Research Paper

Increased HE4 mRNA Expression Correlates with High Level Of eIF3a mRNA And Better Survival in Women with Epithelial Ovarian Cancer

Chen-Hui Luo[1,2], Min Zhao[1], Yu-Xi Tang[3], Shohreh Shahabi[4], Kai-Ning Fang[3], Yan Chen[3], Yan Tang[3], Xiao-Yan Chen[5], Jing Wang[3✉], Hong-Hao Zhou[1✉]

1. Department of Clinical Pharmacology, Institute of Clinical Pharmacology, Hunan Key Laboratory of Pharmacogenetics, Xiangya Hospital, Central South University, Changsha, Hunan, People's Republic of China;
2. Laboratory Animal Research Center, Hunan Cancer Hospital, the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, People's Republic of China;
3. Department of Gynecologic Oncology, Hunan Cancer Hospital, the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, People's Republic of China;
4. Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Prentice Women's Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL, United States;
5. Department of Pathology, Hunan Cancer Hospital, the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, People's Republic of China.

✉ Corresponding authors: Hong-Hao Zhou, MD, Institute of Clinical Pharmacology, Xiangya Hospital, Central South University, 110 Xiangya Road, Changsha 410008, Hunan, People's Republic of China (phone number: +86 731 8480 5379, E-mail: hhzhou2003@163.com); and Jing Wang, MD, PhD, Department of Gynecologic Oncology, Hunan Cancer Hospital, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, 582 Xianjiahu Road, Changsha, 410013, Hunan, People's Republic of China (phone number: +86 731 8865 1849, E-mail: wangjing189@163.com).

Abstract

Human epididymis protein 4 (HE4) is one of the most promising biomarkers for epithelial ovarian cancer (EOC). The majority of previous studies utilized the serum level or tissue protein expression of HE4 based upon immunohistochemistry (IHC) to evaluate the role of HE4 in the diagnosis, prognosis, and surveillance of EOC, but very little is known about HE4 mRNA expression. Eukaryotic translation initiation factor 3a (eIF3a) is implicated in oncogenesis and has been investigated extensively as a potential biomarker for malignancy. We previously reported a positive correlation between IHC expressions of eIF3a and HE4 in EOC. In the present study, we performed RT-PCR to determine mRNA expressions of HE4 and eIF3a in 30 normal ovarian tissues, 45 benign, 20 borderline and 94 malignant ovarian tumors. The association of HE4 and eIF3a mRNA expressions with clinicopathological characteristics and patient survivals was investigated. IHC was also performed in the same participants to investigate the correlation between mRNA and protein levels of HE4. HE4 mRNA level was found to be 48.42 ± 74.55 (mean ± SD, range: 0.01-343.99), significantly higher in primary EOC than in the borderline tumor, benign tumor, and normal ovarian tissue (P<0.001). The cutoff value was 13.99 for HE4 to discriminate malignant from benign tumors at 68.1% sensitivity and 93.0% specificity. By Spearman's correlation test, HE4 mRNA expression was indicated to positively correlate with serum CA125 level (r=0.530, P<0.001). Higher HE4 mRNA expression was associated with decreased frequency of lymph node metastasis (P=0.038) and better overall survival (OS) (P=0.007) in primary EOC. Multivariable analysis showed an independent prognostic value of the relative mRNA level of HE4 greater than one for OS (Hazard Ratio, 0.069, 95%CI, 0.009-0.530, P=0.010). eIF3a mRNA expression in women with primary EOC was 0.95 ± 1.19 (mean ± SD, range: 0.06-7.46), which was in a positive linear correlation with HE4 mRNA expression (r=0.310, P=0.002). In the present study, the HE4 mRNA level was unparalleled with IHC expression of HE4 (P>0.05). Collectively, our study revealed that increased HE4 mRNA expression correlates with high level of eIF3a mRNA and better survival in women with EOC, which calls for further investigations.

Key words: HE4, epithelial ovarian cancer (EOC), mRNA, overall survival, eIF3a

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic cancer in the United States[1]. Human epididymis protein 4 (HE4) is regarded as one of the most promising serum biomarkers for ovarian cancer since 2003[2]. Numerous studies have found that HE4 is a more valuable serum biomarker than CA125 for EOC diagnosis and its recurrence[3-6]. By utilizing the serum levels or protein expressions of HE4, more findings have evaluated the role of HE4 protein in the diagnosis, prognosis, and surveillance of EOC[3-12]. Moreover, serum HE4 level is also reported to be a reliable marker for early diagnosis of endometrial[13], lung [14], and pancreatic cancer[15]. High levels of HE4 in the serum and high staining of HE4 in the tissue by IHC has made it important to determine the distribution of HE4 mRNA expression, to reveal the basis for protein. However, relevant research on HE4 mRNA is far from enough. Thus, additional studies are required to investigate the characteristics of HE4 mRNA expression and its possible association with clinicopathological variables and survivability in EOC.

