10].

Though efforts have been devoted to the study of ccRCC development, progression, and metastatic mechanisms, the molecular features of ccRCC were not clear. The studies on detailed structure, cell biology, biochemistry, and genetic studies of any gene/protein may model their roles in the cells. Advance in high-throughput sequencing technology helps identify key genes associated with tumor prognosis and progression through bioinformatics analysis [11]. However, the role of AGAP2 in ccRCC has not been explored. This study aims to investigate the comprehensive role of AGAP2 in ccRCC and its correlation with immunity, so as to provide new strategies for improving the prognosis of ccRCC.

2. Materials and methods

2.1. Tissue specimens and data acquisition

Eight pairs of ccRCC samples and adjacent renal tissues from patients with primary ccRCC who underwent partial nephrectomy or radical nephrectomy in the Department of Urology, The First Affiliated Hospital of Soochow University were collected. This study was approved by the Institutional Research Ethics Committees of The First Affiliated Hospital of Soochow University (NO.2020/124). The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga) is a project with abundant bioinformation designed to promote tumor-related research. We download mRNA expression profiles and corresponding clinical information of ccRCC and pan-cancer data from TCGA database. Our study followed TCGA's data access policy and publication guidelines and we converted the RNAseq data to FPKM (Fregments Per Kilobase per Million) format.

2.2. The expression analysis and immunohistochemical staining of AGAP2

All the gene expression profile matrices were normalized with tidyverse package to obtain AGAP2 gene expression data and clinical information, and expression level of AGAP2 was compared between normal tissues and tumor tissues using ggplot2 and reshape2 packages. Additionally, immunohistochemical staining was performed on the 8 pairs ccRCC and normal samples to determine the expression of AGAP2.

2.3. Association of AGAP2 expression level with prognosis and clinicopathologic features of ccRCC patients

In this study, we focused on demonstrating the relevance of AGAP2 to clinicopathologic features of ccRCC patients. The AGAP2 expression level was examined at different clinical stage in ccRCC using UALCAN (http://ualcan.path.uab.edu), which is an interactive web resource for RNA - seq and clinical data of 31 cancer types based on the TCGA [12].

The prognostic values of AGAP2 in pan-cancer were assessed by the Kaplan-Meier plotter (http://kmplot.com/analysis) [13]. Tumor samples were classified into high and low expression groups, respectively, depending on the median values of mRNA expression and was presented by K-M survival curves [14,15]. Differences were considered statistically significant when p-values were <0.05.

2.4. Related genes and enrichment analysis of AGAP2

Correlation analysis between AGAP2 and other genes in ccRCC was explored by TCGA data, and the Spearman correlation was calculated. Underlying biological functions of related genes were explored by significant Gene Ontology terms (including biological process (BP), cellular component (CC), and molecular function (MF)) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway on the genes showing the closest association with AGAP2. We first analyzed AGAP2-related genes and presented the top 50 positively

and negatively related genes. Then the top 50 genes positively correlated with AGAP2 were selected for further enrichment analysis.

2.5. Immune cell infiltration analysis

We used GSVA package and Tumor Immune Estimation Resource (TIMER) database (www.timer.cistrome.org) to explore the potential association between the AGAP2 expression level and immune cells. Based on the results of previous survival analysis, the immune cell infiltration score of KIRC from TCGA was downloaded and estimated. To compare the levels of immune cell infiltration, the specimens were divided into high AGAP2 group and low AGAP2 group, according to the median expression level of AGAP2. The molecular characteristics of tumor-immune interactions were analyzed in KIRC using TIMER. And the abundance of six tumor-infiltrating immune cells, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and DCs, was explored [16]. Lollipop chart was plotted to examine the correlation of AGAP2 with 24 types of immune cells infiltration in ccRCC [17]. At the same time, box figure was generated to analyze the relationship of AGAP2 and immune cell recruitment.

2.6. Statistical methods

Chi-square test and Mann-Whitney test were performed for analyzing the basic data of subjects grouped according to the median AGAP2 expression level. Spearman correlation method was used for correlation analysis. Survival analysis was conducted using the *survival* package and the significance was computed using the Cox-Mantel (log rank) test [18]. The difference between the groups was numerically characterized by the hazard rate (HR). To investigate the correlation between AGAP2 expression and the abundance of tumor infiltrating immune cells, Wilcoxon rank sum and Spearman rank correlation tests were used to calculate p values in GSVA package.

