Bioinformatics Analysis of Apolipoprotein H Demonstrates Its Potential as a Prognostic Biomarker in Kidney Renal Clear Cell Carcinoma

Peng Wang

Lishui City People's Hospital

Guan-an Zhao (✉ lyzhaoguanan@163.com)

Lishui City People's Hospital

Research Article

Keywords: renal clear cell carcinoma, APOH, expression, survival, pathway

Posted Date: November 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1105354/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objectives This study aimed to systematically reveal the clinical value of APOH in KIRC.

Methods The expression of APOH and its methylation level in KIRC were explored. Also, the prognostic value of APOH was evaluated. In addition, the correlation of APOH and immune infiltrates in KIRC was analyzed. Finally, enrichment analysis was performed to predict the APOH-associated pathways in KIRC.

Results Compared with the normal group, APOH was down-regulated in KIRC both in mRNA and protein levels. The APOH expression was related to several phenotypes of KIRC patients. Kaplan-Meier analysis showed that APOH high expression correlated with shorter OS, PFI, and DSS time. Cox regression only indicated the independent prognostic impact of APOH for PFI. ROC curve showed the better prediction performance of APOH for PFI and nomogram further revealed its contribution to survival probability. Additionally, a negative correlation between APOH expression and methylation was observed, and APOH methylation did not influence the prognosis of patients. In addition, both expression and methylation of APOH were related to immune infiltrates, suggesting the importance of immune infiltrates in KIRC. Finally, the KEGG and GSEA analyses indicated a positive relationship of APOH with Complement and coagulation cascades pathways in KIRC.

Conclusions APOH was closely related to the prognosis of KIRC patients and might be involved in the Complement and coagulation cascades pathway, implying the involvement of APOH in tumorigenesis and metastasis of KIRC.

1. Introduction

Kidney cancer has become a commonly detected urinary tumor worldwide and accounts for approximately 2% of all cancer cases [1]. Larger than 80% of patients with kidney cancer are renal cell carcinomas, which is originated from renal tubular epithelial cells [2]. Of these, kidney renal clear cell carcinoma (KIRC) is thought as the primary subtype [3], and other histological subtypes are relatively rare. Due to the unapparent clinical performance and characteristics, 30% of KIRC patients present metastasis when they are diagnosed [4]. The metastasis of KIRC increases the difficulty of surgical treatment and causes a poor prognosis of patients [5]. Hence, timely detection and early diagnosis are extremely important for the treatment of KIRC. It is great urgent to elucidate the potential molecular mechanisms underlying the development of KIRC.

Currently, obesity, diabetes, and atherosclerosis are reported as the common carcinogenic factors for KIRC [6]. The KIRC has been considered a metabolic disease [7] with characteristics of robust lipid and glycogen accumulation [8]. It has been reported that apolipoproteins (APOs) play an important role in lipid metabolism and bind to lipids to form lipoproteins. Apolipoprotein H (APOH), namely beta-2-glycoproteins I, is located at chromosome 17q24.2 and is commonly synthesized by the hepatocytes [9]. Up to 35% of APOH is associated with lipoproteins metabolism and it likely exerts an important function in triglyceride-rich lipoprotein clearance by activating the lipoprotein lipase [10]. APOH has been

determined to correlate with hepatitis B virus (HBV) infection [11], systemic lupus erythematosus [12], and venous thrombosis [13]. Previous studies also suggested that APOH was related to the tumor development of non-small lung cancer [14], breast cancer [15], and acute myeloid leukaemia [16]. In terms of kidney-related disease, Norden et al. have identified the over-excretion of APOH in urinary patients with several renal tubular diseases [17]. Giorgia et al. found that APOH levels were considerably higher in the urine of patients with renal cancer history [18]. Additionally, Passam et al. reported the role of APOH in the angiogenesis modulation [19], which may partially disclose its relationship with the onset of renal clear cell carcinoma, a highly vascularized tumor. At present, the detailed function of APOH in KIRC was not described yet.

In this study, we conducted a series of bioinformatics analyses to reveal the potential role of APOH in KIRC. The expression of APOH in KIRC patients and its prognostic value were firstly explored. Then, the methylation of APOH and the effect on the survival of patients were also revealed. In addition, we assessed the correlation of AOPH and immune infiltrates in KIRC. Finally, we predicted the possible pathways associated with APOH involved in the KIRC progression.