Eukaryotic translation initiation factor 3a (eIF3a) is the core subunit of the eIF3 complex, which is crucial for the initiation of mRNA translation, and links translation initiation to transcription[16, 17], to mRNA export[18], and to the nonsense-mediated decay pathway[19]. Aberrant eIF3a expression is implicated in oncogenesis [20] and is reported in a number of cancers including EOC[21-23]. eIF3a as a potential prognostic biomarker for malignancy is under extensive investigation[20-22]. We previously reported a pilot study on increased eIF3a expression detected by IHC in EOC and a positive correlation with HE4[23]. Thus, we hypothesized that there may be a similar correlation between mRNA expressions of eIF3a and HE4.

In the present study, we investigated the mRNA expressions of HE4 and eIF3a in women with primary EOC and their relationships with clinicopathological characteristics and survival of women with primary EOC.

Materials and Methods

Patients and specimens

Specimens and clinical data including serum CA125 levels were collected from ovarian cancer patients who had undergone initial surgery at the Hunan Cancer Hospital (Changsha, China) between August 2011 and April 2014. Inclusion criteria were (a) histologically confirmed EOC including three major histopathologic subtypes (serous, mucinous, and other adenocarcinoma); (b) treatment with platinum/taxane-based chemotherapy after debulking surgery; (c) no radiotherapy or biological therapy before the surgery; and (d) Karnofsky Performance Status (KPS) score ≥80 prior to the surgery. Exclusion criteria included (a) metastatic or non-epithelial tumors in ovary; (b) active pelvic infection; and (c) symptomatic brain or leptomeningeal metastases. All patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system. Moreover, samples and data of normal and benign ovarian tumors were collected from patients who underwent surgery for benign or other malignant pathologies. In total, 189 patients (30 normal, 45 benign neoplasms, 20 borderline and 94 malignant tumors) were identified. Fresh tissue was collected and stored in liquid nitrogen for real-time qPCR, and paraffin samples were obtained for histological diagnosis and IHC analysis.

For prognostic factor evaluation, overall survival (OS) was defined as the interval in months between tumor resection and either death or the last observation. Progression free survival (PFS) was defined as the number of months from surgery to diagnosis of relapse or progression. Patients alive at the end of follow-up were censored. The protocol was approved by the Ethics Committee of Hunan Cancer Hospital (Changsha, China) and all patients provided written informed consent.

Real-Time qPCR

Total RNA was extracted from frozen patient tissues using the Trizol reagent (Thermo Fisher). RNA was reverse transcribed using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara), and RT-qPCR was carried out in a LightCycler® 480 Real-Time PCR System using the SYBR Premix Ex Taq™ (Tli RNaseH Plus) kit (Takara), according to the manufacturers' instructions. The primers were as follows: for eIF3a, 5'-TGATGAGGACAGAGGACCAAGAC-3'(forward) and 5'-TCAGCATTACGCCAGGATGA-3' (reverse); for HE4, 5'-ATAGCACCATGCCTGCTTGT-3' (forward) and 5'-TGCTCCTGTGCCTGAGACTA-3'(reverse); and for GAPDH control, 5'-GAAATCCCATCACCAT CTTCCAGG-3' (forward) and 5'-GAGCCCCAGCCTT CTCCATG-3' (reverse). GAPDH acted as the internal control. The relative mRNA levels of HE4 and eIF3a were defined by using the $2^{-\triangle\triangle Ct}$ cycle threshold method.

Immunohistochemistry (IHC)

Tissue sections (4µm thick) were prepared from paraffin embedded blocks. After deparaffinization and rehydration, IHC was performed using an UltraSensitiveTM SP IHC Kit (Fuzhou Maixin) and a DAB Detection Kit (Streptavidin-Biotin) (Fuzhou

Maixin), following the protocol recommended by the manufacturer and described previously[23]. The primary antibodies were anti-HE4 recombinant rabbit monoclonal antibody (1:1000, Abcam, ab109298). The negative control was samples that were incubated without the primary antibody. Slides were assessed by two independent experienced pathologists blinded to the patient's status. In the case of an inconsistent score between them, a third senior pathologist was consulted for the final evaluation. Staining intensity was scored as 0 to 3 [0 (negative), 1 (weak), 2 (moderate) and 3 (strong)]. The heterogeneity of staining was scored as 0 to 3, depending on the percentage of positively stained tumor cells [0 (0-25%), 1 (25-50%), 2 (50-75%) and 3 (75-100%)]. The intensity and heterogeneity scores were multiplied to generate the total score (TS). TS ≥ 3 was considered as high expression of HE4, while TS < 3 was low expression.