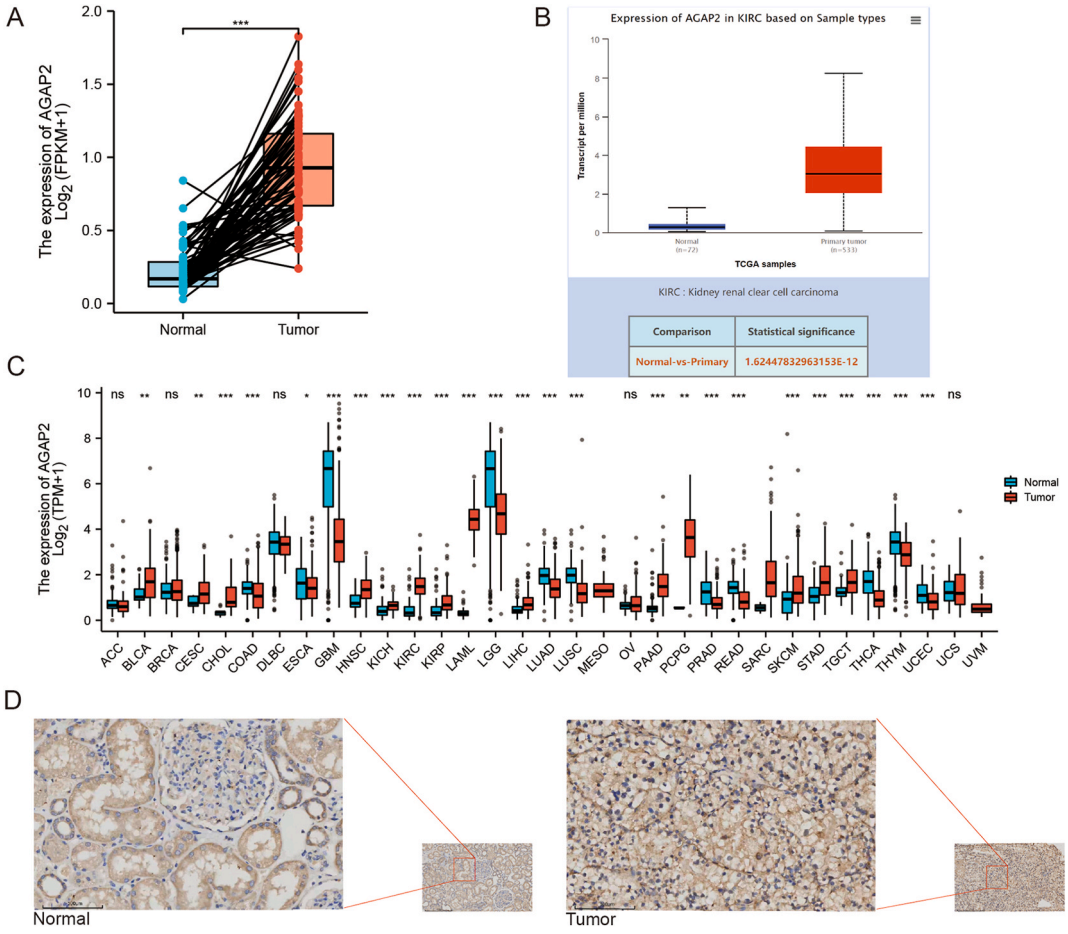

Fig. 1. Expression level of AGAP2 in ccRCC and pan-cancer. (A–B) show the high expression of AGAP2 in ccRCC. (C) shows the high expression of AGAP2 in pan-cancer.

3. Results

3.1. A higher expression level of AGAP2 in ccRCC and pan-cancer samples

The expression difference of AGAP2 between ccRCC and normal tissues was presented (Fig. 1A). The expression level of AGAP2 in ccRCC was significantly higher than that in normal renal tissue. We then used ULCAN to verify the expression difference (Fig. 1B). It was observed that the expression level of AGAP2 was higher in 13 types of cancers when compared to normal tissues, including BLCA, CESC, HNSC, KICH, KIRC, KIRP, LAML, LIHC, PAAD, PCPG, SKCM, STAD, TGCT. But the expression levels of AGAP2 were lower in ESCA, LGG, LUAD, LUSC, PRAD, READ, THCA, THYM, UCEC when compared to normal tissues (Fig. 1C). We further confirmed the expression of AGAP2 in ccRCC using immunohistochemistry (Fig. 1D).

3.2. Association between a higher expression of AGAP2 and the clinicopathologic features in ccRCC

The clinical features and gene expression data of 539 primary ccRCC patients were downloaded from TCGA database. The ccRCC patients were divided into high AGAP2 expression group (N = 270) and low AGAP2 expression group (N = 269), according to the median threshold of AGAP2 expression level. After we detected a higher expression of AGAP2 in ccRCC tissues, the influence of expression level of AGAP2 on the clinicopathologic features of ccRCC patients was analyzed. Comparison between high and low AGAP2 expression level in ccRCC patients showed significant differences between the two groups in clinicopathologic features (Table 1). We also found that high expression group had higher T stage (P = 0.015), M stage (P = 0.014), pathologic stage (P = 0.007) and more dead samples (P = 0.029). Moreover, the association between expression level of AGAP2 and the individual cancer stage was explored by UALCAN. The result demonstrated the highest expression level of AGAP2 in stage IV. The expression level of AGAP2 showed significant difference in comparison between stage I and stage III (P = 0.046), stage I and stage IV (P = 0.019) in ccRCC patients (Fig. 2A).

3.3. Prognostic analysis of AGAP2 in ccRCC

Furthermore, the Kaplan-Meier plotter was used to analyze the prognostic significance of the AGAP2 in pan-cancer. As the results shown in Fig. 2(B, C, D and E), higher expression of AGAP2 reduced the OS of KIRC (HR = 1.43, 95% CI: 1.06–1.94, P = 0.019), whereas higher expression of AGAP2 could improve the OS of CESC (HR = 0.48, 95% CI: 0.3–0.78, P = 0.002), THYM (HR = 0.09, 95% CI: 0.01–0.77, P = 0.006) and UCEC (HR = 0.66, 95% CI: 0.43–1, P = 0.049).