2. Methods

2.1 The expression of APOH in KIRC

We firstly evaluated the protein and mRNA expressions of APOH in KIRC. The protein expression of APOH in normal kidney tissue and tumor tissue was evaluated in the Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>). We also obtained the TCGA-KIRC datasets from the UCSC Xena database (<https://xenabrowser.net/>), which contained the mRNA expression profile of APOH in KIRC and clinical data of KIRC patients. The differential expression of APOH mRNA in normal and KIRC samples was then assessed. Also, the expression difference of APOH mRNA in matched KIRC and adjacent noncancerous samples was compared. In addition, we used the TCGA-KIRC clinical data to explore the correlation between APOH mRNA expression and clinical characteristics of KIRC patients. The clinical characteristics of patients included age, gender, grade, clinical stage, T stage, N stage, M stage, person neoplasm status hemoglobin, platelet qualitative, serum calcium, and WBC. Moreover, the mRNA expression of APOH in other cancer types of the urinary system was also detected in Gene Set Cancer Analysis (GSCA) database (<http://bioinfo.life.hust.edu.cn/GSCA/#/>).

2.2 The effect of APOH expression on the survival of KIRC patient

Based on the TCGA-KIRC data, we evaluated the effect of APOH mRNA expression on the overall survival (OS), progression-free interval (PFI), disease-specific survival (DSS), and disease-free interval (DFI) of patients through Kaplan-Meier survival analysis. We assigned all the patients into low and high expression groups according to the median expression of APOH and compared the survival difference between the 2 groups. We also constructed the receiver operating characteristic (ROC) curve to reveal the prognostic performance of APOH on the survival probability in KIRC. The Cox regression analysis was then performed to predict the prognosis-related factors regarding the above clinical characteristics. Cox

regression results included hazard ratios (HR), 95%CI (confidence interval), and statistical significance. Furthermore, we constructed the nomogram model based on the Cox regression analysis by R rms package to reveal the prognostic contribution of these significant variables. In addition, the prognostic impact of APOH on the other cancer types of the urinary system was also determined in the GSCA database.

2.3 The methylation of APOH and prognostic impact in KIRC

The correlation between APOH mRNA expression and methylation was assessed in the GSCA database. Through DNA Methylation Interactive Visualization Database (DNMIVD) database (<http://119.3.41.228/dnmivd/index/>), we explored the difference of APOH methylation level between normal and KIRC tumor tissues in the promoter region. Further, we disclosed the APOH methylation level of different probes in the SurvivalMeth database (<http://bio-bigdata.hrbmu.edu.cn/survivalmeth/>). Also, the proportional hazards regression model (Cox Regression Model) was used to predict the important prognosis-related probes. Regarding the significant probes, its prognostic impact on the survival of KIRC patients was evaluated by the Kaplan-Meier method.

2.4 The immune infiltrates and APOH in KIRC

In the GSCA database, we analyzed the relationship between immune infiltrates and APOH expression, methylation, and mutation in KIRC. The potential correlation between APOH and immune infiltrates in KIRC was firstly explored. Furthermore, we explored the prognostic value of B cell, CD4_T cell, Macrophage, CD8_T cell, Neutrophil, Dendritic, and APOH in KIRC through Cox Proportional Hazard Model in Tumor IMMune Estimation Resource (TIMER) database (<https://cistrome.shinyapps.io/timer/>). Cox regression results included HR (95%CI) and statistical significance.

2.5 Pathway enrichment analysis

Firstly, we constructed the related gene network associated with APOH and predicted its potential function in the GeneMANIA database (<https://genemania.org/>). Then we used APOH co-expressed genes to explore the related pathways. From the cBioportal database (<http://www.cbioportal.org/>), we detected the co-expressed genes of AOPH in KIRC samples. According to the absolute spearman's correlation and $P\text{-value} < 0.05$, the top 100 co-expressed genes were selected for the potential pathway analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on 100 co-expressed genes was performed in WEB-based Gene SeT AnaLysis Toolkit (WebGestalt) database (<http://www.webgestalt.org/>) to reveal the significant pathways. Furthermore, the gene set enrichment analysis (GSEA) analysis was conducted to disclose the correlation between APOH and significant pathways in KIRC. The enrichment degree was assessed by normalized enrichment score (NES) and nominal P-value.

