European Review for Medical and Pharmacological Sciences 2017; 21: 239-245

High expression of KIF14 is associated with poor prognosis in patients with epithelial ovarian cancer

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Abstract. – OBJECTIVE: Kinesin family member 14 (KIF14) is a mitotic kinesin and plays an important role in tumor progression. KIF14 overexpression has been observed in multiple cancers and has been correlated with a poor prognosis. However, its protein expression and prognostic significance in epithelial ovarian cancer (EOC) remain unclear. In this research, we aimed to explore the relationship of KIF14 expression with clinicopathological parameters and prognosis in EOC.

MATERIALS AND METHODS: In this study, we measured KIF14 expression in 170 EOC carcinoma tissue samples with immunohistochemistry and correlated these data with clinicopathological characteristics.

RESULTS: The expression of KIF14 in EOC tissues was significantly higher than that in normal tissues. Furthermore, KIF14 expression was significantly associated with metastasis (p = 0.047), histological type (p = 0.001), Ki67 expression (p = 0.004) and residual tumor (p = 0.038). Also, Kaplan-Meir survival curves showed that a high level of KIF14 expression was a predictor for worse PFS (p = 0.013) and OS (p = 0.009) in patients with EOC.

CONCLUSIONS: KIF14 expression may be associated with poor prognosis, suggesting that it has potential value as an effective prognostic predictor in EOC patients.

Key Words:
KIF14, EOC, High expression, Poor prognosis.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal of the gynecological tumor and the 5-year survival rate has remained below 40% over the past 30 years[1,2]. The high mortality rate may be attributed to an advanced stage at diagnosis and a high recurrence rate[3]. Therefore, identification of novel molecular prognostic indicators with the potential to identify patients at increased risk of relapse is critical for improving treatment strategies.

The most prevalent genomic change in many cancers is a gain of portions of the long arm of chromosome 1[4-7]. Subsequently, identification of the minimal region of gain in the 1q region of gain (1q32.1) revealed that kinesin family member 14 (KIF14) as an oncogene[6]. KIF14 is a microtubule-dependent molecular motor that uses ATP hydrolysis to power movement along microtubules[8]. During midbody formation and the completion of cytokinesis, KIF14 plays an important role in cytokinesis by interacting with the microtubule-bundling protein PRC1 and citron kinase[9]. Knockdown of KIF14 induces cytokinesis failure, resulting in polyploidy formation and apoptosis[10].

Recently, KIF14 was found to be overexpressed in breast, lung, ovarian, and pancreatic cancer, as well as retinoblastoma, glioma and hepatocellular carcinoma[11-16]. Silencing KIF14 can inhibit cell proliferation and colony formation[12,13], suggesting that the oncogenic role of KIF14. The previous study showed that KIF14 genomic gain and mRNA was elevated in ovarian cancers. In addition, high expression of KIF14 mRNA was correlated with poorer outcome[13]. However, the protein expression of KIF14 was unclear, and the relationship of KIF14 expression with clinicopathological parameters and prognosis remain to be explored in EOC.

Materials and Methods

Tumor Samples

Formalin-fixed paraffin-embedded (FFPE) tissues were obtained from the Harbin Medical University Cancer Hospital. 170 EOC samples were obtained from patients who underwent surgery with the goal of maximal tumor resection between

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March 2006 and September 2009. 40 normal ovaries samples used as controls were obtained from women who underwent surgery for benign gynecological diseases except ovarian diseases during the same period. The pathological diagnoses based on the samples used in this study were re-evaluated by two senior pathologists. The cancer cases selected were based on the availability of resected tissue and follow-up data. Patients whose cause of death remained unknown were excluded. The assessment of tumors was based on the International Federation of Gynecology and Obstetrics (FIGO) and the Silverberg grading system. Histology was according to the World Health Organization (WHO) criteria. Data on survival time and clinic-pathological parameters were collected. None of the patients had received any prior treatment for cancer. The work was approved by the Institute Research Medical Ethics Committee of Harbin Medical University.

