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Research Article

The Influence of Alpha Kinase 2 Expression on Prognosis in Serous Ovarian Cancer Liver Metastasis

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Background. Globally, ovarian cancer is a leading contributor to cancer-related fatalities among women, with a notable concern being the occurrence of liver metastasis as a prevalent clinical complication. This study aims to investigate the potential impact of alpha kinase 2 (ALPK2) on ovarian cancer liver metastasis (OCLM), assessing its implications for patient prognosis and tumor advancement. Our research seeks to examine the prognostic significance of ALPK2 in individuals with OCLM and unravel the consequences of ALPK2 knockdown on the proliferation and invasion of ovarian cancer cells. *Methods*. A retrospective analysis of 49 OCLM cases from our medical center was conducted to evaluate the prognostic significance of ALPK2 through survival analyses. Experimental investigations involved the use of shRNA to knock down ALPK2 in ovarian cancer cell lines, with subsequent scrutiny of cellular changes. *Results*. Survival analyses revealed that ALPK2 functions as an independent adverse prognostic factor in OCLM (HR = 3.74, 95% CI: 1.34–10.42, and $P = 0.012$). Knockdown experiments indicated a reduction in cell proliferation and invasion capacities, potentially associated with the epithelial-mesenchymal transition process. *Conclusions*. ALPK2 emerges as a crucial oncogene promoting tumors in OCLM. Its knockdown exhibits significant therapeutic potential by hindering cancer progression. Further investigations could solidify the role and therapeutic possibilities of ALPK2 in the treatment of ovarian cancer, particularly in cases involving liver metastasis.

1. Introduction

Ovarian cancer represents a particularly elusive form of cancer impacting women globally, constituting around 3% of female cancers yet yielding the highest mortality rate among female reproductive cancers [1]. Early-stage ovarian cancer often lacks noticeable symptoms, posing a challenge for timely diagnosis and resulting in frequent late-stage detection, which significantly diminishes overall prognosis, especially when metastasis occurs [2].

The presence of liver metastasis in ovarian cancer patients introduces additional complexity and severity to the disease [3]. Beyond indicating an advanced disease stage, liver metastasis brings forth a spectrum of clinical challenges. The compromised state of the liver, a crucial organ with multifaceted functions, has broad implications for the patient's overall health and prognosis [4]. Despite advancements in understanding the molecular drivers of ovarian cancer metastasis, a comprehensive understanding remains an ongoing pursuit.

Alpha kinase 2 (ALPK2) is a multifaceted protein with diverse functions in cellular processes [5]. It is notably involved in regulating the cardiogenesis, a critical embryo development process [6]. ALPK2's functional versatility is underscored by its intricate involvement in various signalling mechanisms such as DNA repair and cell cycle, which can have distinct implications in different cellular contexts. While the specific signalling pathways governed by ALPK2 are still under intensive investigation, its participation in pathways related to cell growth, survival, and

motility has been observed in various studies. For example, the variant of ALPK2 rs3809973 was associated with liver fibrosis in HIV/HCV-coinfected cases [7].

In renal carcinoma, ALPK2 appears to exert a tumor-promoting function, as evidenced by its upregulation compared to normal controls [8]. The signalling mechanisms through which ALPK2 operates in this context are yet to be fully elucidated, but its involvement suggests a potential regulatory role in pathways governing cell proliferation and metastasis [9]. Similarly, ALPK2 has been implicated in promoting aggressiveness through multiple signalling pathways in bladder cancer, esophageal cancer, and nonsmall lung cancer [10–12]. The precise molecular mechanisms by which ALPK2 contributes to tumor progression involve intricate crosstalk with signalling cascades associated with increased cell motility, invasion, and resistance to apoptosis.

Recognizing the importance of ovarian cancer, the challenges associated with liver metastasis, and the intriguing yet not fully elucidated role of ALPK2, our study endeavors to bridge this knowledge gap. In ovarian cancer, a previous study also showed a higher expression of ALPK2 in ovarian cancer tissues than that in normal ovarian tissues [13]. We, therefore, seek to unravel the involvement of ALPK2 in the prognosis of serous ovarian cancer liver metastasis (OCLM) through a comprehensive approach, including retrospective analysis, in vitro experiments, and bioinformatics analysis. Through this in-depth exploration, we aspire to offer a clearer understanding of ALPK2's function, paving the way for potential therapeutic avenues in the future.