2.6 Statistical analysis

SPSS 23.0 was used to analyze the data in this study. The quantitative data among groups were compared using t-test or one-way ANOVA. The survival difference between the 2 groups was compared by

log-rank test. The correlation between quantitative variables was estimated by Pearson analysis. Prognosis-related factors were analyzed by the Cox regression model. The P-value less than 0.05 was regarded as the statistical significance.

3. Results

3.1 Expression and prognosis analyses on APOH

We firstly explored the potential value of APOH in human cancers of the urinary system. Expression analysis showed that APOH was just down-regulated in 3 tumor types of kidney cancer (**Fig.1A**). The abnormal expression of APOH was not observed in BLCA and PRAD. We also assessed its prognostic impact, and the result indicated that APOH only influenced the survival time of KIRC patients among 5 cancer types (**Fig.1B**). These findings initially highlighted the importance of APOH in KIRC.

We further investigated the role of APOH in KIRC. The immunohistochemical images showed that the staining of APOH protein in normal cells was high, while it was not detected in tumor cells (**Fig.2A**). In addition, it was found that the APOH protein was mainly located at cytoplasmic/membranous. Qualitative analysis on APOH mRNA expression showed that it was downregulated in KIRC tumor tissues compared with normal tissues (**Fig.2B**). The paired-sample analysis also confirmed the downregulation of APOH mRNA expression in KIRC tissues (**Fig.2C**).

Based on the clinical data of KIRC patients, we assessed the relationship between APOH mRNA expression and clinical characteristics of patients (**Fig.3**). The results showed that age, hemoglobin, platelet qualitative, and WBC did not influence the mRNA expression of APOH. However, higher expression of APOH mRNA was observed in male patients and these patients with grade 4, elevated serum calcium, person neoplasm status with tumor, stage IV, T3 stage, N1 stage, and M1 stage. It followed that APOH mRNA expression was related to several clinical phenotypes of KIRC patients.

Then, we evaluated the effect of APOH mRNA expression on the OS, DFI, PFI, and DSS of KIRC patients by Kaplan-Meier analysis and log-rank test (**Fig.4**). Survival analysis indicated that the DFI showed no difference between APOH high and low expression groups. But, APOH high expression significantly shortened the OS, PFI, and DSS time of KIRC patients compared with low expression.

Due to the vital role of APOH on the OS, PFI and DSS of KIRC patients, we then explored the prediction performance of APOH on these clinical outcomes in KIRC. The ROC analysis in **Fig.5** showed that APOH presented a better prognostic performance on OS, PFI, and DSS in KIRC, and the AUC was 0.598, 0.651, and 0.631, respectively.

Table.1 Cox regression analysis in KIRC based on TCGA clinical data				
	Univariate		Multivariate	
	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)
OS (overall survival)				
APOH expression	0.020	1.200 (1.029-1.400)	0.068	0.778 (0.595-1.018)
age	<0.001	1.031 (1.017-1.044)	0.020	1.028 (1.004-1.052)
hemoglobin	<0.001	0.484 (0.342-0.683)	0.333	0.778 (0.595-1.018)
grade	<0.001	2.722 (2.016-3.677)	0.712	1.085 (0.703-1.676)
stage	<0.001	1.906 (1.668-2.179)	<0.001	1.624 (1.239-2.129)
person neoplasm status	<0.001	4.916 (3.509-6.888)	0.001	2.594 (1.442-4.665)
platelet qualitative	0.004	1.823 (1.215-2.736)	0.751	0.921 (0.552-1.535)
serum calcium	0.003	1.584 (1.164-2.156)	0.591	1.125 (0.732-1.728)
white cell count	0.038	0.706 (0.508-0.981)	0.593	0.879 (0.547-1.411)
gender	0.774	1.047 (0.764-1.434)	0.641	0.893 (0.555-1.437)
PFI (progression-free interval)				
APOH expression	<0.001	1.210 (1.111-1.318)	0.040	0.784 (0.621-0.989)
age	0.481	1.005 (0.992-1.018)	0.482	1.008 (0.985-1.032)
hemoglobin	0.011	0.647 (0.463-0.904)	0.002	2.523 (1.403-4.537)
grade	<0.001	3.660 (2.660-5.037)	0.079	1.550 (0.950-2.527)
stage	<0.001	2.716 (2.324-3.174)	<0.001	2.043 (1.530-2.727)
person neoplasm status	<0.001	46.638 (25.745-84.487)	<0.001	44.957 (15.561-129.883)
platelet qualitative	0.001	1.955 (1.304-2.932)	0.688	1.130 (0.622-2.055)
serum calcium	0.008	1.547 (1.120-2.138)	0.024	0.597 (0.381-0.935)
white cell count	0.008	0.635 (0.454-0.888)	0.271	1.332 (0.799-2.220)
gender	0.020	0.662 (0.468-0.937)	0.055	0.594 (0.350-1.010)
DSS (disease-specific survival)				
APOH expression	0.005	1.180 (1.051-1.326)	0.082	0.784 (0.596-1.031)
age	0.210	1.010 (0.994-1.026)	0.208	0.684 (0.378-1.235)

