Expression of preoperative KISS1 gene in tumor tissue with epithelial ovarian cancer and its prognostic value

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Abstract
Our study aimed to elucidate the role of Kisspeptin (KISS1) in tumor tissues of patients with epithelial ovarian cancer (EOC) and investigate the prognostic value of this biomarker.

Forty EOC patients and 20 uterine fibroids female patients with healthy ovaries undergoing cytoreductive surgery between January 2010 and January 2014 in our hospital were enrolled in this study. KISS1 expression in tumor and normal tissues was detected. Correlations between clinic-pathologic variables and KISS1 expression in EOC tissues and the prognostic value of KISS1 for overall survival were evaluated.

During the follow-up of 11.2 to 62.1 months, the overall survival rate and mean survival time were 28.9% (11/38) and 38.35±2.84 months. Preoperative KISS1 mRNA was higher in tumor tissue than in normal tissue (P<0.001), and it was associated with histologic grade of tumor, surgical FIGO stage, metastasis, and residual tumor size (all P<0.05). Multivariate survival analysis indicated significant influence of residual tumor size (HR = 2.357, P = 0.039) and preoperative KISS1 mRNA (HR = 0.0001, P < 0.001) on mean survival time. Patients with low KISS1 mRNA expression had shorter survival time than those with high expression (P = 0.001).

Preoperative KISS1 mRNA was a potential prognostic biomarker for EOC, and high preoperative KISS1 expression indicated a favorable prognosis.

Abbreviations: BMI = body mass index, EOC = epithelial ovarian cancer, FIGO = Federation of Gynecology and Obstetrics, KISS1 = Kisspeptin, PBS = phosphate buffered solution, SD = standard deviation, UF = uterine fibroids.

Keywords: epithelial ovarian cancer, KISS1 mRNA, metastasis, survival, tumor

1. Introduction
Epithelial ovarian cancer (EOC) is one of the most fatal cancers of female reproductive system.[1] Its lack of early specific signs or symptoms also makes it as one of the most common causes of cancer-related deaths among women, because newly diagnosed patients may be already in advanced stage of cancer and hence miss the optimal opportunity for cytoreductive surgery, as evidenced by a 5-year survival rate <40% for these patients.[2,3]

Despite the efficacy of cytoreductive surgery in patients diagnosed with primary EOC,[4,5] recurrence and metastasis are the major problems influencing patients’ survival.[6] Therefore, it is of great importance to investigate potential prognostic biomarkers that could allow for prevention of cancer invasion and metastasis, with the ultimate goal of improving patient prognosis.

Kisspeptin (KISS1) gene was originally identified in melanoma by Lee et al.[7] in experiments designed to identify the molecules responsible for the antimetastatic effect of human chromosome 6. Previous researches focusing on solid tumors, such as brain, breast, bladder, gastric, and pancreatic cancers, have demonstrated the correlations between reduce level of KISS1 mRNA and increased tumor progression as well as poor prognosis.[8-13] However, the KISS1 expression and its clinical/prognostic relevance in epithelial ovarian cancer still remained not fully investigated.

Hence, the prospective study was designed to assess the expression of preoperative KISS1 in tumor tissue and determine the role of preoperative KISS1 mRNA or other prognostic factors in overall survival for EOC patients after cytoreductive surgery.

2. Methods
2.1. Patients
A total of 40 patients who were scheduled for cytoreductive surgery for EOC between January 2010 and January 2014 in our hospital were enrolled in this study. The inclusion criteria were as follows: a clinical diagnosis of EOC based on overall staging and...
surgical procedure as well as a pathologic diagnosis by more than 2 pathological physicians; patients were Han people aged above 18 years (after pubertal development); a body mass index (BMI) range of 18 to 30 kg/m² with a gravidity of 1 to 3 and parity of 1 to 3 times; patients had no blood relationship with each other; and who were willing to receive platinum-based chemotherapy. Anybody who was in pregnancy, or who with malignant tumor of other organs or tissues, or who had a history of radiotherapy, chemotherapy and an administration of gonadal hormone before operation was excluded.

A total of 20 uterine fibroids (UF) female patients with healthy ovaries were also included in this study as control group under similar conditions including age >18 years, a BMI of 18 to 30 kg/m², a gravidity of 1 to 5, and a parity of 1 to 3. All these patients were willing to undergo surgical resection of their normal ovaries. All the procedures in this study were approved by the Regional Ethics Committee of our hospital and informed consents were obtained from each patient.