Table 1
Association between AGAP2 expression level and clinicopathologic features in ccRCC.

Characteristic	Low expression of AGAP2	High expression of AGAP2	p
n	269	270	
T stage, n (%)			0.015
T1	153 (28.4%)	125 (23.2%)	
T2	39 (7.2%)	32 (5.9%)	
T3	73 (13.5%)	106 (19.7%)	
T4	4 (0.7%)	7 (1.3%)	
N stage, n (%)			0.181
N0	125 (48.6%)	116 (45.1%)	
N1	5 (1.9%)	11 (4.3%)	
M stage, n (%)			0.014
M0	227 (44.9%)	201 (39.7%)	
M1	29 (5.7%)	49 (9.7%)	
Status event, n (%)			0.029
Alive	195 (36.2%)	171 (31.7%)	
Dead	74 (13.7%)	99 (18.4%)	
Histologic grade, n (%)			0.081
G1	7 (1.3%)	7 (1.3%)	
G2	125 (23.5%)	110 (20.7%)	
G3	102 (19.2%)	105 (19.8%)	
G4	27 (5.1%)	48 (9%)	
Pathologic stage, n (%)			0.007
Stage I	151 (28.2%)	121 (22.6%)	
Stage II	34 (6.3%)	25 (4.7%)	
Stage III	51 (9.5%)	72 (13.4%)	
Stage IV	32 (6%)	50 (9.3%)	
Gender, n (%)			0.164
Female	101 (18.7%)	85 (15.8%)	
Male	168 (31.2%)	185 (34.3%)	
Age, n (%)			0.413
≤60	129 (23.9%)	140 (26%)	
>60	140 (26%)	130 (24.1%)	

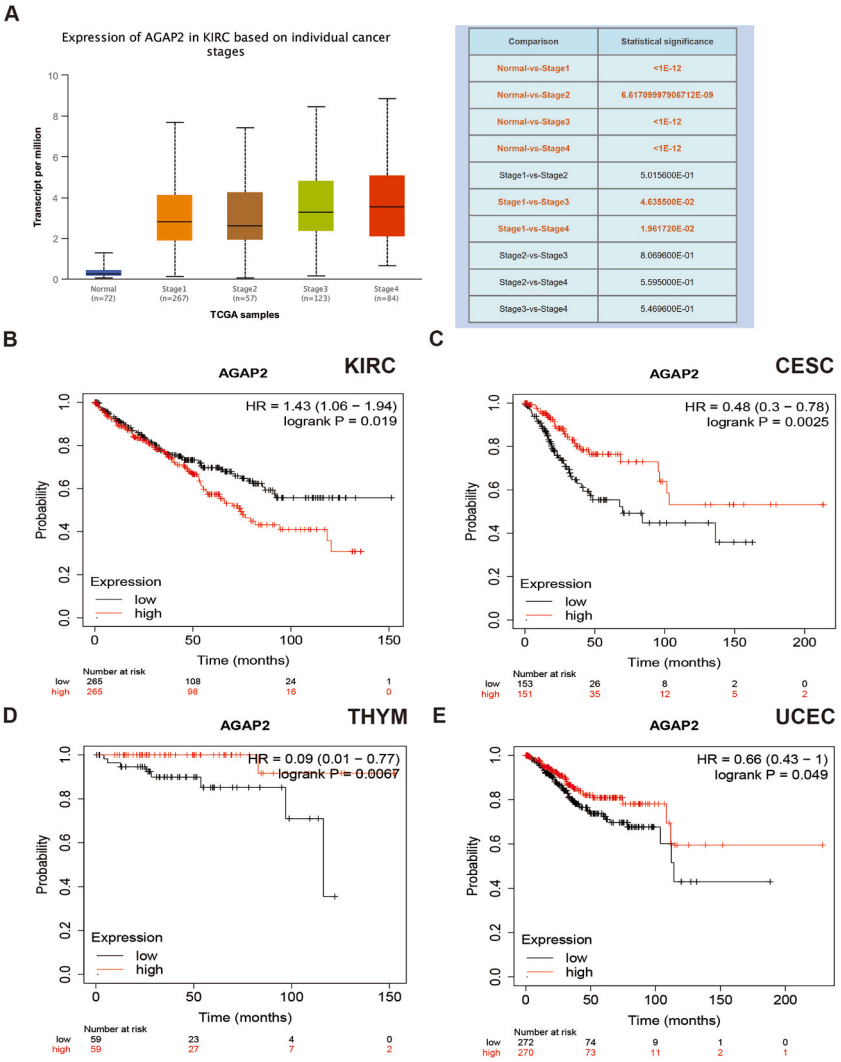

Fig. 2. Association of AGAP2 expression level with clinicopathologic features and overall survival. (A) Association of AGAP2 expression level with individual cancer stages. (B–E) Association of AGAP2 expression level with OS of KIRC, CESC, THYM, and UCEC.