hemoglobin	0.001	0.483 (0.315-0.741)	0.702	0.882 (0.464-1.676)
grade	<0.001	3.878 (2.662-5.650)	0.777	1.071 (0.658-1.752)
stage	<0.001	3.045 (2.473-3.750)	0.001	1.836 (1.297-2.598)
person neoplasm status	<0.001	275.338 (36.005-2105.572)	0.837	1.987e+5 (0-7.565e+55)
platelet qualitative	<0.001	2.802 (1.767-4.444)	0.130	1.643 (0.864-3.121)
serum calcium	0.003	1.763 (1.210-2.570)	0.274	0.756 (0.459-1.247)
white cell count	0.036	0.651 (0.436-0.973)	0.774	0.920 (0.519-1.629)
gender	0.251	0.785 (0.517-1.186)	0.208	0.684 (0.378-1.235)

Subsequently, we performed the Cox regression analysis to explore the prognosis-related factors in KIRC in terms of different clinical outcomes. The univariate analysis showed that APOH was one of the important influencing factors of OS, DFI, and DSS in KIRC (**Table.1**). But the independent prognostic value of APOH was just determined regarding the PFI of KIRC patients.

According to the multivariate Cox regression analysis, APOH only independently predicted the PFI in KIRC patients. Hence, we further enrolled the independent prognostic factors of PFI into the nomogram construction to reveal their contribution. The nomogram analysis (**Fig.6A**) showed that the person neoplasm status made the largest contribution to the survival probability of patients, with a single contribution of 100 points. We also constructed the calibration curve of nomogram predicted survival (**Fig.6B**), suggesting the better prediction performance of APOH on the survival probability of KIRC patients.

3.2 Methylation and prognosis analyses on APOH

Moreover, the Pearson correlation analysis showed a strong negative relationship between APOH mRNA expression and methylation level (**Fig.7A**), suggesting the importance of APOH methylation on its mRNA expression. According to the analysis, we found that the methylation level of APOH in tumor tissue was significantly lower than that in normal tissues (**Fig.7B**). Further investigations showed that the methylation difference of APOH between normal and tumor tissues was related to the methylation probe. Among 6 probes, the methylation level of cg19058765 was higher in the tumor than in the normal group (**Fig.7C**).

Table.2 The Cox regression model analysis on methylation level of APOH					
Probe ID	Coef	HR	Lower (95%)	Upper (95%)	P-value
cg04006839	0.078	1.081	2.44E-01	4.804	0.917
cg09732045	0.568	1.765	2.64E-02	117.908	0.790
cg14334014	-8.78503	0.000153	1.36E-08	1.724	0.064
cg16481618	0.48827	1.62949	2.50E-01	10.639	0.610
cg17095279	-3.1386	0.043343	5.18E-03	0.362	0.003
cg19058765	4.23954	69.375664	1.01E+00	4788.591	0.049

Then the Cox regression model analysis was performed to predict prognosis-related methylation probes of APOH. As shown in **Table.2**, the cg17095279 and cg19058765 showed the correlation with the patient's prognosis among 6 probes. We also performed the Kaplan-Meier analysis on cg17095279 and cg19058765 to explore their prognostic impacts. The survival analysis indicated that the methylation level of these 2 probes did not affect the patient's OS, DFI, and PFI (**Fig.8**).