Immunohistochemistry

To evaluate KIF14 protein expression, immunohistochemical (IHC) staining was performed in all FFPE tissues. Briefly, sections (4 μm, thickness) were baked at 70°C for 90 minutes, deparaffinized with xylene, rehydrated through graded alcohol and rinsed in distilled water. Antigen retrieval was performed by heating the slides in autoclave sterilizer with EDTA for 2 minutes. Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2 for 15 minutes, followed by rinsing in phosphate-buffered saline (PBS). The slides were incubated with a primary antibody overnight at 4°C. Antibody KIF14 was purchased from Bethyl Laboratories (New Delhi, India). Their dilutions were KIF14 (1:200). After washing three times with PBS, the sections were incubated with a peroxidase-conjugated goat anti-rabbit antibody (Zhongshan Biotechnology Co., Beijing, China) for 30 min at 37°C. Sections were stained with 0.02% diaminobenzidine and then counter stained with hematoxylin. A negative control was stained with PBS instead of the primary antibody, and no positive staining was observed. Also, anti-Ki67 (Zhongshan Biotechnology Co., 1:50) antibody was used on consecutive sections as previously described.

Immunohistochemical Evaluation

Two experienced pathologists without knowledge of the information of patients examined all IHC-stained slides under a light microscope. Staining of KIF14 was classified based on the semi-quantitative scoring criteria, which considers combining the percentage and intensity of positively stained tumor cells. The percentage of positively stained tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10%-50% positive tumor cells), and 3 (>50% positive tumor cells). The staining intensity was scored as follows: 0 (no staining), 1 (light yellow, weak staining), 2 (yellow-brown, moderate staining), and 3 (brown, strong staining). The staining intensity score was multiplied by the percentage of positive tumor cells to define the expression level: score of 4 was used to distinguish between low and high expression of KIF14. In addition, Ki67 index was expressed as the percentage of positively stained cells in a section.

Statistical Analysis

The Mann-Whitney U test was used to compare KIF14 levels in EOC and normal tissues. Pearson χ^2-test was used to test the correlation between KIF14 protein levels and clinical parameters, Ki67 index. Progression-free survival (PFS) and overall survival (OS) described the survival function for both Kaplan-Meier survival analyses and Cox proportional hazard univariate and multivariate regression analyses. Statistical significance was set at $p < 0.05$.

Results

Patients Characteristics

Of the 170 patients, 63 (37.1%) were ≤ 55 years, 111 (65.3%) were serous ovary carcinoma, 142 (83.5%) were metastasis, 115 (67.6%) were menopausal, and 109 (64.1%) patients received chemotherapy (Table I).

KIF14 Expression in Ovary Cancer

We evaluated the cytoplasmic localization of KIF14 in EOC and control samples (Figure 1). KIF14 expression was high in 118 (69.4%) of the 170 tumor tissues and in 11 (27.5%) of 40 normal control tissues, and the difference was statistically significant ($p < 0.001$).

Associations between the protein expression of KIF14 and clinicopathological parameters high levels of KIF14 expression were significantly correlated with metastasis ($p = 0.047$), histological type ($p = 0.001$), Ki67 expression ($p = 0.004$) and residual tumor ($p = 0.038$). No correlations between KIF14 expression, age, tumor grade, FIGO stage, ascites, menopausal status, CA125 level and chemotherapy were observed (Table I).

Table I. Association between KIF14 protein expression and clinicopathologic characteristics of EOC patients.

Characteristic	No. of patients (%)	KIF14 protein expression [n (%)]		*p*-value
		Low	High	
Age (years)				
≤ 55	63 (37.1)	18 (28.6)	45 (71.4)	0.661
> 55	107 (62.9)	34 (31.8)	73 (68.2)	
Metastasis				
Negative	28 (16.5)	13 (46.4)	15 (53.6)	0.047*
Positive	142 (83.5)	39 (27.5)	103 (72.5)	
Histological type				
Serous	111(65.3)	24 (21.6)	87 (78.4)	0.001*
Others	59 (37.4)	28 (47.5)	31(52.5)	
Grade				
G1-2	101 (59.4)	29 (28.7)	72 (71.3)	0.521
G3	69 (40.6)	23 (33.3)	46 (66.7)	
FIGO stage				
I-II	45 (26.5)	17 (37.8)	28 (62.2)	0.222
III-IV	125 (73.5)	35 (28.0)	90 (72.0)	
Ki67				
Low	73 (42.9)	31 (42.5)	42 (57.5)	0.004*
High	97(57.1)	21(21.6)	76(78.4)	
Residual tumor (cm)				
≤ 1	81 (47.6)	31 (38.3)	50 (61.7)	0.038*
> 1	89 (52.4)	21 (23.6)	68 (76.4)	
Ascites (ml)				
≤ 500	62 (36.5)	20 (32.3)	42 (67.7)	0.720
> 500	108 (63.5)	32 (29.6)	76 (70.4)	
Menopausal status				
No	55 (32.4)	17 (30.9)	38 (69.1)	0.950
Yes	115 (67.6)	35 (30.4)	80 (69.6)	
Preoperative CA125 level (U/ml)				
≤ 35	14 (8.2)	2 (14.3)	12 (85.7)	0.281
> 35	156 (91.8)	50 (32.1)	106 (67.9)	
Chemo.				
No	61 (35.9)	18 (29.5)	43 (70.5)	0.819
Yes	109 (64.1)	34 (31.2)	75 (68.8)	

