Adenylate kinase 7 is a prognostic indicator of overall survival in ovarian cancer

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Abstract

Ovarian cancer (OC), a common malignant heterogeneous gynecological tumor, is the primary cause of cancer-related death in women worldwide. Adenylate kinase (AK) 7 belongs to the adenylate kinase (AK) family and is a cytosolic isoform of AK. Recent studies have demonstrated that AK7 is expressed in several human diseases, including cancer. However, there is a scarcity of reports on the relationship between AK7 and OC. Here, we compared the expression of AK7 in normal and cancerous ovarian tissues from The Cancer Genome Atlas database and used the c^2 test to assess the correlation between AK7 levels and the clinical symptoms of OC. Finally, the prognostic significance of AK7 in OC was determined using the Kaplan–Meier analyses and Cox regression and performed gene set enrichment analysis to detect any relevant signaling pathways. We found that AK7 levels were substantially downregulated in OC than that in normal ovarian tissues ($P < .001$). Low AK7 levels were related to the patients' age ($P = .0093$) in OC. The median overall survival (OS) of patients with low AK7-expressing OC was shorter than patients with high AK7-expressing OC ($P = .019$). The Cox regression analysis (multivariate) identified low AK7 levels were independently related to the prognosis of OC (HR 1.34; $P = .048$). Our study demonstrated that the downregulated levels of AK7 could serve as an independent prognostic indicator for the OS in OC. Additionally, gene set enrichment analysis revealed that EMT, apical junction, TGF-b signaling, UV response, and myogenesis were associated in the low AK7 expression phenotype (NOM $P < .05$).

Abbreviations: AK = adenylate kinase, AK7 = adenylate kinase 7, EOC = epithelial ovarian cancer, OC = ovarian cancer, PCD = primary ciliary dyskinesia, TCGA = the Cancer Genome Atlas.

Keywords: adenylate kinase 7, ovarian cancer, prognosis, the Cancer Genome Atlas

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1. Introduction

Ovarian cancer (OC) is 1 of the 3 most common gynecological tumors in women. It is the fifth most common cause of cancer death in women worldwide.[1] The prodromal manifestations of OC are always nonspecific, which makes it difficult to distinguish from other carcinomatosis.[2] Additionally, the current screening strategies for the diagnosis of early-stage OC, including transvaginal ultrasound, computed tomography, detection of tumor marker CA125, and detection of BRCA gene mutation, are apparently ineffective in reducing the mortality rate of OC.[2,3] Consequently, early stage diagnosis of OC is uncommon leading to a poor prognosis with a 5-year OS rate of $< 30\%$.[4,5] Thus, there is an urgent requirement to detect a biomarker for tumor prognosis.

The mechanism of pathogenesis of OC is complex. Recently, a study found that the ciliated epithelial cells in the fallopian tube underwent periodic proliferation and differentiation during the menstrual cycle, which could be 1 of the mechanisms involved in the pathogenesis of OC.[6] In recent years, researchers have become interested in the role of cilia in various human diseases, including tumorigenesis. It has been shown that primary cilia are involved in cell-cycle regulation and further implicated in tumor progression.[7,8] Adenylate kinase 7 (AK7), a cytosolic isoform of adenylate kinase (AK) isoenzymes located on chromosome 14q32, is known to have a tissue-restricted expression pattern and is expressed in cilia-rich tissues in the epithelium.[9]

However, there is no direct evidence showing an association between AK7 and tumor progression and prognosis, possibly via the regulation of cilia function. In this study, we retrospectively analyzed the data from The Cancer Genome Atlas (TCGA)

cohort and assessed the correlation between AK7 levels and clinicopathological symptoms of OC to evaluate the prognostic value (PV) of AK7 in OC. We also performed GSEA to explore relevant signaling pathways.

2. Materials and methods

2.1. Data extraction from the TCGA database

We extracted cancerous ovarian tissues (n = 308; OC group) and normal ovarian tissues (n = 88; CON group) from the TCGA database to determine AK7 levels and the PV by RNAseq (Illumina HiSeq). The high and low AK7 expression groups were classified based on the median value of AK7.

2.2. Statistical analysis

Statistical analysis was performed using R software (version 3.5.2). The c2 test assessed the correlation between AK7 levels and the clinical symptoms of OC. Kaplan–Meier curve was used to compare the OS between the AK7 expression groups. The independent PV of AK7 in OC was determined via Cox regression analyses. A value of $P < .05$ implied statistical significance.