2. Methods

2.1. Patient Enrollment and Data Collection. From 2015 to 2019, a retrospective evaluation was undertaken at our medical center to identify eligible patients diagnosed with serous ovarian cancer liver metastasis (OCLM) simultaneously at the time of diagnosis. Exclusion criteria comprised patients with metastasis to distant organs other than the liver and those with a survival duration of less than one month postdiagnosis. Following these criteria, a cohort of 49 patients was selected for this study. Comprehensive demographic and clinical information, encompassing age at diagnosis, tumor laterality, lymph node status, and chemotherapy administration, was meticulously documented.

2.2. Immunohistochemistry (IHC). Tumor tissue samples of hepatic metastasis from the identified 49 patients underwent IHC staining to evaluate ALPK2 protein expression levels, following previously described protocols using anti-ALPK2 antibody (Abcam, ab111284, 1 : 150 dilution) [14, 15]. The categorization of samples into low and high ALPK2 expression was based on both staining intensity and distribution percentages.

2.3. Cell Culture and Transfection. Cell culture and transfection procedures were conducted using OVCAR-5 and OVCAR-3 serous ovarian cancer cell lines sourced from ATCC. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin under standard conditions (37°C, 5% CO_2) in a humidified incubator. Tissue culture flasks or plates were employed for cell seeding when the confluence reached 70–80%, and overnight adhesion was allowed. Regular medium replacement occurred every 2-3 days, and subculturing with trypsin-EDTA solution was performed when the cells reached 70-80% confluence. Transfection procedures involved the use of shRNAs targeting ALPK2 or scramble control shRNA, following the manufacturer's instructions. Immunoblotting was subsequently employed to confirm knockdown efficiency and quantify protein expression levels of E-cadherin, N-cadherin, and vimentin.

2.4. Western Blotting. We employed RIPA (RIPA buffer from Beyotime Biotechnology, China) for protein extraction from the cellular samples. The concentrations of the extracted proteins were quantified using a BCA Protein Assay Kit (obtained from Beyotime Biotechnology, China). Subsequently, 30 μg of proteins per lane was loaded onto gels with appropriate concentration levels. The cellular proteins were subjected to size fractionation through 10–12% SDS-polyacrylamide gel electrophoresis and, thereafter, transferred onto PVDF membranes. These membranes were then subjected to a blocking step with PBS containing 5% BSA for 2 hours at room temperature. Following this, the membranes were incubated with specific primary antibodies at 4°C overnight. This incubation was succeeded by treatment with HRP-conjugated anti-mouse or anti-rabbit IgG antibodies (catalog numbers ab6728 and ab6721, respectively, at a dilution of 1 : 1,000, sourced from Abcam) for 2 hours at room temperature. The protein bands were finally visualized using an enhanced chemiluminescence detection system, following the manufacturer's recommended protocol. The anti-ALPK2 (Abcam, ab111909), anti-E-cadherin (Cell Signalling Technology, 24E10), anti-N-cadherin (Cell Signalling Technology, D4R1H), anti-vimentin (Cell Signalling Technology, R28), and anti-GAPDH (Cell Signalling Technology, 14C10) were used for Western blot analysis.

2.5. Cell Proliferation Assay. For the cell proliferation assay, transfected cells were placed in 96-well plates at a density of 5000 cells per well and allowed to grow for specific time intervals. A CCK-8 assay was carried out according to the manufacturer's guidelines. In brief, the culture medium was replaced with fresh medium containing CCK-8 reagent, and cells were incubated for 2 hours at 37°C. The spectrophotometric measurement of absorbance at 450 nm using a microplate reader determined the formazan dye's intensity,

which is proportional to viable cell numbers. The percentage of cell viability or proliferation was calculated by normalizing to the control group. Results were expressed as the mean ± SD to ascertain statistical significance.

2.6. Cell Invasion Assay. Transwell inserts featuring a porous membrane with an 8 μm pore size and coated with Matrigel were meticulously positioned within a 24-well plate. In the upper chamber of these transwell inserts, cells were diligently seeded at a density of 20,000 cells per well using serum-free medium. Meanwhile, the lower chamber was filled with the medium enriched with a 20% FBS concentration to induce cell invasion. Over the course of 48 hours, these cells were incubated at 37°C to facilitate the invasion process. Upon completion of the incubation period, non-invaded cells residing on the upper surface of the membrane were gently eliminated using a cotton swab. Subsequently, the cells that had successfully invaded the lower side of the membrane were firmly fixed in place and subsequently stained with crystal violet, allowing for the visualization of the invaded cells. The quantification of invaded cells was performed across multiple randomly chosen fields. The results were then expressed as the mean ± standard deviation (SD) in order to determine the statistical significance.