2.2. Procedure
Demographic and clinical data consisting of age, BMI, gravidity, parity were collected from all the patient. Pathological and imaging examinations were performed for each EOC patient, including histologic type, the surgical stage of EOC according to International Federation of Gynecology and Obstetrics (FIGO) staging system,[14] and the histologic grade of tumor based on the WHO grading system.[15]

During surgery, ovarian tissue samples of tumor were collected from all the EOC patients, and normal ovarian tissue samples were obtained from each UF patient. Then tissue sample (3 g) was rinsed with phosphate buffered solution (PBS) and stored in liquid nitrogen. Residual tumor size after surgery was detected for EOC patients and was defined as the long diameter of macroscopic focus. All these patients received platinum-based adjuvant chemotherapy for 2 to 10 courses after the surgical procedure as well as a pathologic diagnosis by more than 2 pathological physicians; patients were Han people aged above 18 years (after pubertal development); a body mass index (BMI) range of 18 to 30 kg/m² with a gravidity of 1 to 3 and parity of 1 to 3 times; patients had no blood relationship with each other; and who were willing to receive platinum-based chemotherapy. Anybody who was in pregnancy, or who with malignant tumor of other organs or tissues, or who had a history of radiotherapy, chemotherapy and an administration of gonadal hormone before operation was excluded.

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Level of preoperative KISS1 mRNA in tissues was quantitatively determined by real-time PCR. Briefly, total RNAs were isolated from 50 mg of fragmented frozen tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. After concentrations and quality measurements of RNA by BioPhotometer plus (Eppendorf, Hamburg, Germany), cDNA was reverse transcribed from 1 μg of total RNA using Roche Reverse Transcription kit (Roche, Basel, Switzerland) and oligo (dT) primers (Takara). The KISS1 primers were: 5'-CCACCCCTGGAGACATCCTCA-3' (forward primer) and 5'-GCCGAAGGAGTTCCAGTT-3' (reverse primer). The house-keeping gene, β-actin, was used as an internal control for normalization of the results. The β-actin primers were: 5'-ATCATGTTTGGACCTCTCAAC-3' (forward primer) and 5'-CATCTCTAGGTCAGTCA-3' (reverse primer). PCR procedure was performed as follows: 95°C for 5 minutes; 35 cycles of 94°C for 45 seconds, 58°C for 40 seconds, and 72°C for 40 seconds. Subsequently, PCR products were examined by 2% agarose gel electrophoresis, and then scanned by an automatic electrophoresis gel imaging analysis system (Chemi Imager 5300, Alpha Innotech, San Leandro, CA, USA).

2.3. Follow-up
The EOC patients were followed up for lymph node or abdominopelvic cavity metastasis and survival every 3 months with 2 years after surgery, and thereafter every 6 months. The follow-up data were obtained from Medical History Taking and clinic service records, or by telephone and letters from patients or their familialities. Survival time was defined as the length of time from the date of surgery to the date of death or the last follow-up. The ending point was defined as death or the date of the last follow-up, and the cause of death was also recorded.

2.4. Statistical analyses
Data were shown as mean±standard deviation (SD). Comparisons of clinical characteristics between patients with tumorous ovaries and healthy ovaries and correlations between clinical-pathologic variables and preoperative KISS1 expression in EOC patients were analyzed by an independent sample t test. Cox regression analysis (Forward LR method) was used to assess prognostic factors associated with survival time and for the multivariate analysis. Overall survival rates were calculated using the Kaplan–Meier method and Kaplan–Meier survival curve was generated by log-rank test. All statistical analyses were performed using SPSS 21.0 (SPSS Inc, Chicago, IL) and P<0.05 was considered statistically significant difference.

3. Results
Among the 40 EOC patients, pathological and imaging examinations revealed 24 (60.0%) serous cystadenocarcinoma, 6 (15.0%) mucous cystadenocarcinoma, 7 (17.5%) endometrioid carcinoma, and 3 (7.5%) clear cell carcinoma. According to surgical FIGO (2006) staging system, 7 (17.5%) cases of EOC in stage I, 5 (12.5%) cases in stage II, 24 (60.0%) cases in stage III, and 4 (10.0%) cases in stage IV were included in this study. As to histologic grade, tumors were divided into 12 (30%) cases of G1, 8 (20%) cases of G2, and 20 (50%) cases of G3.

During the follow-up time of 11.2 to 62.1 months, 11 of the 40 EOC patients were alive. Two patients were lost to follow-up due to the wrong telephone and address, and the follow-up rate was 95% (38/40). According to the Kaplan–Meier analysis, the overall survival rate and mean survival time were 28.9% (11/38) and 38.35±2.84 months (95% CI, 32.78–43.92 months), respectively. Besides, 33 patients (including cases in FIGO stage II-IV) had metastasis and 7 cases (FIGO stage I) had no metastasis.