Statistical analysis

The data are presented as mean ± standard deviation. To determine statistical significance, the ANOVA test, Student's t-test, Mann-Whitney test, chi-squared test, Pearson's correlation test, and Spearman test were performed as appropriate using SPSS 22.0 software. Figures were made with Graph-Pad Prism 6.0. OS and PFS were evaluated using the Kaplan-Meier method and log-rank test. Cox proportional hazards model was used to evaluate prognostic values of HE4 and eIF3a. All p-values were two-tailed.

Results

Clinicopathologic variables of patients

The cohort included 189 women. Specimens included 30 normal ovaries resected during surgery for other gynecologic malignancies, 45 benign ovarian neoplasms, 20 borderline tumors, and 94 malignant ovarian tumors. The histological diagnosis of samples was reconfirmed by a pathologist with expertise in gynecologic cancer. The age of patients at the time of diagnosis was ranged from 25 to 76 years old. The median age was 50 years old, and the mean was 50.69 years old. The mean ages in nonmalignant and malignant groups were 49.32 ± 10.78 years and 51.44 ± 10.04 years, respectively (P=0.183). All EOC patients received platinum/taxane-based postoperative chemotherapy with a mean of 4.94 cycles of chemotherapy. General clinical and pathological information of EOC patients were shown in Table 1. The mean follow-up for the malignant group was 36.19 ± 14.00 months.

Table 1. mRNA expressions of HE4 and eIF3a and serum CA125 levels in women with primary EOC.

	HE4 mRNA[a] (n=94)	P value	eIF3a mRNA[a] (n=94)	P value	Serum CA125[a] (U/ml) (n=90)	P value
Age						
<50 y	54.3 ± 81.4 (45)	0.470	1.1 ± 1.5 (45)	0.288	1811 ± 2216 (43)	0.061
≥50 y	43.1 ± 68.1 (49)		0.8 ± 0.9 (49)		1084 ± 1223 (47)	
Histological subtype						
Mucinous	23.0 ± 27.1 (5)	0.206	1.5 ± 1.3 (5)	0.266	761 ± 1402 (5)	0.514
Serous	33.4 ± 33.6 (35)		0.7 ± 0.9 (35)		1339 ± 1378 (34)	
Others	59.2 ± 91.5 (47)		1.1 ± 1.4 (47)		1655 ± 2178 (44)	
Grade						
Grade 1	22.1 ± 27.7 (7)	0.363	1.1 ± 1.3 (7)	0.582	692 ± 998 (7)	0.270
Grade 2	57.1 ± 83.5 (54)		0.8 ± 1.0 (54)		1321 ± 1582 (53)	
Grade 3	39.8 ± 64.0 (33)		1.1 ± 1.5 (33)		1798 ± 2215 (30)	
Stage						
I+II	32.2 ± 28.6 (13)	0.402	1.5 ± 2.1 (13)	0.324	277 ± 494 (13)	0.000
III+IV	51.0 ± 79.3 (81)		0.9 ± 1.0 (81)		1626 ± 1863 (77)	
Residual tumor						
<1cm	46.9 ± 73.4 (78)	0.688	0.9 ± 1.2 (78)	0.836	1419 ± 1870 (75)	0.591
>1cm	38.7 ± 46.2 (14)		0.9 ± 1.0 (14)		1713 ± 1392 (13)	
LN-metastasis						
Absent	65.3 ± 101.9 (34)	0.038	1.1 ± 1.5 (34)	0.350	926 ± 1231 (34)	0.014
Present	34.1 ± 37.4 (58)		0.8 ± 0.9 (58)		1801 ± 2024 (54)	
Ascites						
Absent	48.9 ± 63.6 (30)	0.856	1.1 ± 1.2 (30)	0.692	798 ± 1123 (29)	0.031
< 500ml	47.1 ± 79.4 (17)		0.8 ± 0.8 (17)		1569 ± 1957 (15)	
> 500ml	40.4 ± 62.0 (40)		1.0 ± 1.4 (40)		1966 ± 2076 (40)	
Menopause						
Negative	48.7 ± 76.8 (52)	0.928	0.8 ± 0.9 (52)	0.229	1169 ± 1379 (51)	0.116
Positive	50.1 ± 73.9 (40)		1.2 ± 1.5 (40)		1819 ± 2210 (38)	
NACT						
Negative	47.3 ± 74.2 (78)	0.625	0.9 ± 1.1 (78)	0.205	1524 ± 1908 (77)	0.030
Positive	57.7 ± 79.8 (15)		1.4 ± 1.5 (15)		879 ± 688 (13)	

[a]: The data are shown as Mean ± SD.