*$p < 0.05$.

Prognostic significance of KIF14 expression in ovary cancer Kaplan-Meier survival analysis showed thathigh KIF14 expression group had worse OS (Log-rank 6.905, $p = 0.009$) and PFS (Log-rank 6.103, $p = 0.013$) than that of the low group (Figure 2).

We also performed univariate and multivariate analyses to evaluate the impact of KIF14 expression and other clinicopathological parameters on prognosis. In the univariate analysis, metastasis ($p = 0.002$), FIGO stage ($p = 0.002$), residual tumor ($p < 0.001$), ascites ($p = 0.018$) and KIF14 expression ($p = 0.010$) were correlated with PFS. In addition, Cox univariate analysis showed that metastasis ($p < 0.001$), FIGO stage ($p < 0.001$), residual tumor ($p < 0.001$), ascites ($p = 0.001$), chemotherapy ($p = 0.004$) and KIF14 expression ($p = 0.010$) were correlated with OS (Table II).

In the multivariate analysis, residual tumor ($p = 0.044$) and KIF14 expression ($p = 0.031$) were found to have statistically significant associations with PFS. Moreover, Cox multivariate analysis showed that metastasis ($p = 0.041$), FIGO stage ($p = 0.043$), residual tumor ($p = 0.041$), and KIF14 expression ($p = 0.010$) were correlated with OS (Table III).

Discussion

In this research, we assessed KIF14 protein expression in 170 EOC patients using immu-