The clinical and histological characteristics between EOC patients with tumorous ovaries and UF with healthy ovaries were shown in Table 1. There were no differences in age, BMI, gravidity, and parity between the 2 groups of patients (all P>0.05), whereas the levels of preoperative KISS1 mRNA in tumor tissue were higher than that in normal tissue (P<0.001). Interestingly, Table 2 shows that in EOC patients the preoperative KISS1 mRNA expression in tumor tissue was associated with histologic grade of tumor, surgical FIGO stage, metastasis, and residual tumor size. KISS1 expression was higher in the low histologic grade (G1) of tumor tissue and early surgical FIGO stage (I-II) of EOC compared with that in high grade (G2-G3) and advanced stage (III-IV), respectively (P=0.006 and P<0.001, respectively). Patients with no metastasis or small residual tumor had higher levels of preoperative KISS1 mRNA levels in tumor tissue than that with metastasis or large residual tumor (P<0.001 and P=0.007, respectively). There were no correlations between preoperative KISS1 mRNA expression and other suppositional factors else (P>0.05).
BMI = body mass index, KISS1 = Kisspeptin.

Furthermore, the variables with \( P < 0.05 \) were included in the Cox regression analysis (Forward LR method). Multivariate survival analysis indicated that both residual tumor size (hazard ratio = 2.357, \( P = 0.039 \)) and preoperative KISS1 mRNA in tumor tissue (hazard ratio = 0.0001, \( P = 0.001 \)) significantly influenced mean survival time (Table 3), suggesting that EOC patients with residual tumor <1 cm or high preoperative KISS1 mRNA in tissue were more likely to live longer. However, histologic grade of tumor (\( P = 0.233 \)), surgical FIGO stage (\( P = 0.772 \)), and metastasis (\( P = 0.723 \)) were not statistically associated with survival time. According to the Kaplan–Meier analysis, the mean survival times of patients with high (>0.81) and low (<0.81) KISS1 expression in tissues were 45.67 ± 3.48 and 27.84 ± 3.16 months, respectively (Fig. 1, log-rank test, \( P = 0.002 \)).

### Table 1
Comparisons in clinical characteristics between female patients with epithelial ovarian cancer (tumorous ovaries) and uterine fibroids (healthy ovaries).

| Patients with tumorous ovaries (n = 40) | Patients with healthy ovaries (n = 20) | \( P \) value |
|----------------------------------------|----------------------------------------|--------------|
| Age, y                                 |                                        |              |
| Mean                                   | 53.28 ± 11.10                          | 56.75 ± 9.95 | 0.12         |
| Range                                  | 30–83                                  | 44–72        |
| BMI, kg/m\(^2\)                        | 23.20 ± 2.88                           | 23.64 ± 2.67 | 0.57         |
| Gravida, times                         | 19.9–29.4                              | 20.1–29.5    |
| Parity, times                          |                                        |              |
| Mean                                   | 3.10 ± 1.36                            | 2.96 ± 1.15  | 0.67         |
| Range                                  | 1–5                                    | 1–5          |
| Preoperative KISS1 mRNA in tissue      |                                        |              |
| Mean                                   | 0.81 ± 0.10                            | 0.59 ± 0.08  | <0.001       |

Data were shown as mean ± standard deviation (SD). The relative expression of KISS1 mRNA was calculated using the β-actin as internal reference. \( P < 0.05 \) was considered statistical significance.

### Table 2
The levels of preoperative KISS1 mRNA in tumor tissue in female patients with epithelial ovarian cancer.

| Patients number | KISS1 mRNA | \( P \) value |
|-----------------|------------|--------------|
| Age             |            |              |
| <50 y           | 15         | 0.80 ± 0.11  | 0.65         |
| ≥50 y           | 25         | 0.81 ± 0.10  |             |
| Histologic type |            |              |
| Serous          | 24         | 0.79 ± 0.10  | 0.21         |
| Nonserous       | 16         | 0.83 ± 0.11  |             |
| Histologic grade* |        |              |
| G1              | 12         | 0.87 ± 0.08  | 0.006        |
| G2–G3           | 28         | 0.78 ± 0.10  |             |
| Surgical FIGO stage† |  |              |
| I–II            | 12         | 0.91 ± 0.04  | <0.001       |
| III–IV          | 28         | 0.76 ± 0.08  |             |
| Lymph node and abdominopelvic cavity metastasis |  |              |
| Negative        | 7          | 0.94 ± 0.02  | <0.001       |
| Positive        | 33         | 0.78 ± 0.10  |             |
| Residual tumor size |      |              |
| ≤1 cm           | 28         | 0.83 ± 0.10  | 0.007        |
| >1 cm           | 12         | 0.74 ± 0.09  |             |

Data were shown as mean ± standard deviation (SD). The relative expression of KISS1 mRNA was calculated using the β-actin as an internal reference. \( P < 0.05 \) was considered statistical significance.