Abbr.: NACT: neoadjuvant chemotherapy; LN: lymph node.

HE4 mRNA expression in women with primary EOC

HE4 mRNA expression in 94 women with primary EOC was 48.42 ± 74.55 (range: 0.01-343.99), significantly higher than women in the other three groups (Figure 1A, P<0.001). HE4 mRNA expression in women with borderline tumors was 12.47 ± 13.72, similar to that in the benign tumor group (4.93 ± 9.20, P=0.260) but significantly higher than that in the normal ovary group (1.03 ± 2.17, P=0.021). The cutoff value of HE4 relative level was 13.99 to discriminate malignant from benign tumors at 68.1% sensitivity and 93.0% specificity and was 15.93 to discriminate malignant from borderline tumors at 61.7% sensitivity and 76.5% specificity (Figure 1B, P<0.01).

As shown in Table 1, HE4 mRNA expression significantly correlated with lymph node metastasis in primary EOC. Its relative level in the lymph node metastasis-absent group was much higher than in the lymph node metastasis-present group (65.31 ± 101.94 *vs.* 34.13 ± 37.43, P=0.038). Although it seemed that HE4 expression increased in the sequence of mucinous, serous, and other adenocarcinoma (22.99 ± 27.08 *vs.* 33.44 ± 33.55 *vs.* 59.24 ± 91.48), the difference did not reach significance (Table 1, P=0.206). Moreover, no correlation was observed between HE4 mRNA expression and other clinicopathologic characteristics which included age, FIGO stage, tumor grade, residual tumor, ascites, neoadjuvant chemotherapy (NACT), and menopause (Table 1).

Serum CA125 levels are listed in Table 1. Its level in the malignant tumor group was highest compared to the other three groups (Figure 1A, P<0.001), and significantly correlated with FIGO stage, lymph node metastasis, ascites, and NACT (Table 1). Furthermore, Spearman correlation analysis revealed that HE4 mRNA level was positively linear related with serum CA125 (Figure 1C, r=0.530, P<0.001).

eIF3a mRNA expression in women with primary EOC

eIF3a mRNA expression in women with primary EOC was 0.95 ± 1.19 (range: 0.06-7.46). There was no significant difference among malignant tumor, borderline tumor, benign tumor, and the normal ovary group (Figure 1A, P=0.668). Furthermore, no significant association was found between eIF3a mRNA expression and clinicopathologic features of primary EOC patients (Table 1). There was also no correlation to be found between eIF3a mRNA level and serum CA125 (r= -0.116, P=0.158).

The mRNA expressions of HE4 and eIF3a were in a positive linear correlation among women with primary EOC (Figure 1C, r=0.310, P=0.002).

Correlations of mRNA expressions of HE4 and eIF3a with primary EOC survival

To demonstrate a potential difference in survival according to the quantitative levels of HE4 and eIF3a, we performed the Kaplan-Meier method and log-rank test of PFS and OS with the respective levels 1) divided into four quartiles and 2) grouped by greater than one or not (>1 or <1).
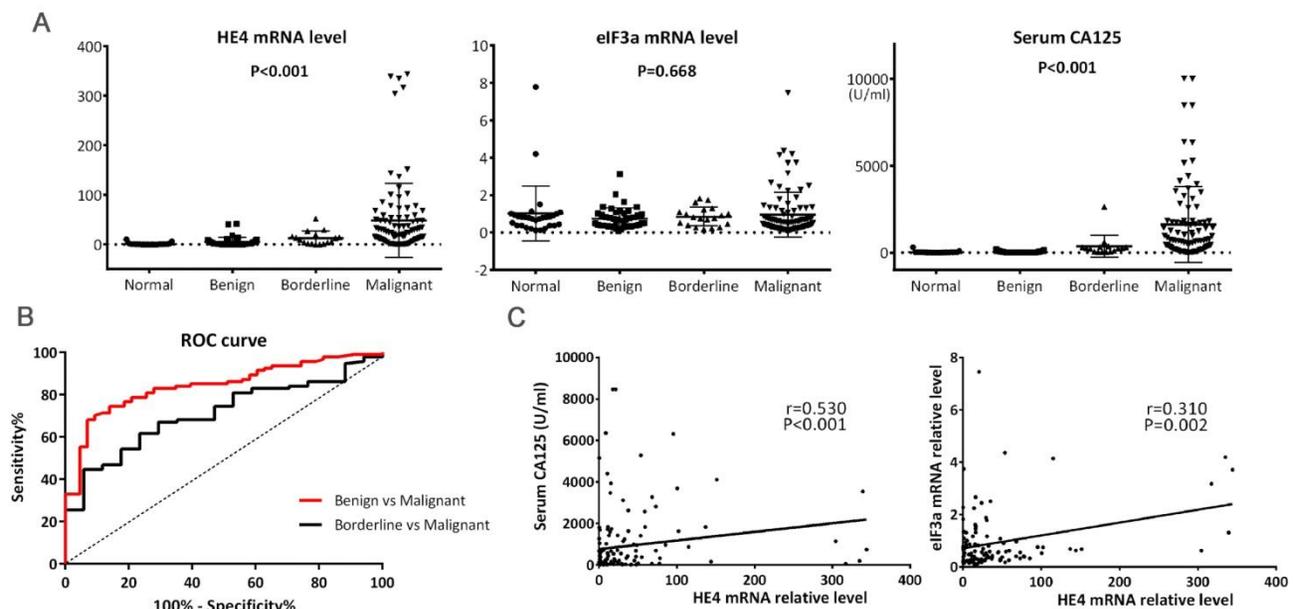