GSEA. In GSEA, target genes are ranked according to predetermined gene sets based on the differential expression between the 2 sample groups, followed by the assessment of the position of the predetermined gene sets in the sorting table.[10] Here, we used GSEA 3.0 for patient data analysis. Permutation analysis was done to obtain normalized enrichment score (NES).

2.3. Ethics approval

This study did not require ethics approval since all clinical data were from public databases.

3. Results

3.1. Patient characteristics

Table 1 shows the demographic, clinical symptoms, and gene expression data of patients in the OC group.

3.2. AK7 expression and association with clinicopathological variables

We found a substantially downregulated AK7 levels in the OC group than the CON group ($P < .05$). Furthermore, there was a marked difference in AK7 levels based on patient age (Fig. 1). Patients with OC were classified into high and low AK7 expression groups. Table 2 describes their clinicopathological parameters and OS. We found that low AK7 levels were correlated with patient age ($P = .0093$).

3.3. Low AK7 expression as an independent prognostic factor for poor OS

Low AK7 levels were related to poor OS ($P = .019$; Fig. 2), especially in those with late-stage OC ($P = .014$) but not early-stage OC ($P = .62$); G3/G4 grade ($P = .011$) but not G1/G2 grade ($P = .97$); old age ($P = .018$) but not young age ($P = .83$; Fig. 2). The results of the univariate analysis showed that patient age and AK7 levels were related to poor OS (Table 3). Further multivariate analysis estimated the independent PV of low

Table 1

Demographic and clinical characteristics of TCGA cohort.

Characteristics	Numbers of cases
Age	
<55	113 (36.69)
>=55	195 (63.31)
Subdivision	
NA	17 (5.52)
Bilateral	212 (68.83)
Left	37 (12.01)
Right	42 (13.64)
Stage	
NA	2 (0.65)
I	1 (0.32)
II	22 (7.14)
III	245 (79.55)
IV	38 (12.34)
Longest dimension	
Large	124 (46.1)
Small	145 (53.9)
Lymphatic invasion	
NA	180 (58.44)
NO	44 (14.29)
YES	84 (27.27)
Histologic grade	
NA	2 (0.65)
G1	1 (0.32)
G2	37 (12.01)
G3	261 (84.74)
G4	1 (0.32)
GB	2 (0.65)
GX	4 (1.3)
New type	
NA	145 (47.08)
Locoregional	4 (1.3)
Metastatic	1 (0.32)
Progression	12 (3.9)
Recurrence	146 (47.4)
Sample type	
Primary Tumor	303 (98.38)
Recurrent Tumor	5 (1.62)
Vital status	
Deceased	184 (59.74)
Living	124 (40.26)
AK7	
High	154 (50)
Low	154 (50)

NA = not available, AK7 = Adenylate kinase, TCGA = the Cancer Genome Atlas.

AK7 levels for poor OS of OC (HR: 1.34, 95% CI: 1-1.8, $P = .048$; Table 3).

3.4. AK7-related signaling pathway

The results of GSEA showed a marked difference in the enrichment of MSigDB Collection (NOM $P < .05$; Table 4). The essential signaling pathways, including EMT, apical junction, TGF-b signaling, UV response, and myogenesis, were chosen based on NES. These signaling pathways were all enriched in low AK7 expression phenotype (Table 4 and Fig. 3).