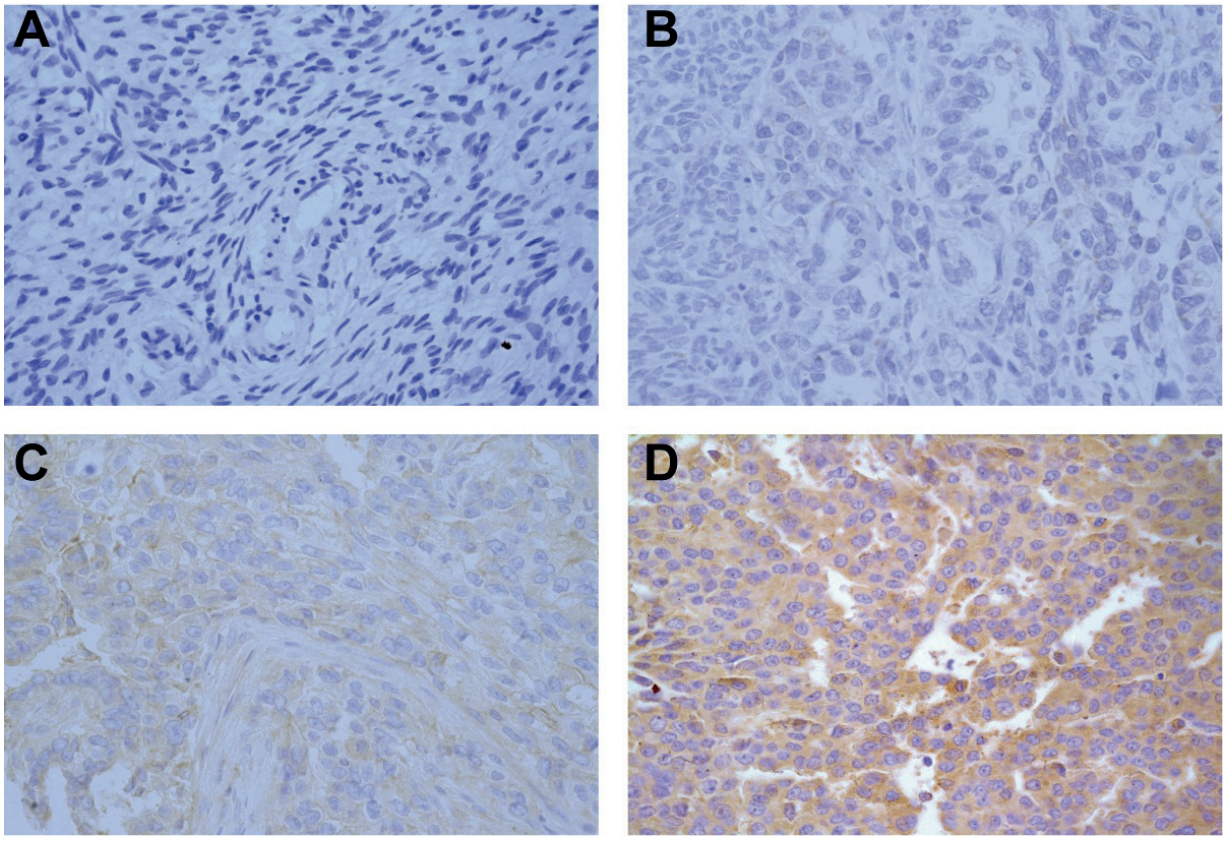

Figure 1. Representative examples of KIF14 immunohistochemical staining showing high and low expression. ***A,*** Low KIF14 expression in normal tissue. ***B,*** Low KIF14 expression in EOC. ***C,*** Moderate KIF14 expression in EOC. D, High KIF14 expression in EOC. (×400 original magnification).

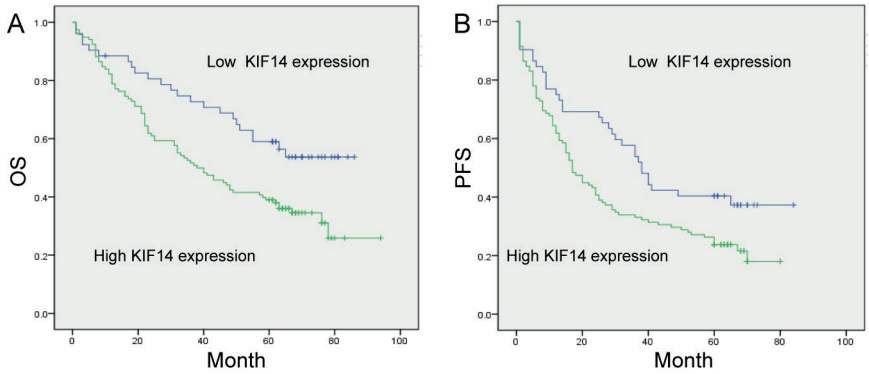

Figure 2. Kaplan-Meier survival curves according to KIF14 expression. Significant survival difference was observed between patients with high and low KIF14 expression in (A) OS ($p = 0.009$) and (B) PFS ($p = 0.013$).

nohistochemistry. The results showed that KIF14 was overexpressed in EOC tissues and that high KIF14 expression correlated with metastasis and worse prognosis. Also, KIF14 proved to be an independent risk factor for poorer survival in EOC.

We observed that KIF14 protein levels were significantly higher in EOC tissues than normal tissues, which was consistent with previous studies[13,17]. Theriault et al[18] found that nearly 30% of ovarian cancers displayed genomic gain of KIF14mand that the KIF14 promoter is highly hypomethylated. Furthermore, the transcription factors Sp1 and YY1 could bind to the KIF14 promoter, and a low level of miR382 was significantly correlated with KIF14

Table II. Univariate analyses of factors affecting PFS and OS in patients with EOC.

Characteristic	PFS		OS	
	HR (95% CI)	p	HR (95% CI)	p
Age				
≤55	1.064 (0.738-1.535)	0.740	1.250 (0.827-1.890)	0.290
>55				
Metastasis				
Negative	2.452 (1.401-4.289)	0.002*	6.800 (2.497-18.515)	0.000*
Positive				
Histological type				
Serous	0.956 (.659-1.387)	0.812	0.801 (0.523-1.229)	0.310
Others				
Grade				
G1-2	1.081 (0.756-1.546)	0.668	1.087 (0.731-1.616)	0.679
G3				
FIGO stage				
I-II	1.963 (1.271-3.032)	0.002*	3.650 (1.994-6.682)	0.000*
III-IV				
Residual tumor (cm)				
≤1	1.973 (1.375-2.831)	0.000*	2.482 (1.643-3.749)	0.000*
>1				
Ascites (ml)				
≤500	1.574 (1.081-2.291)	0.018*	2.055 (1.327-3.183)	0.001*
>500				
Menopausal status				
Yes	1.022 (0.699-1.494)	0.911	0.951 (0.627-1.443)	0.815
No				
Preoperative CA125 level (U/ml)				
≤35	1.168 (0.612-2.231)	0.638	1.287 (0.596-2.778)	0.520
>35				
Chemo.				
Yes	0.899 (0.620-1.302)	0.572	0.556 (0.374-0.827)	0.004
No				
Ki67				
≤10%	1.209 (0.844-1.732)	0.300	1.134 (0.762-1.687)	0.537
>10%				
KIF14				
Low	1.643 (1.097-2.461)	0.016*	1.844 (1.156-2.939)	0.010*
High				