Figure 1. (A) Levels of HE4 mRNA, eIF3a mRNA, and serum CA125 among tumors. HE4 mRNA and serum CA125 levels were significantly increased in malignant tumors. (B) ROC curves for discrimination of malignant tumors from benign and borderline tumors using HE4 mRNA levels. The area under curve (AUC) for Benign *vs.* Malignant and Borderline *vs.* Malignant were 0.842 (95%CI, 0.775-0.909) and 0.710 (95%CI, 0.599-0.821), respectively. Both P<0.01. (C) Correlations of HE4 mRNA with serum CA125 and eIF3a mRNA. Spearman's and Pearson's correlation test were used, respectively.

For HE4, we did not find any statistical correlation with PFS or OS among four quartiles, but the relative level of HE4 greater than one (>1) was significantly associated with increased OS (Figure 2A). Since the median OS was not reached yet, the mean OS increased from 31.36 months (95% CI, 16.34-46.39 months) for HE4 less than one (<1) to 48.04 months (95% CI, 44.04-52.04 months) for HE4 greater than one (>1) (P=0.009). Furthermore, we even found that OS in patients with HE4 greater than 10, 50, and 100 were also significantly higher than that less than one (47.49, 51.34, 52.70 months, P=0.016, 0.009, 0.049 respectively). No significant difference existed with PFS for HE4.

For eIF3a, we found the median PFS increased from 22.93 months (95% CI, 12.63-33.23 months) for patients in the first quartile to 38.54 months (95% CI, 14.63-62.45 months) in the upper fourth quartile (Figure 2B, P=0.075), but we did not find a similar correlation between PFS and eIF3a level greater than one or not. It was also found that no correlation existed between OS and eIF3a mRNA level among any groups.

Multivariable analysis further confirmed an independent prognostic value of the relative mRNA level of HE4 greater than one for OS (Hazard Ratio, 0.069, 95%CI, 0.009-0.530, P=0.010), but this was not the case for PFS (Table 2, P=0.445). Multivariable analysis did not indicate the prognostic significance of eIF3a mRNA level for both PFS and OS. As shown in Table 2, CA125 and optimal cytoreduction (residual tumor <1cm) were also found to significantly correlate with OS (P=0.018 and 0.001, respectively).

HE4 protein level by IHC detection and the correlation with HE4 mRNA level

We further compared the mRNA and protein levels of HE4 in this study. IHC detection was performed using paraffin samples from the same participants to validate HE4 protein levels (Figure 3). Primary EOC group had the highest expression of HE4 compared to the other three groups (malignant *vs.* normal, benign, and borderline group, 67.2% *vs.* 7.7, 35.7, and 46.7%, P<0.001). Among the clinicopathologic characteristics in the primary EOC group, only residual tumor correlated with the IHC result of HE4. HE4 protein level was significantly higher in residual tumor >1cm group than <1cm group (high expression ratio, 100% *vs.* 61.4%; TS, 6.00 ± 2.00 *vs.* 3.61 ± 2.74, P<0.05). By analyzing PFS, we found that high HE4 expression decreased the mean PFS from 38.65 months (95% CI, 29.58-47.73 months) to 28.18 months (95% CI, 22.91-33.44 months) compared to low HE4 expression (Figure 2C, P=0.093). Furthermore, the mean OS of EOC patients with high HE4 expression was significantly worse than those with low HE4 expression (Figure 2D, 37.59 *vs.* 53.47 months; 95% CI, 32.57-42.61 *vs.* 48.88-58.06 months, P=0.022).

In the present study, the relative level of HE4 mRNA did not correlate with TS of HE4 in malignant patients (r=0.066, P=0.596). HE4 mRNA levels also did not show a significant difference between high and low IHC expressions of HE4 (44.11 ± 59.26 *vs.* 55.71 ± 95.98, P=0.544).