4. Discussion

Several complex factors interact to influence the pathogenesis and progression of OC, including genetic factors, reproductive
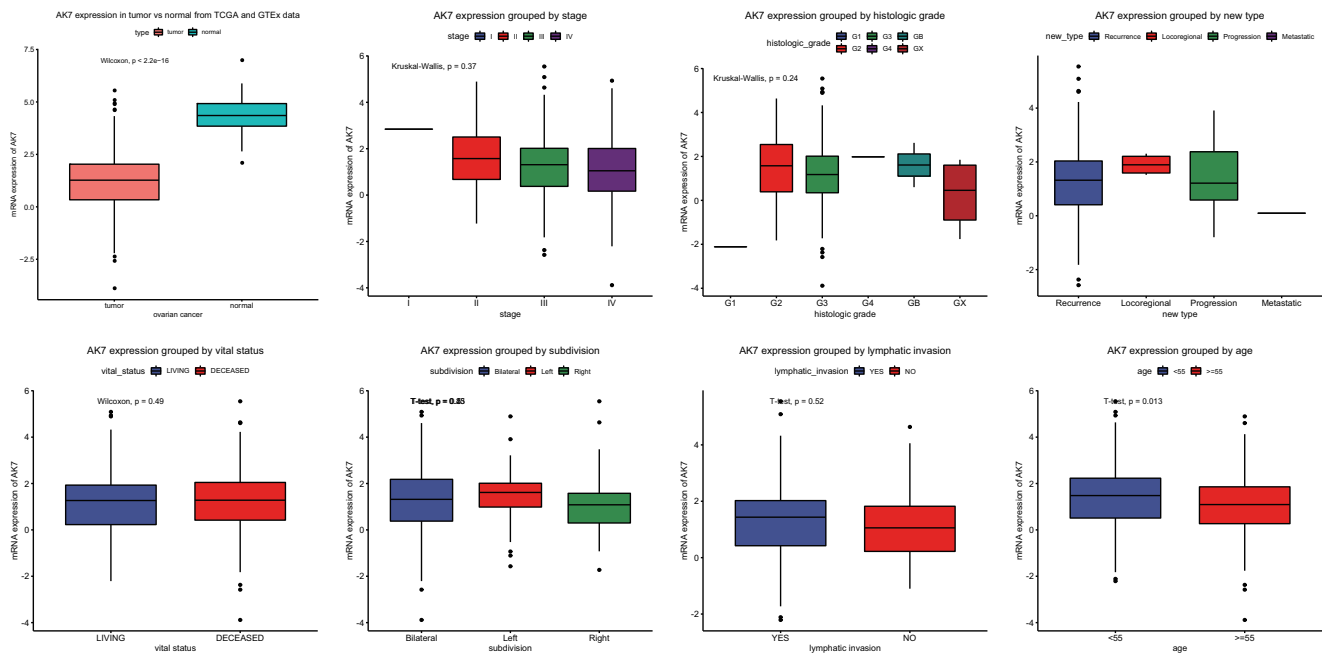
Figure 1. AK7 expression in OC. Boxplots show the difference in AK7 expression grouped by stage, histological grade, new type, vital status, subdivision, lymphatic invasion, and patient age. AK7 = adenylate kinase 7.

factors, environmental factors, and so on.[11] Among these pathogenic factors, cilia were found to be involved in ovarian tumorigenesis. Cilia are sensory and motor organelles extending from the cellular surface and have long been considered as a

degraded organelle.[12] Accumulating evidence has shown that primary cilia structure dysfunction may cause a series of multisystemic developmental disorders known as ciliopathies and multifactorial human diseases, including cancer.[13,14] It has

Table 2

Association of AK7 mRNA expression in ovarian cancer tissues with clinicopathologic variables.

Parameter	Variable	N	AK7 mRNA expression				$\chi 2$	P value
			High	%	Low	%		
Age	<55	113	68	(44.16)	45	(29.22)	6.7652	.0093
	>=55	195	86	(55.84)	109	(70.78)		
Subdivision	Bilateral	212	111	(74.5)	101	(71.13)	4.0109	.1346
	Left	37	22	(14.77)	15	(10.56)		
	Right	42	16	(10.74)	26	(18.31)		
Stage	I	1	1	(0.65)	0	(0)	2.2183	.5284
	II	22	12	(7.79)	10	(6.58)		
	III	245	125	(81.17)	120	(78.95)		
	IV	38	16	(10.39)	22	(14.47)		
Longest dimension	Large	124	60	(43.8)	64	(48.48)	0.4212	.5164
	Small	145	77	(56.2)	68	(51.52)		
Lymphatic invasion	No	44	18	(27.27)	26	(41.94)	2.4315	.1189
	Yes	84	48	(72.73)	36	(58.06)		
Histologic grade	G1	1	0	(0)	1	(0.66)	5.5678	.3506
	G2	37	24	(15.58)	13	(8.55)		
	G3	261	126	(81.82)	135	(88.82)		
	G4	1	1	(0.65)	0	(0)		
	GB	2	1	(0.65)	1	(0.66)		
	GX	4	2	(1.3)	2	(1.32)		
New type	Locoregional	4	4	(4.82)	0	(0)	5.3073	.1506
	Metastatic	1	0	(0)	1	(1.25)		
	Progression	12	5	(6.02)	7	(8.75)		
	Recurrence	146	74	(89.16)	72	(90)		
Sample type	Primary Tumor	303	151	(98.05)	152	(98.7)	0	1
	Recurrent Tumor	5	3	(1.95)	2	(1.3)		
Vital status	Deceased	184	92	(59.74)	92	(59.74)	0	1
	Living	124	62	(40.26)	62	(40.26)		