2.7. Statistical Analysis. Survival analyses, employing the Kaplan–Meier method, and statistical significance assessments between groups through the log-rank test were conducted. Multivariate analyses were performed to identify prognostic factors influencing survival outcomes. All statistical computations were considered significant at $P < 0.05$.

2.8. Ethical Approval. All procedures and experiments involving patients and animals adhered strictly to the ethical standards of the Affiliated Taian City Central Hospital of Qingdao University and followed the 1964 Helsinki Declaration [16]. Written informed consent was obtained from all individual participants included in the study.

3. Results

3.1. Patient Enrollment and Data Collection. In the timeframe spanning 2015–2019, our medical center initially identified 66 patients diagnosed with serous ovarian cancer liver metastasis (OCLM). Following the exclusion of patients with concurrent distant metastases ($n = 12$) and those with a survival duration of less than one month ($n = 5$), a final cohort of 49 patients was established for this study (Table 1). Their follow-up duration ranged from 1 to 64 months, with a median of 16 months. At diagnosis, patient ages ranged from 29 to 90 years, with a median age of 63. Among these, 21 patients were aged 60 or younger, while 28 were older. Tumor laterality revealed 13 patients with unilateral and 36 with bilateral tumors. Lymph node involvement was observed in 30 patients (positive), while 19 were negative. Chemotherapy was administered to 40 patients and 9 did not undergo this treatment.

3.2. ALPK2 Expression and Association with Patients' Characteristics. Analysis through immunohistochemistry (IHC) revealed that 24 patients exhibited low ALPK2 protein expression, while 25 showcased high levels in the tumor samples from hepatic metastasis. (Figure 1). Correlating these expression levels with clinical variables showed no statistically significant associations. ALPK2 expression remained evenly distributed across age groups (≤60 years and >60 years, $P = 0.869$). Tumor laterality (unilateral or bilateral) presented a nonsignificant association with ALPK2 expression ($P = 0.376$). Similarly, the lymph node status (negative or positive) did not significantly correlate with ALPK2 levels ($P = 0.176$). Chemotherapy administration demonstrated an evenly split ALPK2 expression ($P = 0.662$). Consequently, in our OCLM cohort, ALPK2 expression appeared independent of the assessed clinical parameters (Table 1).

3.3. Prognostic Significance of ALPK2 Expression for Overall Survival. Kaplan–Meier analysis revealed distinct overall survival patterns (Figure 2 and Table 2). The 3-year overall survival rate for the entire cohort was 48.7%, with a median survival time of 36 months (Figure 2(a)). Notably, age emerged as a determinant, with younger patients (≤60 years) displaying a mean overall survival of 43.9 ± 4.4 months and a 3-year survival rate of 67.5%. Conversely, those aged >60 years faced reduced survival outcomes as follows: 25.8 ± 4.3 months with a 3-year survival rate of 29.3% (Figure 2(b), $P = 0.019$). Tumor laterality, lymph node status, and chemotherapy exhibited no substantial differences in overall survival (Figures 2(c)–2(e), all $P > 0.05$). However, a significant difference arose in ALPK2 expression. Patients with low ALPK2 expression demonstrated a favorable survival of 41.4 ± 3.9 months and a 3-year rate of 62.5%. In contrast, those with high ALPK2 expression had a drastically reduced survival time of 25.1 ± 5.2 months, with a 3-year survival rate of 24.1% (Figure 2(f), $P = 0.027$).

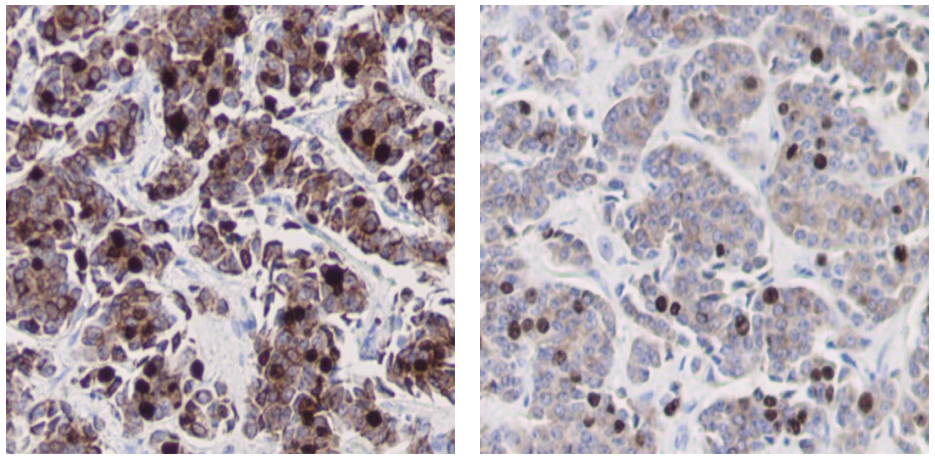
In multivariate analysis, age and ALPK2 expression emerged as significant prognostic markers. Older age (>60 years) and high ALPK2 expression were both associated with a reduced survival rate, with hazard ratios of 3.33 ($P = 0.009$) and 3.74 ($P = 0.012$), respectively. This underscores ALPK2's pivotal role as an unfavorable prognostic factor in OCLM (Table 3).