3.3 Correlation analysis between APOH and immune infiltrates in KIRC

We also explored the relationship between APOH and immune infiltrates in KIRC. As shown in **Fig.9**, APOH expression was strongly related to several immune cells such as CD4_cell, CD_8 cell, and monocyte. In addition, it was observed that the methylation of APOH was also related to immune infiltrates such as B_cell, CD_4 cell, neutrophils, and CD8_cell. However, no correlation between APOH mutation and immune infiltrates was observed in KIRC.

Table.3 Cox regression analysis on immune infiltrates and APOH in KIRC				
	Univariate		Multivariate	
	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)
B cell	0.961	1.408 (0.155-7.091)	0.553	0.368 (0.014-10.000)
CD4_T cell	0.715	1.391 (0.237-8.177)	0.749	0.644 (0.043-9.569)
Macrophage	0.291	0.412 (0.080-2.136)	0.015	0.053 (0.005-0.560)
CD8_T cell	0.259	0.581 (0.227-1.491)	0.042	0.196 (0.041-0.940)
Neutrophil	0.575	1.902 (0.201-17.96)	0.144	22.268 (0.347, 1427.911)
Dendritic	0.788	1.095 (0.567-2.113)	0.191	3.333 (0.548-20.279)
APOH	0.050	1.097 (1.000-1.203)	0.022	1.121 (1.017-1.237)

The Cox regression analysis was also performed to predict prognosis-related factors (**Table.3**). In the univariate Cox regression, we found no prognostic impacts of all immune infiltrates and APOH. However, when adding these variables into multivariate Cox analysis, the macrophage, CD8_T cell, and APOH showed the independent prognostic value. According to the results, the potential correlation between APOH and several immune infiltrates needed further assessment.

3.4 Pathway enrichment analysis

We used GeneMANIA to predict the related genes that may have the same function and interaction with APOH and predicted the potential functions of APOH. The function prediction (**Fig.10**) indicated that APOH was closely related to hemostasis, coagulation, complement activation, and negative regulation of blood coagulation, referring to the regulation of blood coagulation.

In order to reveal the potential function of APOH, we selected the top 100 co-expressed genes of APOH in cBioportal for pathway enrichment analysis. The KEGG analysis (**Fig.11A**) showed that these co-expressed genes were mainly enriched in Complement and coagulation cascades, and *Staphylococcus aureus* infection pathways. In addition, the Folate biosynthesis, Cholesterol metabolism, and Pathogenic *Escherichia coli* infection pathways were also predicted. GSEA analysis was then used to explore the potential correlation between APOH and significant pathways in KIRC, and the results presented that APOH showed a significant positive correlation with Complement and coagulation cascades, and *Staphylococcus aureus* infection pathways (**Fig.11B**). We speculated that APOH largely participated in Complement and coagulation cascades involved in KIRC.

4. Discussion

The APOH is an important member of the apolipoproteins (APOs) family. Crook et al. indicated a significant relationship between APOH and fasting glucose, lipids, and lipoprotein levels in healthy samples [20]. Previous studies have reported the involvement of APOH in metabolism-related disease. Antoni et al. showed that both APOH mRNA and protein in the liver of patients with diabetes and metabolic syndrome (MS) were significantly higher than those patients with non-diabetic and non-MS [21]. Oka et al. also determined the regulation of APOH on diet-induced obesity in zebrafish and mammals [22]. Sandra et al. identified the APOH as an obesity-resistance gene and relevant to healthy thinness [23]. The function of APOH has been paid more and more attention.