AK7 = Adenylate kinase 7, N = number.
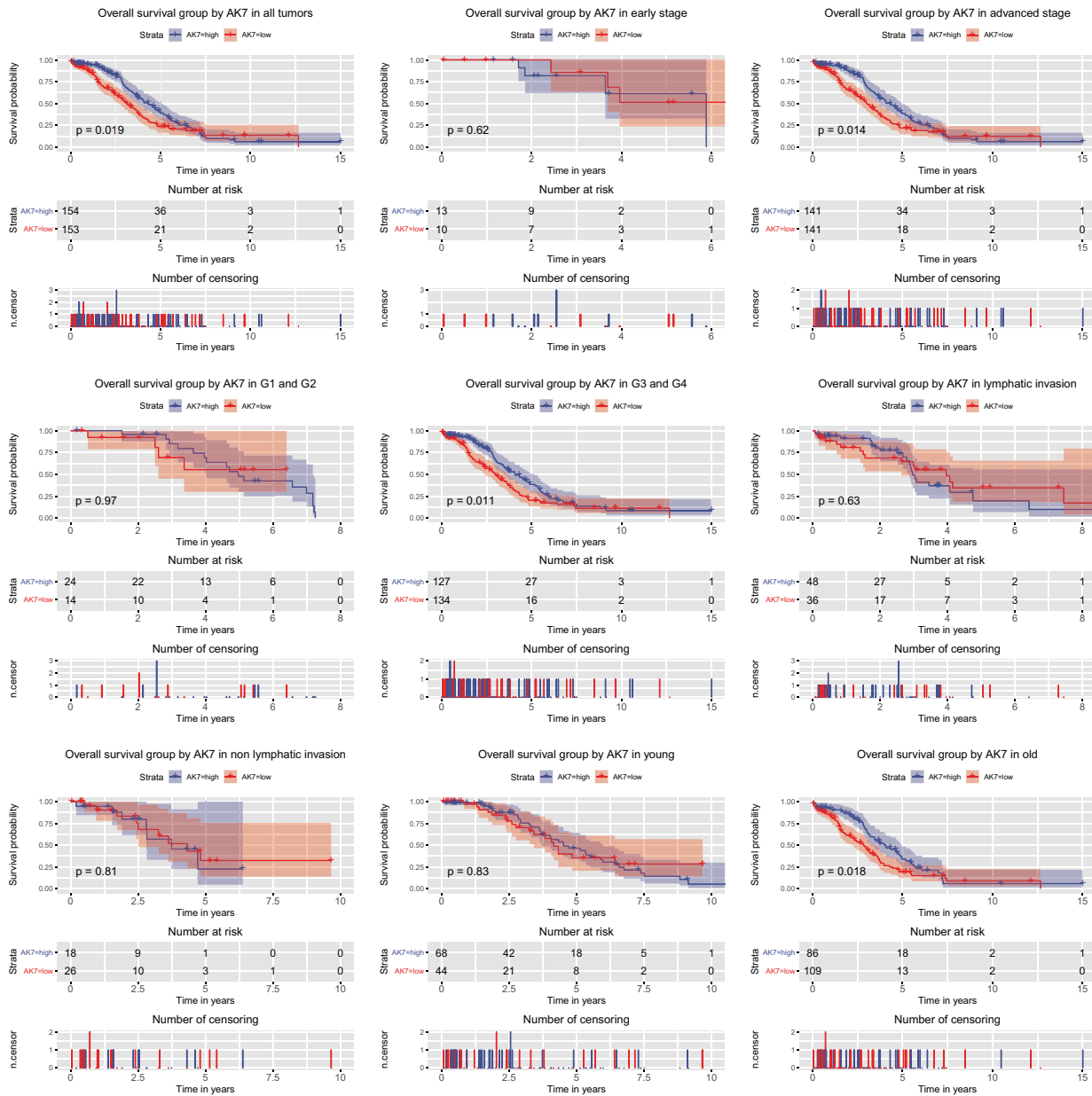
Figure 2. The PV of AK7 in patients with OC. Kaplan-Meier curves for the survival of patients with OC based on AK7 expression in cancerous ovarian tissues. AK7 = adenylate kinase 7.