3.4. Identification and function analysis of AGAP2-related genes

Then, the TCGA database was used to study the correlated significant genes with the AGAP2. The top 50 positively or negatively correlated genes were shown in the heatmap plot (Fig. 3A and B). We found that the most positively correlated genes with AGAP2 were TMC8, RASAL3, and ZAP70, while the negatively correlated genes with AGAP2 were SYPL1, SCOC, and MRPS35. Moreover, the top 50 positive-related genes were used to conduct function analysis. In the GO analysis (Fig. 4A–C), the expression of top 50 positive-related genes was found to be closely associated with T cell activation, immunological synapse, GTPase regulator activity. Enrichment analysis showed that they were closely related to immune activity. Most of the genes were correlated with T cell activation involved in immune response and T cell-mediated immunity. The KEGG pathway analysis demonstrated an enrichment and crosstalk in T cell receptor signaling pathway, natural killer cell-mediated cytotoxicity, PD-L1 expression and PD-1 checkpoint pathway, Th1 and Th2 cell differentiation, and Th17 cell differentiation (Fig. 4D).

3.5. Correlation analysis between AGAP2 expression and immune cell infiltration in ccRCC

According to previous enrichment analysis results, we found that AGAP2-related genes were mainly associated with T cell activation, immunological synapse, PD-L1 expression and PD-1 checkpoint pathway. We speculated that AGAP2 may have an internal correlation with immune cells. Firstly, we explored the correlation between AGAP2 with infiltration levels of immune cells in KIRC (Fig. 5A). here, the results showed that AGAP2 was significantly correlated with the infiltration level of CD4+T cells. To reveal the correlation between AGAP2 and the immune infiltrating cells, the correlation analysis between AGAP2 and 24 types immune

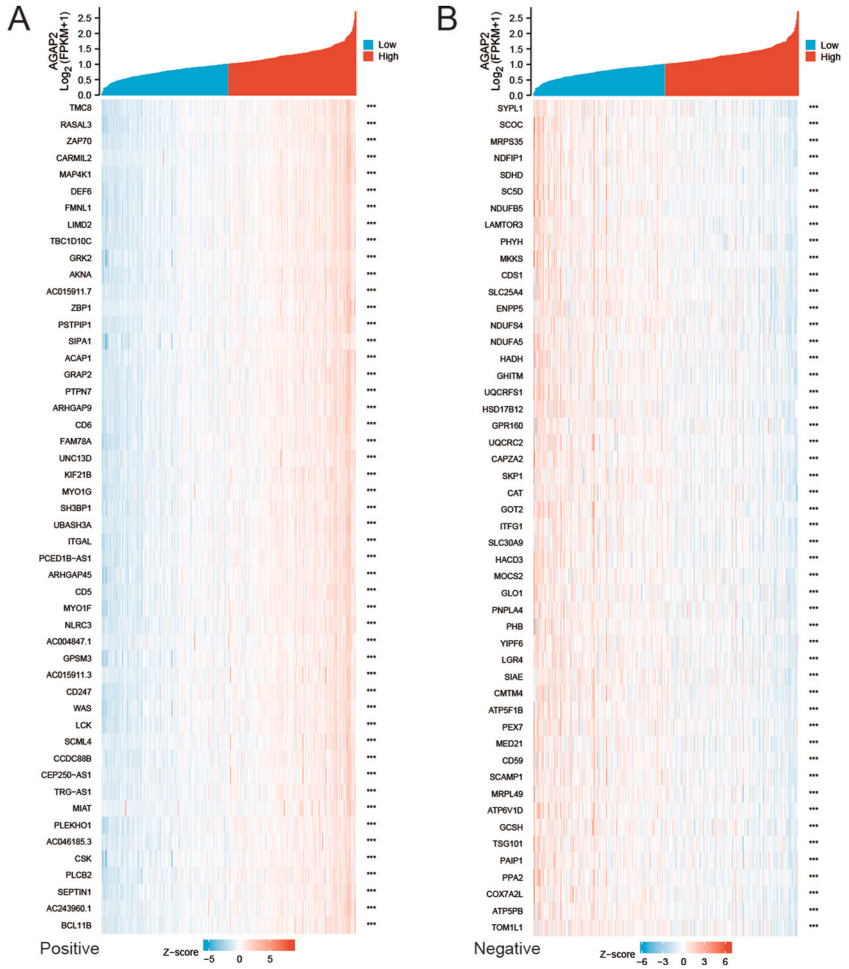

Fig. 3. Differential genes associated with AGAP2 were identified. (A): top 50 positively correlated genes of AGAP2. (B) top 50 negatively correlated genes of AGAP2.

infiltrating cells was performed. It was found that AGAP2 was significantly associated with T cells, Cytotoxic cells, Treg, Th1 cells, CD8 T cells, T helper cells, Term, B cells, NK CD56bright cells, aDC, TFH, NK CD56dim cells, DC, Th2 cells, Tcm, pDC, Macrophages, NK cells, iDC, Eosinophils in ccRCC (Fig. 5B). We also validated the relationship between AGAP2 and most immune marker sets after adjusting the purity (Table 2). The data revealed that AGAP2 was significantly correlated with Th1 cell markers, such as TBX21, STAT4, STAT1, IFN-γ and TNF-α. Th2 cell markers, TFH cell markers and Th17 cell markers also showed significant correlations with AGAP2 expression. Moreover, we observed that a strong positive correlation between AGAP2 and marker genes of T cell exhaustion, especially the correlation of AGAP2 with CTLA4 and PD-1.