To further validate the prognostic relevance of ALPK2 in ovarian cancer in a larger cohort, we enrolled the TCGA ovarian cancer dataset. Based on the mRNA level of ALPK2, TCGA cases were divided into the low-ALPK2 group and the high ALPK2 group. Accordingly, patients with higher ALPK2 showed worse overall survival ($P < 0.001$, Figure 3(a)). The median overall survival month of the high-ALPK2 group was 34.4 months, while it was 50.0 months for the low-ALPK2 group. Moreover, in ovarian cancer patients with FIGO stage III-IV, a similar longer median overall survival time was observed in patients with low-ALPK2 levels (45.7 months vs. 34.3 months, $P < 0.001$; Figure 3(b)). Besides overall survival, the progression-free survival of the abovementioned cohort was also analyzed. As a result, patients with higher ALPK2

Table 1: Clinical characteristics of OCLM patients.

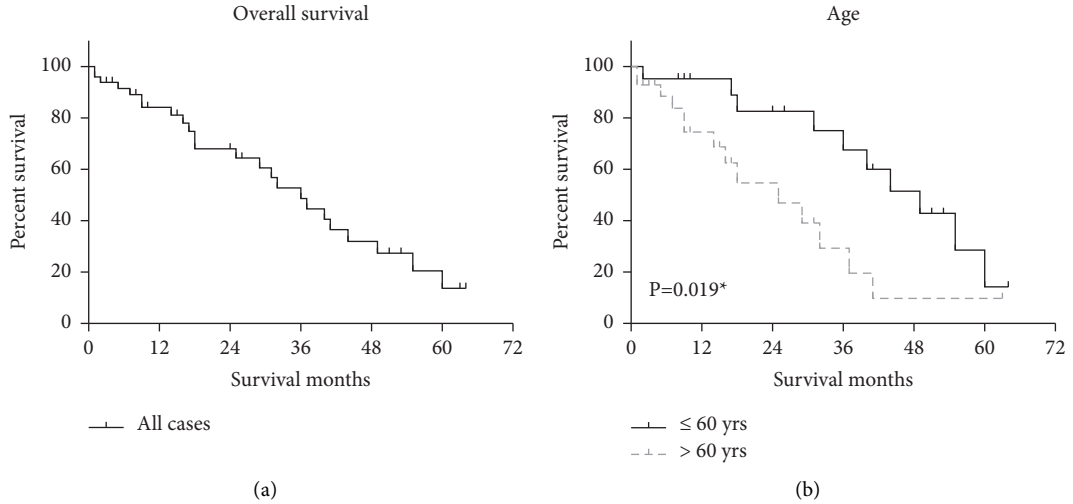
Variables	Cases ($n = 49$)	ALPK2 expression level		P value
		Low ($n = 24$)	High ($n = 25$)	
Age (years)				1.000
≤60 yrs	21	10	11	
>60 yrs	28	14	14	
Laterality				0.520
Unilateral	13	5	8	
Bilateral	36	19	17	
Lymph node status				0.244
Negative	19	7	12	
Positive	30	17	13	
Chemotherapy				0.725
Yes	40	19	21	
No	9	5	4	

OCLM, ovarian cancer liver metastases; ALPK2, alpha kinase 2. *Note.* Data were tested by the two-sided Fisher exact test.



(a) (b)

Figure 1: Protein expression of ALPK2 in ovarian cancer tissues from liver metastasis. (a) Representative illustration of high ALPK2 protein expression through immunohistochemistry staining. Magnification: 400x. (b) Representative illustration of low ALPK2 protein expression through immunohistochemistry staining. Magnification: 400x.



(a) (b)

Figure 2: Continued.