Growing evidence also demonstrated the involvement of APOH in human cancers. Chung et al. revealed APOH as a novel serum protein biomarker that distinguished breast cancer and healthy subjects with high sensitivity and specificity [15]. Ma et al. defined APOH as an oncofetal biomarker in colorectal cancer [24]. In addition, APOH was reported as a hub gene associated with liver metastasis in colorectal cancer [25]. In this study, we found that APOH was down-regulated in 3 tumor types of kidney cancer, and expression of APOH was just related to the prognosis of KIRC patients, which suggested the clinical significance of APOH in KIRC. We also predicted the independent prognostic value of APOH in KIRC. Our study initially showed that APOH was involved in the progression of KIRC. APOH was also determined as

an immune-related gene to predict the prognosis of patients with gastric cancer [26] and lung squamous cell carcinoma with early stage [27]. In this study, the importance of immune infiltrates on APOH expression in KIRC was highlighted, as a significant correlation between them was observed. Mandili et al. suggested the APOH as a urinary marker of renal cancer in von Hippel-Lindau patients [18]. These researches showed that APOH was closely related to the progression of human cancers.

Pathway analysis in this study indicated that APOH was positively associated with Complement and coagulation cascades in KIRC. The pathway has been recognized to play an important role in the host immune response and hemostasis by elimination of pathogens and prevention of life-threatening bleeding, but the cross-talk of the complement pathway and coagulation cascades might be involved in many other important biological behaviors [28]. Complement is generally regarded as a protective mechanism against the formation of tumors, but complement activation in the tumor microenvironment can promote malignant biological behavior such as tumor growth enhancement and metastasis increase. [29]. The dual role of complement in destroying and enhancing cancer has been reviewed by a previous study [30]. The coagulation cascades may exert a vital role in tumorigenesis and development through the promotion of blood clotting and alteration of gene expression affecting key intracellular molecular events [31]. The function analysis on APOH in GeneMINIA showed that APOH participated in complement activation and blood coagulation, thus we speculated that abnormally high expression of APOH may play a vital role in KIRC tumorigenesis and metastasis. A detailed mechanism should be further conducted to verify these findings.

5. Conclusion

This study revealed the down-regulation of APOH in KIRC both in mRNA and protein levels compared with the normal group. The expression of APOH affected the clinical outcome of KIRC patients in terms of OS, PFI, and DSS. Further, APOH expression independently predicted the patient's PFI and showed better prediction performance. APOH was determined as an important prognostic biomarker in KIRC. In addition, APOH expression showed a negative correlation with its methylation level, but methylation of APOH did not influence the prognosis of patients. We also found a strong relationship between immune infiltrates and APOH expression and methylation. Through enrichment analysis, we found that APOH expression showed a significant positive correlation with the Complement and coagulation cascades pathway, which implied that APOH might be involved in the progression and metastasis of KIRC.

Declarations

Availability of data and materials: The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests: The authors have no conflicts of interest to declare.

Author contributions:

- (I) Conception and design: Peng Wang and Guan-an Zhao
- (II) Collection and assembly of data: Peng Wang
- (III) Data analysis and interpretation: Peng Wang and Guan-an Zhao
- (IV) Manuscript writing: All authors
- (V) Final approval of manuscript: All authors

Consent for publication: Not applicable.

Ethics approval and consent to participate: Not applicable.

Funding: No funding was received for conducting this study.

Acknowledgement: Not applicable.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. 2018**Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.***CA Cancer J Clin*, **68**:394-424. doi:10.3322/caac.21492.
2. Bobulescu IA, Pop LM, Mani C, Turner K, Rivera C, et al. 2021**Renal Lipid Metabolism Abnormalities in Obesity and Clear Cell Renal Cell Carcinoma.***Metabolites*, **11**. doi:10.3390/metabo11090608.
3. Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, et al. 2020**Epidemiology of Renal Cell Carcinoma.***World J Oncol*, **11**:79-87. doi:10.14740/wjon1279.
4. Bhatt JR, Finelli A. 2014**Landmarks in the diagnosis and treatment of renal cell carcinoma.***Nat Rev Urol*, **11**:517-525. doi:10.1038/nrurol.2014.194.
5. Campbell S, Uzzo RG, Allaf ME, Bass EB, Cadeddu JA, et al. 2017**Renal Mass and Localized Renal Cancer: AUA Guideline.***J Urol*, **198**:520-529. doi:10.1016/j.juro.2017.04.100.
6. Psutka SP, Stewart SB, Boorjian SA, Lohse CM, Tollefson MK, et al. 2014**Diabetes mellitus is independently associated with an increased risk of mortality in patients with clear cell renal cell carcinoma.***J Urol*, **192**:1620-1627. doi:10.1016/j.juro.2014.06.014.
7. Sudarshan S, Karam JA, Brugarolas J, Thompson RH, Uzzo R, et al. 2013**Metabolism of kidney cancer: from the lab to clinical practice.***Eur Urol*, **63**:244-251. doi:10.1016/j.eururo.2012.09.054.
8. Du W, Zhang L, Brett-Morris A, Aguila B, Kerner J, et al. 2017**HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism.***Nat Commun*, **8**:1769. doi:10.1038/s41467-017-01965-8.
9. Day JR, O'Hara PJ, Grant FJ, Lofton-Day C, Berkaw MN, et al. 1992**Molecular cloning and sequence analysis of the cDNA encoding human apolipoprotein H (beta 2-glycoprotein I).***Int J Clin Lab Res*,