Table 2. Univariable and multivariable Cox regression analysis of HE4 mRNA expression with PFS and OS in EOC.

Variables	PFS				OS			
	Univariable HR (95% CI)	*P* value	Multivariable[a] HR (95% CI)	*P* value	Univariable HR (95% CI)	*P* value	Multivariable[a] HR (95% CI)	*P* value
FIGO Stage (I+II *vs.* III+IV)	2.297 (0.904-9.478)	0.073	— —	0.171	3.595 (0.485-26.639)	0.211	— —	0.425
Tumor grade (G1 *vs.* G2 *vs.* G3)	1.210 (0.710-2.062)	0.484	— —	0.678	1.008 (0.499-2.036)	0.982	— —	0.550
Histological subtype (mucinous *vs.* serous *vs.* others)	0.710 (0.430-1.173)	0.181	— —	0.395	0.485 (0.276-0.855)	0.012	— —	0.078
HE4 relative level (>1 *vs.* <1)	1.141 (0.353-3.693)	0.826	— —	0.445	0.288 (0.106-0.779)	0.014	0.069 (0.009-0.530)	0.010
eIF3a relative level (<25% *vs.* >75%)	0.523 (0.232-1.179)	0.118	— —	0.461	0.416 (0.129-1.346)	0.143	— —	0.841
CA125	1.000 (1.000-1.001)	0.000	1.000 (1.000-1.001)	0.001	1.000 (1.000-1.000)	0.002	1.000 (1.000-1.001)	0.018
Residual tumor (>1 *vs.* <1)	1.220 (0.515-2.890)	0.652	— —	0.573	3.041 (1.259-7.349)	0.013	11.029 (2.774-43.842)	0.001
Lymph node metastasis (present *vs.* absent)	2.268 (1.118-4.602)	0.023	— —	0.057	1.595 (0.631-4.029)	0.324	— —	0.522
Neoadjuvant chemotherapy (present *vs.* absent)	1.286 (0.572-2.890)	0.542	— —	0.160	0.562 (0.132-2.393)	0.436	— —	0.490
Menopause (present *vs.* absent)	1.062 (0.587-1.921)	0.843	— —	0.708	0.483 (0.200-1.166)	0.106	— —	0.649
Ascites (present *vs.* absent)	1.710 (0.901-3.247)	0.101	— —	0.980	4.213 (1.250-14.199)	0.020	— —	0.627

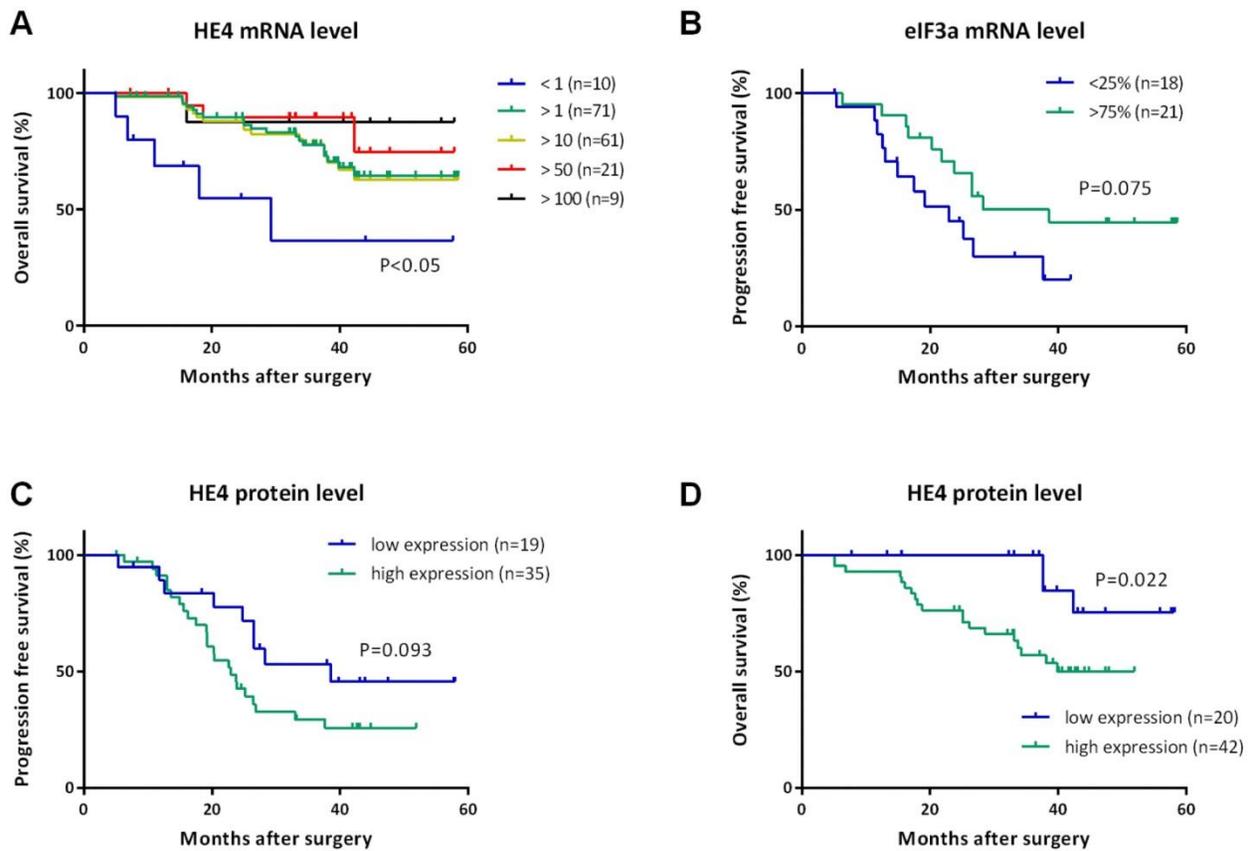[a]: Method = Forward Stepwise (Likelihood Ratio)