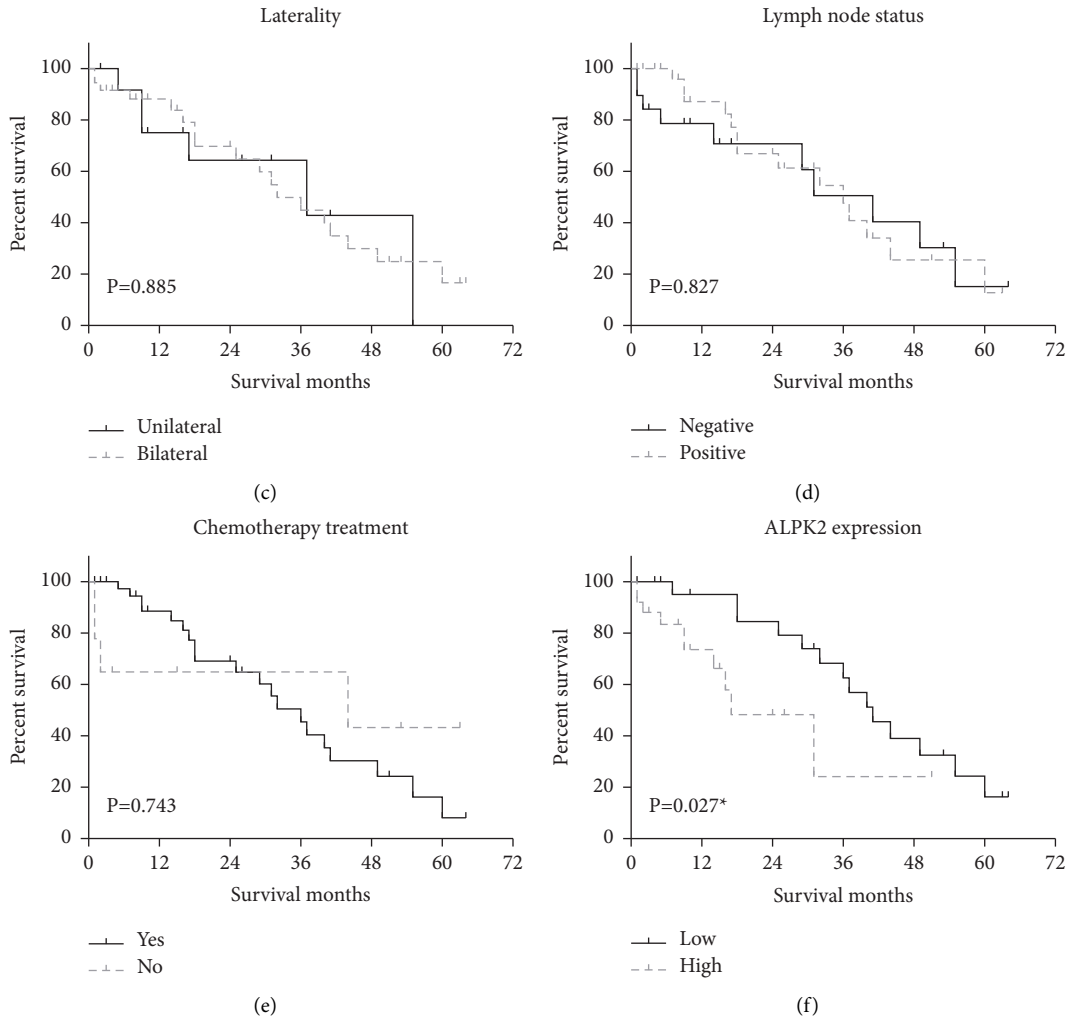


Figure 2: Overall survival analyses of our cohort. Overall survival curves for all enrolled OCLM patients (a), or based on patients' age (b), laterality (c), lymph node status (d), chemotherapy treatment (e), and ALPK2 expression level (f). Data were plotted using the Kaplan–Meier method and compared via the log-rank test. $^{*}P < 0.05$.

Table 2: Overall survival analyses of OCLM patients.

Variables	Cases ($n = 49$)	OS (months) Mean ± S.D.	3 year OS (%)	P value
Age (years)				0.019*
≤60 yrs	21	43.9 ± 4.4	67.5	
>60 yrs	28	25.8 ± 4.3	29.3	
Laterality				0.885
Unilateral	13	35.2 ± 7.0	64.3	
Bilateral	36	35.3 ± 4.1	44.8	
Lymph node status				0.827
Negative	19	34.8 ± 5.8	50.5	
Positive	30	35.3 ± 4.4	47.7	
Chemotherapy				0.743
Yes	40	34.8 ± 3.6	45.4	
No	9	37.2 ± 9.7	64.8	
ALPK2 expression				0.027*
Low	24	41.4 ± 3.9	62.5	
High	25	25.1 ± 5.2	24.1	

OCLM, ovarian cancer liver metastases; ALPK2, alpha kinase 2. *Note.* Data were tested by the two-sided log-rank test $^{*}P < 0.05$ with statistical significance.