- 21:256-263. doi:10.1007/BF02591656.
10. Takada D, Ezura Y, Ono S, Iino Y, Katayama Y, et al. 2003 **Apolipoprotein H variant modifies plasma triglyceride phenotype in familial hypercholesterolemia: a molecular study in an eight-generation hyperlipidemic family.** *J Atheroscler Thromb*, **10**:79-84. doi:10.5551/jat.10.79.
 11. de Groot PG, Meijers JC. 2011 **beta(2) -Glycoprotein I: evolution, structure and function.** *J Thromb Haemost*, **9**:1275-1284. doi:10.1111/j.1538-7836.2011.04327.x.
 12. Lee JS, Lee EJ, Yeom J, Oh JS, Hong S, et al. 2021 **Urine beta-2-glycoprotein 1 as a biomarker for diagnosis of systemic lupus erythematosus.** *Lupus*, **30**:1306-1313. doi:10.1177/09612033211014268.
 13. Tang L, Zeng W, Lu X, Wang QY, Liu H, et al. 2014 **Identification of APOH polymorphisms as common genetic risk factors for venous thrombosis in the Chinese population.** *J Thromb Haemost*, **12**:1616-1625. doi:10.1111/jth.12679.
 14. Pietrowska M, Jelonek K, Michalak M, Ros M, Rodziewicz P, et al. 2014 **Identification of serum proteome components associated with progression of non-small cell lung cancer.** *Acta Biochim Pol*, **61**:325-331.
 15. Chung L, Moore K, Phillips L, Boyle FM, Marsh DJ, et al. 2014 **Novel serum protein biomarker panel revealed by mass spectrometry and its prognostic value in breast cancer.** *Breast Cancer Res*, **16**:R63. doi:10.1186/bcr3676.
 16. Lee SW, Kim IJ, Jeong BY, Choi MH, Kim JY, et al. 2012 **Use of MDLC-DIGE and LC-MS/MS to identify serum biomarkers for complete remission in patients with acute myeloid leukemia.** *Electrophoresis*, **33**:1863-1872. doi:10.1002/elps.201200047.
 17. Norden AG, Fulcher LM, Lapsley M, Flynn FV. 1991 **Excretion of beta 2-glycoprotein I (apolipoprotein H) in renal tubular disease.** *Clin Chem*, **37**:74-77.
 18. Mandili G, Notarpietro A, Khadjavi A, Allasia M, Battaglia A, et al. 2018 **Beta-2-glycoprotein-1 and alpha-1-antitrypsin as urinary markers of renal cancer in von Hippel-Lindau patients.** *Biomarkers*, **23**:123-130. doi:10.1080/1354750X.2016.1269132.
 19. Passam FH, Qi JC, Tanaka K, Matthaei KI, Krilis SA. 2010 **In vivo modulation of angiogenesis by beta 2 glycoprotein I.** *J Autoimmun*, **35**:232-240. doi:10.1016/j.jaut.2010.06.013.
 20. Crook M, Chng SI, Lumb P, Reid F. 2001 **Serum apolipoprotein H and its relationship to blood pressure, serum lipids, fasting plasma glucose and insulin in normal individuals.** *Ann Clin Biochem*, **38**:494-498. doi:10.1177/000456320103800506.
 21. Castro A, Lazaro I, Selva DM, Cespedes E, Girona J, et al. 2010 **APOH is increased in the plasma and liver of type 2 diabetic patients with metabolic syndrome.** *Atherosclerosis*, **209**:201-205. doi:10.1016/j.atherosclerosis.2009.09.072.
 22. Oka T, Nishimura Y, Zang L, Hirano M, Shimada Y, et al. 2010 **Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity.** *BMC Physiol*, **10**:21. doi:10.1186/1472-6793-10-21.