*$p < 0.05$.

mRNA levels. Thus, these mechanisms could enhance KIF14 expression in EOC.

In the present work, high expression of KIF14 was significantly associated with metastasis, histological type, Ki67 expression and residual tumor. Ehrlichova et al[17] also found that KIF14 was correlated with Ki67 expression in ovarian carcinoma. Furthermore, high KIF14 expression was correlated with tumor grade, Ki-67 expression, lymph node metastasis in breast cancer[11]. In glioma, high KIF14 expression was associated with advanced pathological grade and Ki-67 expression[14], indicating that KIF14 potentially plays a role in proliferation and metastasis.

KIF14 is an independent prognostic marker in many cancers[11-14,16,19,20], Theriault et al[13] found that high KIF14 expression was significantly associated with poor PFS in serous ovarian cancers. In the Kaplan-Meier survival analysis, patients with high KIF14 expression had significantly shorter PFS and OS compared with patients with low KIF14 expression. Moreover, univariate and multivariate analyses showed that high KIF14 expression in EOC was significantly associated with reduced PFS and OS.

KIF14 has been implicated in cancer progression and chemotherapy resistance in multiple

Table III. Multivariate analyses of factors affecting PFS and OS in patients with EOC

Characteristic	PFS		OS	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age				
≤55	1.044 (0.722-1.510)	0.818	1.222 (0.806-1.854)	0.346
>55				
Metastasis				
Negative	1.656 (0.816-3.358)	0.162	3.244(1.047-10.054)	0.041*
Positive				
Grade				
G1-2	1.173 (0.817-1.685)	0.387	1.263 (0.845-1.889)	0.254
G3				
FIGO stage				
I-II	1.241 (0.704-2.187)	0.455	2.052 (1.024-4.112)	0.043
III-IV				
Residual tumor (cm)				
≤1	1.520 (1.011-2.286)	0.044*	1.575 (1.019-2.436)	0.041*
>1				
Ki67				
≤10%	0.927 (0.635-1.353)	0.693	0.769 (0.506-1.168)	0.219
>10%				
KIF14				
Low	1.591 (1.044-2.423)	0.031*	1.903 (1.163-3.113)	0.010*

*$p < 0.05$.

cancers. In previous investigations, KIF14 knockdown reduced proliferation and colony formation and enhanced apoptosis in ovarian cancer cells[13,21]. KIF14 significantly enhanced docetaxel chemotherapy resistance through the PI3K/AKT pathway in triple-negative breast cancer cells[19,22]. Tao et al[16] found that silencing KIF14 could induce apoptosis by inactivating AKT signaling in hepatocellular carcinoma. The depletion of KIF14 could inhibit Skp1/Cul1/F-box complex, which targets p27 for degradation by the 26S proteasome, resulting in the accumulation of p27 and cytokinesis failure[23]. Also, KIF14 tethers the Rap1 effector Radil to microtubules, leading to activation of integrin signaling[24,25]. Thus, suppression of KIF14 expression may inhibit tumor migration and invasion[24,26]. Together, these data suggest that KIF14 may play a key role in tumor progression. Further studies will be required to fully characterize the mechanisms by which KIF14 contribute to cancer, and to elucidate its potential role as a target molecule for therapeutic intervention.

Conclusions

KIF14 is overexpressed in EOC, and high KIF14 expression is strongly associated with metastasis, histological type, Ki67 and residual tumor. Furthermore, KIF14 is an independent predictor of poor outcomes and may be a novel EOC biomarker and a potential therapeutic target in this disease.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (no. 81372785).

Conflict of interest

The authors declare no conflicts of interest.

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