Table 3: Multivariate analysis for overall survival of OCLM patients.

Variables	Hazard ratio	95% CI	*P* value
Age (>60 vs. ≤60 ys)	3.33	1.35–8.20	0.009*
ALPK2 expression (High vs. low)	3.74	1.34–10.42	0.012*

95% CI, 95% confidence interval; OCLM, ovarian cancer liver metastases; ALPK2, alpha kinase 2. *Note.* Data were analyzed by the Cox hazard regression test
*P < 0.05 with statistical significance.

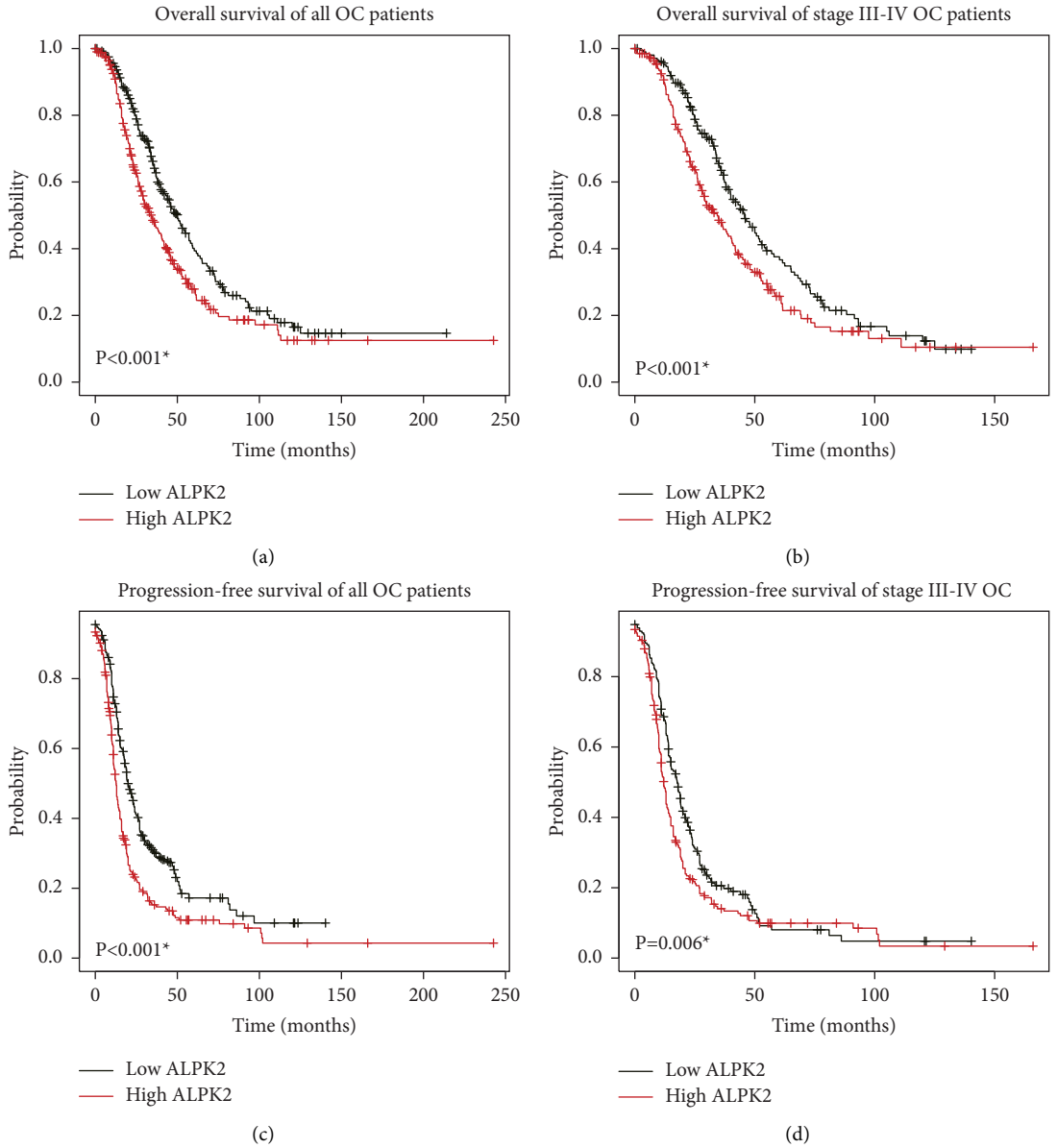


Figure 3: Survival analyses of the TCGA cohort. Overall survival curves of all ovarian cancer patients (a) in TCGA cohort or stage III-IV patients (b) were plotted according to the mRNA level ALPK2. Similarly, progression-free survival curves of all ovarian cancer patients (c) in the TCGA cohort or stage III-IV patients (d) were also plotted. Data were plotted using the Kaplan–Meier method and compared via the log-rank test. $^*P < 0.05$.