### Table 3
Multivariate analyses of mean survival time in female patients with epithelial ovarian cancer.

| Variables                     | \( P \) value | Hazard ratio (HR) | 95% CI       |
|-------------------------------|--------------|------------------|--------------|
| Histologic grade (G1 vs G2–G3) | 0.233        | —                | —            |
| Surgical FIGO stage (I–II vs III–IV) | 0.772        | —                | —            |
| Metastasis (negative vs positive) | 0.723        | —                | —            |
| Residual tumor size (<1 cm vs >1 cm) | 0.039        | 2.557            | 1.042–5.332   |
| Preoperative KISS1 mRNA in tissue | <0.001        | 0.0001           | 0.0000–0.0070 |

Cox regression analysis (Forward LR method) was used to assess prognostic factors associated with survival time. \( P < 0.05 \) was considered statistical significance.

### 4. Discussion
Despite many reports demonstrating that KISS1 gene had an effect on tumor progression, metastasis, and patient survival among various of cancers,[16] the clinical or prognostic role of the KISS1 gene expression in EOC had been rarely reported yet. In the present study, we conducted a prospective analysis of 40 cases with epithelial ovarian cancer and found that the overall survival rate and mean survival time were 28.9% and 38.35 ± 2.84 months, respectively. Additionally, it was confirmed that both residual tumor size and preoperative KISS1 mRNA were significantly associated with prognosis in overall survival.

Martin et al[19] have reported high expression of KISS1 mRNA in patients with breast cancer, particularly in those with aggressive tumors. Also, it was also found in this study that KISS1 mRNA level was significantly higher in tumor tissues of EOC compared with that in normal tissue. However, these results were directly converse to several recent studies.[12,17,18]

In this study, preoperative KISS1 mRNA in tumor tissue appeared dependent on histologic grade of tumor and surgical FIGO stage, similar with a previous study.[12] However, Zhu et al[19] reported that both residual tumor size and preoperative KISS1 mRNA were significantly associated with prognosis in overall survival.

KISS1 mRNA levels were directly converse to several recent studies.[12,17,18] Furthermore, KISS1 mRNA expression in tumor tissue was associated with metastasis and residual tumor size. Conversely, Ikeguchi et al[20] reported similar KISS1 mRNA expression in esophageal squamous cell carcinoma as in the noncancerous epithelia, and that no relationship between KISS1 mRNA expression and tumor size or degree of tumor invasion but with lymph node metastasis. Besides, the low number of patients included in this study might be another reason for the correlation of KISS1 mRNA expression with histologic grade, surgical FIGO stage, metastasis, and residual tumor size, as the group is not distributed symmetrically with respect to the fore-mentioned variables (12 G1 vs 28 G2–3; 12 stage I–II vs 28 stage III–IV; 7 negative vs 33 positive; 28 ≤ 1 cm vs 12 > 1 cm, respectively).

According to the result, the presence of metastasis and residual tumor size was negatively associated with KISS1 expression were more likely to live longer.
epithelial ovarian cancer, and high preoperative KISS1 mRNA expression had shorter survival time than those with high-censored KISS1 mRNA expression (log-rank test, \( P = 0.001 \)). KISS1 = Kisspeptin.

mRNA, suggesting patients with low KISS1 expression were more subjected to metastasis or residual tumors. This might be explained by the inhibitive effect of KISS1 overexpression on tumor cell migration.[12] As recurrence and metastasis were mainly responsible for the death in cancer,[6] these patients usually had a poor prognosis. This could be supported by a hazard ratio of 0.0001 for KISS1 gene expression indicating an improved patient prognosis, as evidenced by longer survival time in patients with high KISS1 mRNA expression than those with low expression according to Kaplan–Meier survival curves (\( P = 0.001 \)). Besides, residual tumor size also negatively influenced mean survival time.

Several limitations to this study must be addressed. First, the patient included in this study was not sufficient. Second, comparisons between primary EOC tumor and the secondary metastatic site and detection of KISS1 mRNA from further multivariate survival analysis, that was, high KISS1 gene expression indicating an improved patient prognosis, as evidenced by longer survival time in patients with high KISS1 mRNA expression than those with low expression according to Kaplan–Meier survival curves (\( P = 0.001 \)). Besides, residual tumor size also negatively influenced mean survival time.

This study indicated that preoperative KISS1 mRNA in tumor tissue appeared dependent on histologic grade of tumor and surgical FIGO stage. It was confirmed that preoperative KISS1 mRNA could be a potential prognostic biomarker for epithelial ovarian cancer, and high preoperative KISS1 mRNA expression in tumor tissue indicated a favorable prognosis. Besides, residual tumor size might also have a reverse effect on survival time.

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