Figure 2. Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) of women with EOC. (A) OS curves indicated the prolonged OS of patients with HE4 mRNA level greater than 1, 10, 50, and 100 compared to less than 1. All P<0.05. (B) PFS curve indicated patients with eIF3a mRNA level in the first quartile (<25%) recurred earlier than those in the upper fourth quartile (>75%). P=0.075. PFS curve (C) and OS curve (D) for HE4 protein levels detected by IHC indicated worse survival of patients with high expression of HE4 protein than those with low expression of HE4 protein. P=0.093 and 0.022, respectively.
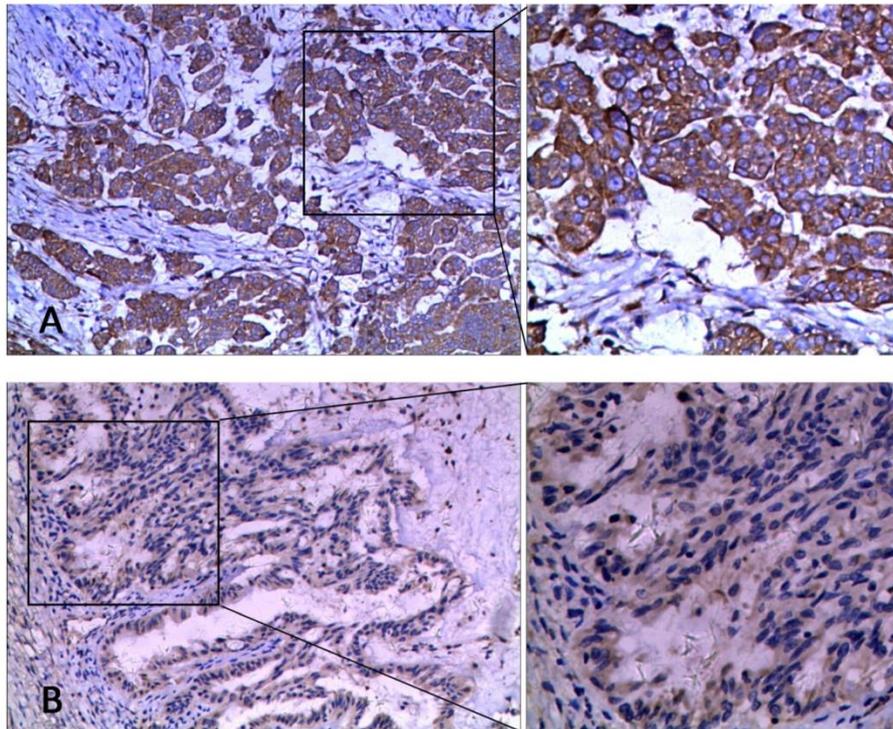

Figure 3. The representative images of HE4 staining by IHC in ovarian tissues at 100× and 200× magnification. (A) High expression, (B) Low expression.