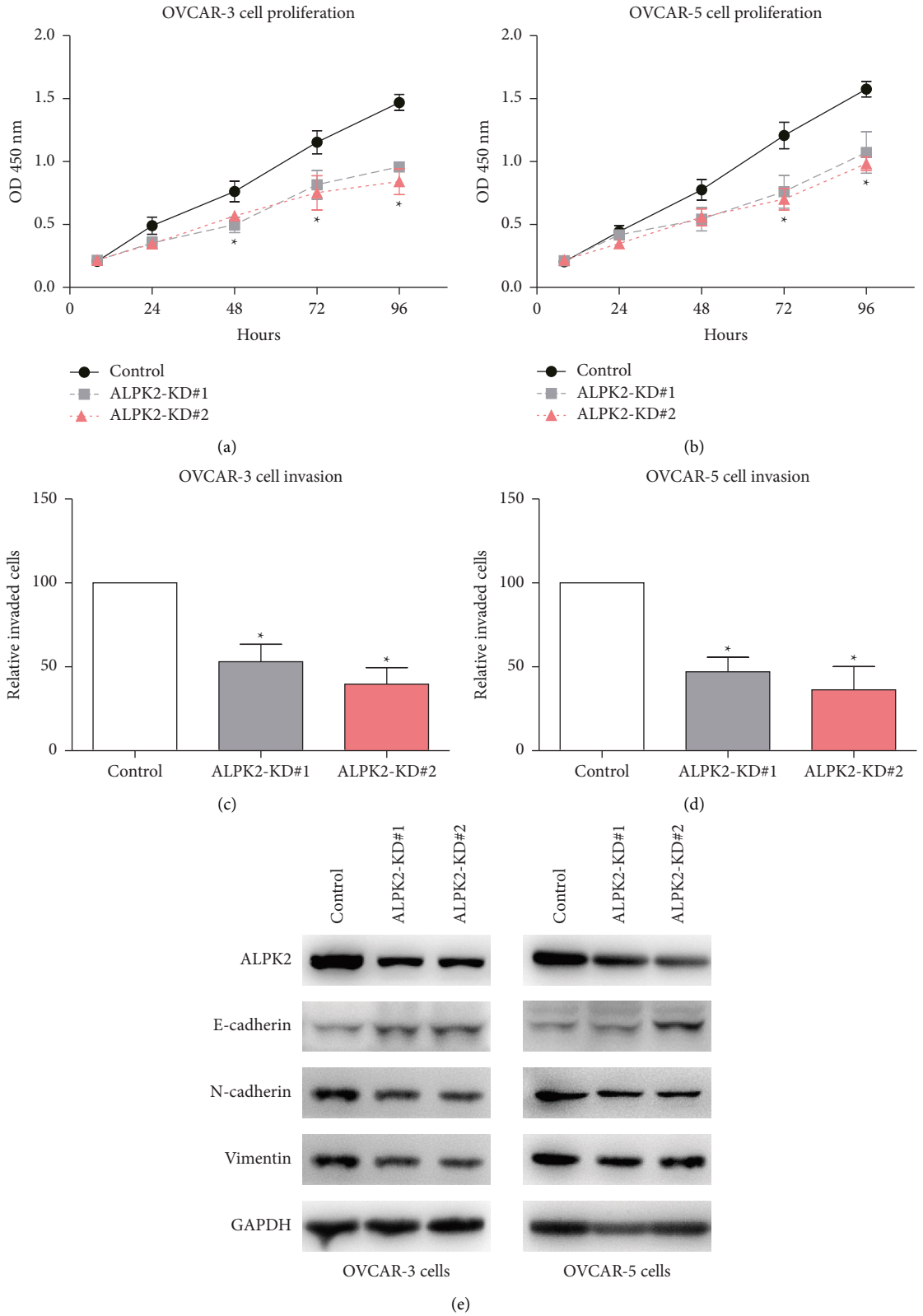


Figure 4: Cellular effects of ALPK2-knockdown in ovarian cancer cells. (a-b) ALPK2-knockdown by shRNAs results in decreased cell proliferation viability in both OVCAR-3 and OVCAR-5 cell lines compared to control cells treated with scrambled-shRNA, as shown by the MTT assay. (c-d) ALPK2-knockdown by shRNAs impairs the invasion capacities of both OVCAR-3 and OVCAR-5 cell lines, as demonstrated by the Matrigel transwell assay. (e) Immunoblotting data illustrate that ALPK2-knockdown significantly upregulates E-cadherin levels while downregulating the levels of N-cadherin and vimentin. Student's *t* tests were used to compare the results between the KD#1 and the control group as well as between the KD#2 and the control group. The significance level for these tests was set at $P < 0.05^*$. KD#1 means knockdown group 1, and KD#2 means knockdown group 2.

exhibited shorter progression-free survival time on the context of all ovarian cancer patients (12.7 months vs. 20.0 months, $P < 0.001$; Figure 3(c)) and stage III-IV patients (12.0 months vs. 17.7 months, $P = 0.006$; Figure 3(d)), respectively.

3.4. Impacts of ALPK2-Knockdown on Ovarian Cancer Cells. Post ALPK2-knockdown, several pronounced effects on cellular dynamics were observed, as depicted in Figure 4. There was a marked reduction in cell proliferation viability in OVCAR-5 and OVCAR-3 ovarian cancer cell lines, evident in both CCK-8 assay results when compared to control cells treated with scrambled-shRNA (Figures 4(a) and 4(b)). In addition, the invasive potential of both OVCAR-5 and OVCAR-3 cell lines was substantially compromised with ALPK2-knockdown, as demonstrated by the Matrigel transwell assay (Figures 4(c), 4(d)). This attenuation in invasiveness post ALPK2 inhibition indicates its essential role in ovarian cancer cell motility. Notably, insights into the molecular effects of the knockdown were obtained through immunoblotting. The data revealed a notable upregulation in the level of E-cadherin, a hallmark of epithelial phenotype, post ALPK2-knockdown. Simultaneously, there was a marked downregulation in the mesenchymal markers, N-cadherin and vimentin (Figure 4(e)). These shifts suggest a potential reversal of the epithelial-to-mesenchymal transition (EMT) process, a critical pathway in cancer metastasis.

4. Discussion