Table 3

Univariate and multivariate analyses of overall survival in patients with ovarian cancer.

Parameters	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI 95	P value	Hazard Ratio	CI 95	P value
Age	1.63	1.19–2.24	.003	1.57	1.14–2.16	.005
Subdivision	0.84	0.67–1.04	.101			
Stage	1.09	0.8–1.5	.581			
Longest dimension	1.12	0.82–1.52	.485			
Lymphatic invasion	1.02	0.85–1.23	.798			
Histologic grade	1.12	0.88–1.42	.349			
New type	0.99	0.63–1.55	.951			
Sample type	0.43	0.11–1.73	.235			
AK7	1.41	1.06–1.89	.02	1.34	1–1.8	.048

AK7 = adenylate kinase 7.

Table 4

GSEA enrichment plot in low ABCB9 phenotype.

Gene set	ES	NES	NOM *P*-value
HALLMARK_epithelial mesenchymal transition	0.64390194	1.8245728	.019880716
HALLMARK_UV response	0.41891807	1.5772356	.029940119
HALLMARK_TGF-beta signaling	0.48144048	1.5725749	.03952569
HALLMARK_myogenesis	0.43896723	1.5351363	.043052837
HALLMARK_apical junction	0.3967964	1.5181613	.042

ES = enrichment score, NES = normalized enrichment score, NOM = nominal.

been shown that primary cilia have a dual role in regulating tumorigenesis, whereas the loss of primary cilia and abnormally activated cilia regulation of hedgehog signaling pathway has been associated with the progression and prognosis of various tumors, including pancreas, breast, prostate, ovarian cancer, and so on.[15–17] Shpak performed bioinformatics analysis to study the gene expression patterns of cilia in OC and identified 354 cilia genes abnormally expressed in OC tissues, indicating an important role of ciliary disruption in the development of OC.[14] However, there is still a lack of in vivo experimental data to verify this hypothesis.

The maintenance of proper cilia structure and function requires ATP hydrolysis, which is done by the AK family.[9] Based on recent studies, AK7, a cytosolic human AK isoform, was found to be associated with ciliary homeostasis. The mutation of AK7 in primary ciliary dyskinesia (PCD) was found in both humans and murine species.[18,19] However, there is still a lack of research on the association between AK7 and human cancer. It has been speculated that ciliary dysfunction mediates the effects of AK7 on the genesis, progress, and prognosis of different types of cancer, including OC.