Difference in the level of immune cell infiltration between the AGAP2 high expression group and the AGAP2 low expression group was studied as well. Interestingly, we found significant differences in the infiltrating levels of immune cells between the AGAP2 high-expression and low-expression groups, including T cells, aDC, B cells, CD8 T cells, Cytotoxic cells, DC, NK CD56bright cells, NK CD56dim cells, NK cells, pDC, T helper cells, Treg, Th1 cells, Macrophages, Th2 cells (Fig. 6A-O). The results showed that AGAP2 high expression group had higher infiltrating immune cell levels in 15 types immune cells when compared to AGAP2 low expression group.

4. Discussion

The prognosis of ccRCC has been improved significantly by the advances in targeted therapy, such as PD-1 immune checkpoint inhibitors [19]. However, PD-1 immune checkpoint inhibitors were not fully effective and therapeutic process often ends with side-effects [20]. Therefore, the therapy should be further improved. Previous studies have identified the prognostic significance of Arf GAP family in cancers. For instance, ASAP2, ArfGAP with SH3 domain, ankyrin repeat and PH domain 2, have been found to drive the progression of pancreatic ductal adenocarcinoma (PDAC) [21]. In addition to ASAP, ARAP1 was observed to be related to pediatric acute lymphoblastic leukemia (ALL) because its over-expression was related to drug resistance. However, the expression of AGAP, ArfGAP with GTPase domain, ankyrin repeat and PH domain is still unclear in ccRCC. Our study aimed to explore the expression level

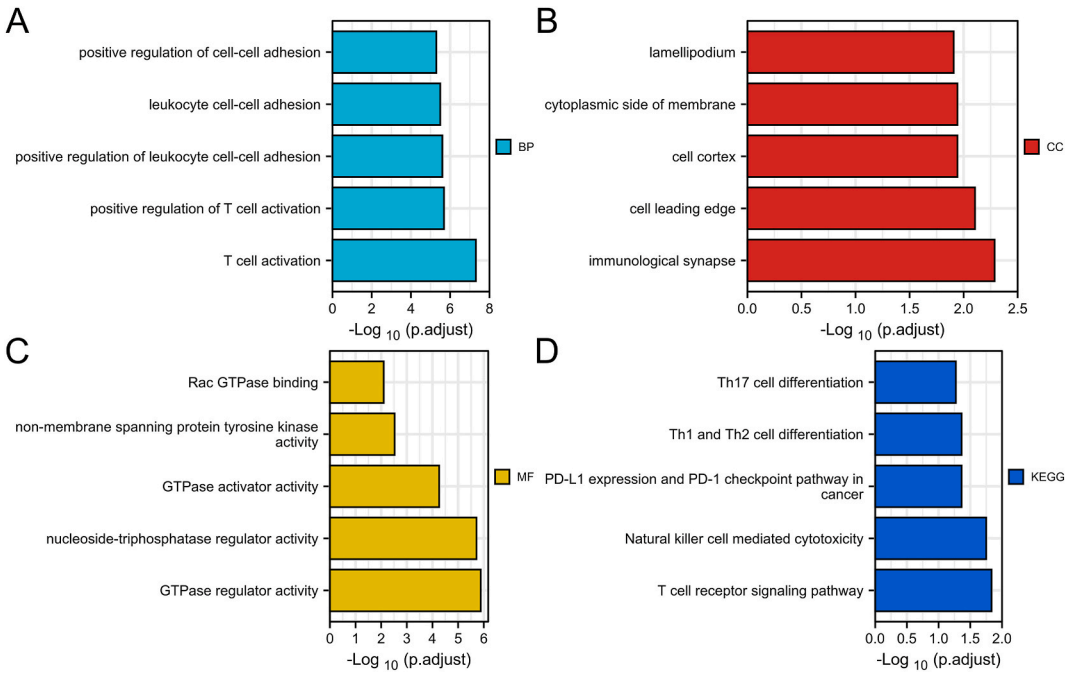

Fig. 4. Function analysis of AGAP2 in ccRCC. (A) BP of the top 50 genes the most positively associated with AGAP2. (B) CC of the top 50 genes the most positively associated with AGAP2. (C) MF of the top 50 genes the most positively associated with AGAP2. (D) KEGG pathway of the top 50 genes the most positively associated with AGAP2.