Ovarian cancer liver metastasis poses a substantial clinical challenge, contributing significantly to the high mortality rates associated with the disease [17]. Through a comprehensive examination of serous ovarian cancer liver metastasis (OCLM) patients, this study revealed crucial insights into the involvement of alpha kinase 2 (ALPK2) in both in vivo and in vitro settings. A notable finding was the heightened expression of ALPK2 in ovarian cancer liver metastasis, suggesting its potential as a therapeutic target.

The observed overexpression of ALPK2 was intricately linked to unfavorable prognostic outcomes. Elevated ALPK2 levels significantly diminished overall survival in OCLM patients, aligning with prior studies associating increased ALPK2 expression with aggressive tumor behavior in other cancer types [18]. This suggests that ALPK2 may not only serve as a prognostic marker but could also potentially act as a mechanistic driver of tumor progression [10–12]. The significance of age as a factor affecting overall survival emphasizes the importance of early detection and targeted interventions.

In vitro findings provided a cellular perspective on clinical outcomes, revealing that ALPK2-knockdown led to reduced cell proliferation and invasion in ovarian cancer cell lines. This was accompanied by notable changes in epithelial-mesenchymal transition (EMT) markers, consistent with findings in a previous study which implicated ALPK2 in driving EMT, fostering invasiveness and metastasis [13]. The altered E-cadherin, N-cadherin, and vimentin levels after ALPK2 silencing underscore the kinase's role in this cellular transformation, offering a potential molecular pathway for therapeutic exploitation.

However, despite valuable insights, the study has limitations, including its retrospective nature and a relatively small sample size, potentially introducing biases. Future prospective studies with larger cohorts are necessary for validating these findings. In addition, detailed mechanistic studies elucidating how ALPK2 regulates EMT and influences the immune microenvironment would enhance our understanding.

5. Conclusions

In conclusion, this study underscores the pivotal role of ALPK2 in ovarian cancer liver metastasis, spanning from cellular mechanisms to clinical outcomes. ALPK2 not only serves as a marker but also potentially as a therapeutic target, laying the groundwork for future studies aimed at precision interventions and personalized oncology in ovarian cancer.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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