23. Hasstedt SJ, Coon H, Xin Y, Adams TD, Hunt SC. 2016 **APOH interacts with FTO to predispose to healthy thinness.** *Hum Genet*, **135**:201-207. doi:10.1007/s00439-015-1629-3.
24. Ma Y, Zhang P, Wang F, Liu W, Yang J, et al. 2012 **An integrated proteomics and metabolomics approach for defining oncofetal biomarkers in the colorectal cancer.** *Ann Surg*, **255**:720-730. doi:10.1097/SLA.0b013e31824a9a8b.
25. Zhang T, Guo J, Gu J, Wang Z, Wang G, et al. 2019 **Identifying the key genes and microRNAs in colorectal cancer liver metastasis by bioinformatics analysis and in vitro experiments.** *Oncol Rep*, **41**:279-291. doi:10.3892/or.2018.6840.
26. Qiu XT, Song YC, Liu J, Wang ZM, Niu X, et al. 2020 **Identification of an immune-related gene-based signature to predict prognosis of patients with gastric cancer.** *World J Gastrointest Oncol*, **12**:857-876. doi:10.4251/wjgo.v12.i8.857.
27. Fan T, Lu Z, Liu Y, Wang L, Tian H, et al. 2021 **A Novel Immune-Related Seventeen-Gene Signature for Predicting Early Stage Lung Squamous Cell Carcinoma Prognosis.** *Front Immunol*, **12**:665407. doi:10.3389/fimmu.2021.665407.
28. Markiewski MM, Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. 2007 **Complement and coagulation: strangers or partners in crime?** *Trends Immunol*, **28**:184-192. doi:10.1016/j.it.2007.02.006.
29. Afshar-Kharghan V. 2017 **The role of the complement system in cancer.** *J Clin Invest*, **127**:780-789. doi:10.1172/JCI90962.
30. Mamidi S, Hone S, Kirschfink M. 2017 **The complement system in cancer: Ambivalence between tumour destruction and promotion.** *Immunobiology*, **222**:45-54. doi:10.1016/j.imbio.2015.11.008.
31. Wang X, Wang E, Kavanagh JJ, Freedman RS. 2005 **Ovarian cancer, the coagulation pathway, and inflammation.** *J Transl Med*, **3**:25. doi:10.1186/1479-5876-3-25.

Figures

Figure 1

Related analysis on APOH in human cancers of the urinary system. (A) Differential expression of APOH mRNA between normal and tumor tissues. (B) Prognostic impact of APOH in terms of various clinical outcomes.

Figure 2

The expression of APOH in KIRC. The expression of (A) APOH protein and (B) mRNA in normal and KIRC tumor tissues. (C) mRNA expression levels of APOH in matched KIRC and adjacent noncancerous samples.

Figure 3

The correlation analysis between APOH mRNA expression and clinical characteristics of KIRC patients.

Figure 4

The effects of APOH mRNA expression on the survival of KIRC patients. OS, overall survival; PFI, progression-free interval; DSS, disease-specific survival; DFI, disease-free interval.

Figure 5

The prognostic performance of APOH in KIRC. OS, overall survival; PFI, progression-free interval; DSS, disease-specific survival. AUC, area under curve.

Figure 6

The prognostic performance analysis in terms of PFI. (A) Nomogram construction based on the Cox regression model. (B) Calibration curve of nomogram predicted survival. PFI, progression-free interval.

Figure 7

The methylation analysis on APOH in KIRC. (A) The correlation between APOH mRNA expression and methylation level. (B) The differential methylation level of APOH between normal and KIRC tissues. (C) The methylation level of different probes.

Figure 8

The prognostic effect of APOH methylation level in KIRC based on important probes.

Figure 9

The correlation analysis between APOH and immune infiltrates in KIRC.

Figure 10

The APOH related signal network and function prediction.

Figure 11

The pathways analysis associated with APOH using (A) KEGG and (B) GSEA analysis.