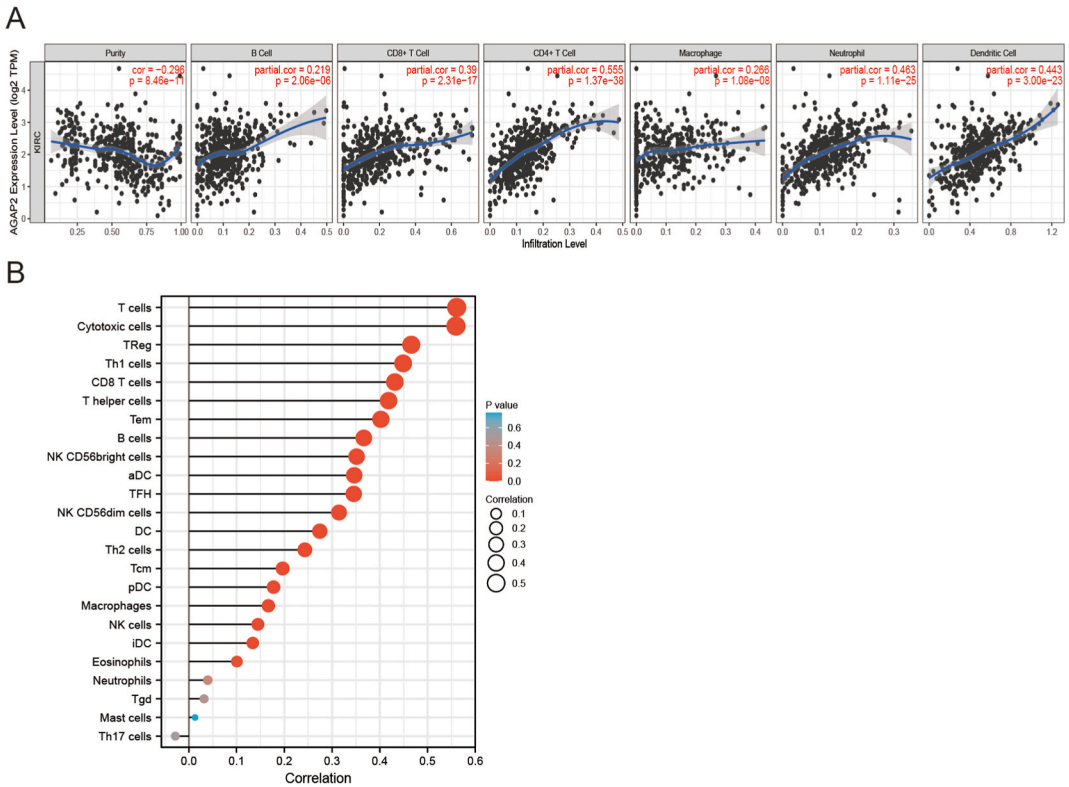

Fig. 5. (A) Associations between AGAP2 expression level and immune infiltration in KIRC. (B) Lollipop chart of AGAP2 expression level in 24 immune cells.

Table 2
Correlation analysis between AGAP2 and markers of immune cells in TIMER.

Description	Gene markers	AGAP2			
		NONE		PURITY	
		COR	P	COR	P
CD8 + T cell	CD8A	0.512	5.17E-37	0.445	8.24E-24
	CD8B	0.496	1.64E-34	0.44	3.37E-23
T cell (general)	CD3D	0.585	2.70E-50	0.526	3.99E-34
	CD3E	0.619	1.21E-57	0.561	1.21E-39
	CD2	0.595	1.99E-52	0.535	1.62E-35
B cell	CD19	0.441	8.26E-27	0.378	3.86E-17
	CD79A	0.435	4.59E-26	0.368	3.08E-16
Monocyte	CD86	0.423	1.40E-24	0.361	1.22E-15
	CSF1R	0.476	1.67E-31	0.429	4.55E-22
TAM	CCL2	0.18	2.88E-05	0.116	1.25E-02
	CD68	0.269	2.94E-10	0.245	9.54E-08
	IL10	0.408	7.78E-23	0.348	1.39E-14
M1 Macrophage	NOS2	0.247	7.54E-09	0.196	2.35E-05
	IRF5	0.345	2.21E-16	0.307	1.57E-11
	PTGS2	0.126	3.70E-03	0.105	2.37E-02
M2 Macrophage	CD163	0.346	1.82E-16	0.312	7.06E-12
	VSIG4	0.364	3.45E-18	0.308	1.32E-11
	MS4A4A	0.361	7.01E-18	0.307	1.71E-11
Neutrophils	CEACAM8	0.095	2.84E-02	0.109	1.95E-02
	ITGAM	0.43	1.91E-25	0.37	2.07E-16
	CCR7	0.55	1.73E-43	0.498	2.91E-30
	KIR2DL1	0.212	7.58E-07	0.184	7.16E-05
	KIR2DL3	0.197	4.82E-06	0.184	6.87E-05
	KIR2DL4	0.311	1.90E-13	0.281	7.75E-10
	KIR3DL1	0.193	7.00E-06	0.2	1.50E-05
	KIR3DL2	0.271	1.86E-10	0.249	5.70E-08
	KIR3DL3	0.161	1.93E-04	0.135	3.64E-03
	KIR2DS4	0.196	5.37E-06	0.19	4.05E-05
Dendritic cell	HLA-DPB1	0.469	1.69E-30	0.421	3.52E-21
	HLA-DQB1	0.345	2.67E-16	0.283	6.55E-10
	HLA-DRA	0.405	1.91E-22	0.352	6.54E-15
	HLA-DPA1	0.406	1.31E-22	0.34	6.57E-14
	CD1C	0.313	1.29E-13	0.266	7.06E-09
	NRP1	0.166	1.17E-04	0.125	7.15E-03
	ITGAX	0.471	7.86E-31	0.436	7.26E-23
Th1	TBX21	0.604	3.39E-54	0.579	1.23E-42
	STAT4	0.632	7.54E-61	0.578	1.64E-42
	STAT1	0.402	4.25E-22	0.341	5.30E-14
	IFNG(IFN-γ)	0.483	1.85E-32	0.408	6.74E-20
	TNF(TNF-α)	0.419	4.87E-24	0.402	2.55E-19
Th2	GATA3	0.256	1.93E-09	0.26	1.49E-08
	STAT6	0.242	1.63E-08	0.258	1.82E-08
	STAT5A	0.468	2.55E-30	0.402	2.38E-19
	IL13	0.291	6.84E-12	0.284	5.59E-10
Tfh	BCL6	0.207	7.37E-06	0.203	2.41E-06
	IL21	0.155	3.34E-04	0.123	7.98E-03
Th17	STAT3	0.248	6.46E-09	0.221	1.59E-06
	IL17A	0.073	9.23E-02	0.037	4.27E-01
Treg	FOXP3	0.546	1.06E-42	0.484	1.85E-28
	CCR8	0.482	2.74E-32	0.414	1.74E-20
	STAT5B	0.196	5.19E-06	0.192	3.42E-05
	TGFB1(TGFβ)	0.314	1.20E-13	0.285	4.29E-10
T cell exhaustion	PD-1 (PDCD1)	0.553	5.43E-44	0.501	1.27E-30
	CTLA4	0.539	1.74E-41	0.475	2.88E-27
	LAG3	0.529	8.35E-40	0.469	1.29E-28
	TIM-3	0.144	8.84E-04	0.079	9.10E-02
	GZMB	0.476	1.92E-31	0.431	3.02E-22

TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; COR, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

and prognostic value of AGAP2 in ccRCC.

The results of expression level analysis in pan-cancer suggested that the expression level of AGAP2 in 13 types of cancers was higher in tumor tissues compared to normal tissues. Our results were consistent with previous studies. Upregulation of AGAP2 was also found in immunohistochemical results. Previous studies showed that ccRCC tissues had higher expression level of AGAP2 compared to normal tissues, including in breast, lung, ovary, bladder, vulva, uterus, and cervical cancers [22]. In terms of greater affiliation, AGAP2

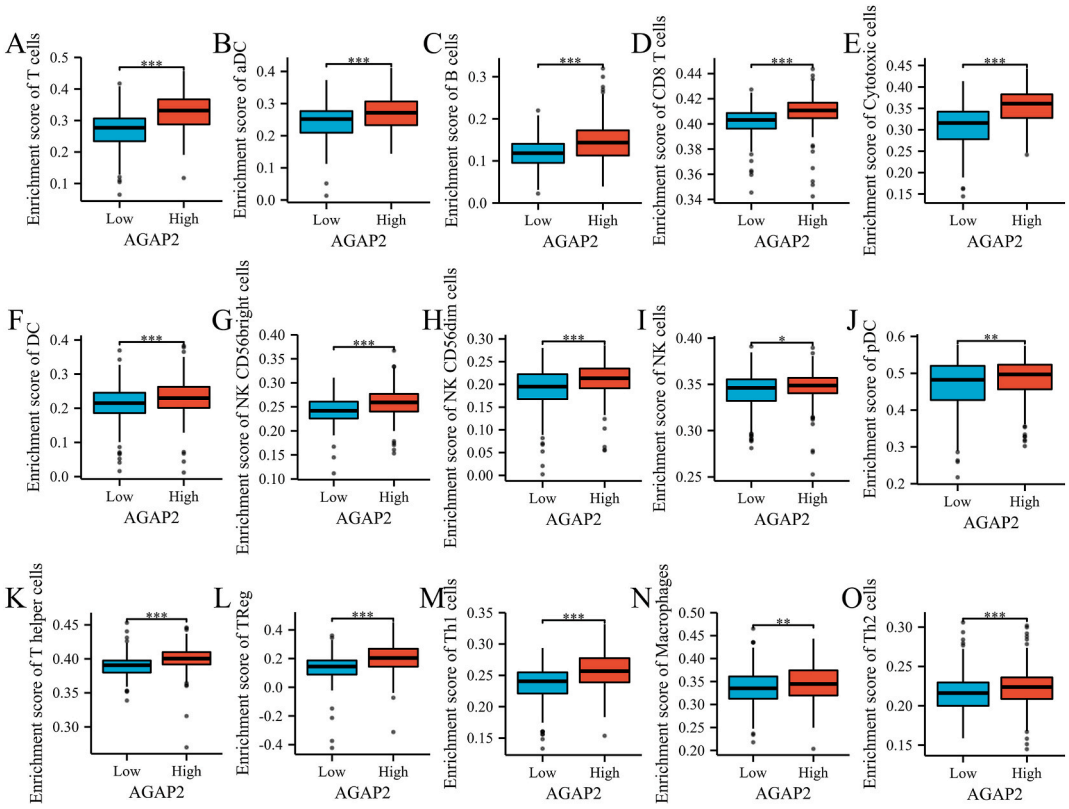

Fig. 6. Comparison of immune cells between high and low AGAP2 expression groups. (A–O) Histogram showing the difference of T cells, aDC, B cells, CD8 T cells, Cytotoxic cells, DC, NK CD56bright cells, NK CD56dim cells, NK cells, pDC, T helper cells, Treg, Th1 cells, Macrophages, and Th2 cells infiltration level between high and low AGAP2 expression groups.