Here, we detected a substantially downregulated expression of AK7 in cancerous ovarian tissues than with normal ovarian tissues, which was also associated with patient age based on the analysis of high and low AK7 groups. Milara identified a downregulated AK7 expression at both RNA and protein levels and found that it was associated with PCD.[18] Consistently, Angeles observed the phenotypes of PCD in AK7-deficient mice.[19] These findings are consistent with our hypothesis that lower AK7 expression may cause ciliary structure disorder or dysfunction, further affecting the progression and prognosis of OC.
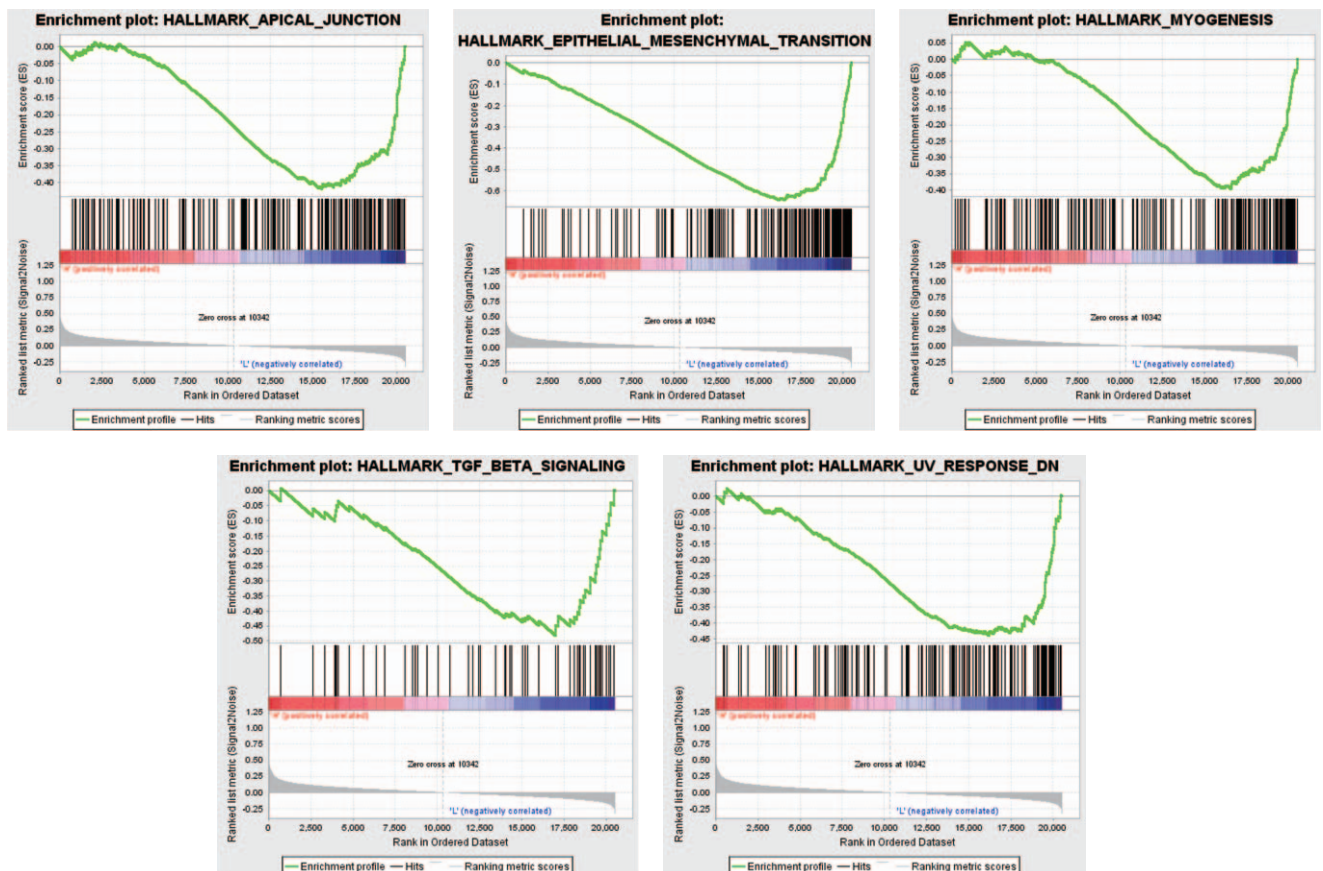

Figure 3. Enrichment plots from gene set enrichment analysis.

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Also, patients who expressed lower levels of AK7 in OC tissues were more prone to have a poorer prognosis, particularly those with late-stage disease, G3/G4 grade, and old age. Also, late-stage diagnosis is related to a poor prognosis. Thus, AK7 might act as a novel indicator of OC prognosis. Moreover, the onset age of epithelial ovarian cancer, the most common and malignant type of OC, was 62 years and older patients with lower AK7 levels had a worse prognosis, suggesting a valuable role of AK7 in the diagnosis of epithelial ovarian cancer.[2] Cox regression analysis revealed that low AK7 levels had a significant PV in patients with OC.

Our investigations are focused on the identification of novel biomarkers of cancers to track their onset and development. This is the first study to detect a relationship between AK7 levels and OS in patients with OC. The results of our study have shown that downregulated AK7 levels are related to poor OS in OC and has an independent PV in OC. These results provide a new insight that AK7 plays a valuable role in the prognosis of OC, which might be mediated by ciliary structure disorder and function and lays a foundation for further investigation.

Author contributions

Conceptualization: XZ, LZ.
Data curation: LZ, YL.
Formal analysis: XZ, YJ.
Funding acquisition: LZ.
Investigation: LZ, XZ, YJ, ZY.
Methodology: YL, YJ.
Project administration: XZ, YL.
Resources: YL, ZY, YG, YZ.
Software: YJ.
Supervision: YZ.
Validation: YZ, YG.
Visualization: ZY, YG.
Writing – original draft: XZ.
Writing – review & editing: LZ.

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