Discussion

Numerous studies have reported expressions of HE4 protein in serum and in tumor specimens of women with EOC[3-12], but little was reported about the expression of HE4 mRNA[24, 25]. Prior studies only reported a significantly increased mRNA level of HE4 in EOC[24, 25]. In the present study, we not only reported the increased mRNA expression of HE4, but also indicated cutoff values for the relative level of HE4 mRNA to discriminate EOC from benign and borderline tumors at comparatively high sensitivity and specificity. Furthermore, we also found that high levels of HE4 mRNA predict a better overall survival in primary EOC. To our knowledge, this is the first study to report the prognostic value of HE4 mRNA levels in malignancy. Similarly, no previous report was about eIF3a mRNA in malignancy, although a number of studies revealed the role of eIF3a protein in many types of cancer[20-23]. The first report about eIF3a protein levels in women with EOC and the correlation between eIF3a protein and HE4 protein was published by our group[23]. To our knowledge, the present study is also the first investigation about the expression and clinical importance of eIF3a mRNA in EOC, as well as the association of HE4 mRNA with eIF3a mRNA.

A negative correlation of HE4 with lymph node metastasis is reported by this study. We have not found any association between the HE4 mRNA level and age, FIGO stage, tumor grade, residual tumor, ascites, NACT, and menopause which were previously shown to correlate with the serum level or IHC expression of HE4[3, 6]. Our data indicated that HE4 mRNA levels have a significant prognostic value in primary EOC, but interestingly, its negative correlation with the survival of patients is contrary to those results on protein level in previous reports[4, 5, 7-10] as well as in our own study. Consequently, to investigate the relationship between mRNA and protein levels of HE4 in this study, we performed IHC and demonstrated that HE4 mRNA level is not associated with its protein level. Our results arouse the curiosity regarding the mechanism of HE4's function, especially the role of HE4 in the malignant biological behaviors of ovarian cancer.

Although a number of reports have shown that increased HE4 protein level correlates with poor survival in EOC[3, 6], the underlying mechanism is unclear and the effect of HE4 protein in the tumorigenesis of EOC remains controversial[9, 26-28]. Several recent studies confirmed that HE4 overexpression promotes proliferation, invasion and metastasis of ovarian cancer cells[9, 26], yet some earlier work showed decreased growth and invasiveness of ovarian cancer cells induced by HE4 overexpression[27, 28]. Interestingly, Huan Wang et al. found that at low concentrations (0.083-0.2µg/ml), exogenous HE4 protein was able to significantly increase viability, proliferation, and colony formation of SKOV-3 cells, but higher concentration (1µg/ml) of exogenous HE4 protein did not further enhance the above effects[29]. It seemed that the effect of HE4 protein on malignant cells varied depending on its levels. It was previously reported that some proteins, such as Nox4[30] and histone H3[31], could cause feedback inhibition on their own mRNA expressions. Here, we speculate that HE4 may have a similar effect. HE4 mRNA and protein expressions initially increase with the malignant transformation of ovarian cells. As HE4 protein reaches a higher level and accumulates constantly, it causes feedback inhibition on its own mRNA, leading to an unparalleled expression profile of HE4 mRNA and protein. This could explain our observations that poor survival was associated with high levels of HE4 protein and low levels of HE4 mRNA, and that HE4 mRNA negatively correlated with lymph node metastasis in the present study. The speculation calls for further investigations.

Our study indicated that HE4 mRNA played a unique role in predicting the prognosis of EOC, which was different from that of its protein. Furthermore, the high correlation between HE4 mRNA level and serum CA125 level increases the prognostic significance of HE4 mRNA in EOC.

Unfortunately, we do not find any significance about eIF3a mRNA distribution among ovarian tumors and its correlation with clinicopathologic features of primary EOC. The varying range of eIF3a mRNA level is much smaller than that of HE4 mRNA level in this study, which implies a stable role of eIF3a in EOC. However, there is still a positive linear correlation between mRNA expressions of HE4 and eIF3a, similar to their protein levels[23], suggesting some connections between them in their molecular mechanism.

As retrospective research, there are regretting limitations. One is that there is no data on HE4 serum levels available to investigate its correlation with the HE4 mRNA level. Another is that the period of follow-up was relatively short to observe the differences in survival of EOC. Longer follow-up and an increased number of patients may lead to further confirmation of our study.

In summary, our study demonstrated that high levels of HE4 mRNA could discriminate malignant from benign tumors in primary EOC and show a better prognosis than low levels of HE4 mRNA. Unlike HE4, eIF3a was not found to have a similar role in EOC. Further investigations are required to confirm the unique independent role of HE4 mRNA

and offer new insight into the molecular mechanism between HE4 and eIF3a.

Abbreviations

HE4: human epididymis protein 4; EOC: epithelial ovarian cancer; eIF3a: eukaryotic translation initiation factor 3a; IHC: immunohistochemistry; KPS: Karnofsky Performance Status; FIGO: Federation of Gynecology and Obstetrics; OS: overall survival; PFS: progression free survival; NACT: neoadjuvant chemotherapy.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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