belongs to Arf GAP proteins as ras superfamily members belong to G-protein-coupled receptors (GPCRs). GPCRs make up the largest integral membrane receptors family in humans responsible for cell signaling events involved in nearly every physiological processes. They play an important role in communication systems. AGAP family members are interfaces between signaling pathways, which induce the hydrolysis of GTP binding to Arf and was identified as GTPase switches of Arf family GTP-binding proteins in previous studies [22]. As the GTPase switches are activated, conversion of Arf from the GTP to GDP bound form will occur [23]. The AGAP2 may regulate cytoskeletal remodeling by controlling the hydrolysis of GTP binding to Arf proteins and is involved in a variety of cellular functions [24]. In April of this year, Qu et al. reported that the three-dimensional structures of the complexes of two adhesive GPCR (ADGRD1 and ADGRF1) binding to G protein, and the functional correlation study was carried out [25]. The molecular mechanism of spontaneous activation of these orphan receptors was elucidated for the first time, providing an important basis for studying the signal transduction mechanism of these receptors and future drug design.

As the expression level of AGAP2 in tumor tissues was significantly higher than that in normal tissues, we further investigated the association between AGAP2 and clinicopathological features of ccRCC. Our results revealed that AGAP2 expression level was closely associated with T stage, M stage, pathologic stage. The highest expression level of AGAP2 was found in stage IV. Moreover, the expression level of AGAP2 showed significant difference in comparison between stage I-II and stage III-IV. These results demonstrated that AGAP2 may play an important role in ccRCC progression. As shown by a previous study, AGAP2 expression were closely related to tumor and other pathological progression [26]. And the study of AGAP2 revealed that AGAP2 was overexpressed in human glioblastoma cell lines, and regulated focal adhesion kinase (FAK) activity and focal adhesion disassembly during cell migration. Also, overexpression of AGAP2 promoted Akt activity and the invasion of two glioblastoma cell lines [27]. Therefore, elevating AGAP2 expression may increase focal adhesion remodeling and cancer cell migration. In the prognostic analysis of our study, AGAP2 expression level appeared to significantly affect the overall survival time of ccRCC patients. A higher expression level of AGAP2 may be a risk factor for the OS of ccRCC patients, while a higher expression level of AGAP2 may be a favorable factor for the OS of CESC, THYM and UCEC.

In addition, we analyzed the function and pathway of AGAP2 and its 50 related genes in ccRCC patients. The results indicated that most of the genes were associated with T cell activation involved in immune response and T cell mediated immunity. The KEGG pathway analysis demonstrated an enrichment and crosstalk in T cell receptor signaling pathway, natural killer cell mediated cytotoxicity, PD-L1 expression and PD-1 checkpoint pathway, Th1 and Th2 cell differentiation, and Th17 cell differentiation. The tumor immune microenvironment plays an important role in regulating tumor growth, invasion and metastasis, and invasive immune cells

are the key part of tumor immune microenvironment [28,29]. As a highly immune-related tumor, the development of ccRCC is closely related to immune cell activation. Our study revealed that AGAP2 was significantly correlated with markers of $CD4^+$ T cells. Considering the correlations of AGAP2 with CTLA4 and PD-1, AGAP2 expression may play a crucial role in T cell exhaustion. Therefore, these results further confirmed that AGAP2 was specifically correlated with immune infiltrating cells in ccRCC, suggesting that AGAP2 may play a crucial role in immune escape in the ccRCC microenvironment and regulating the function of T cells in ccRCC.

There were some limitations in our study. Although upregulation of AGAP2 was associated with shorter OS in ccRCC, the data analyzed in this study came from public databases, and more evidence from experimental and clinical study is needed to verify our findings and explore the application of AGAP2. The mechanisms of AGAP2 are so complex that more exploration will be required in the future.

5. Conclusion

In summary, bioinformatics and immunohistochemical staining studies showed that increased AGAP2 expression in ccRCC was associated with clinicopathological stage progression, poor prognosis, and immune invasion. This may provide useful information for further understanding of the biological aggressiveness of ccRCC and the development of new therapies.

In the future, we plan to conduct experiments, such as overexpression or knockdown of AGAP2 to detect tumor characteristics, for example, tumor invasiveness, proliferation and EMT of ccRCC.

Conflict of interests

The authors declared no conflicts of interest with this article.

Authors' contributions

(I) Conception and design: Zekun Xu, Jiangnan Xu and Yuxuan Wang; (II) Administrative support: Changsong Pei and Min Xu; (III) Provision of study materials: Xiaojie Ang, Jiangnan Xu; (III) Collection and assembly of data: Zekun Xu, Yuxuan Wang, and Jiangnan Xu; (IV) Data analysis and interpretation: Zekun Xu, Xiaojie Ang, Nianxin Ge.

Data availability statement

Available in "figshare" online website.

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