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Original Article

Prognostic value of STC1 expression in ovarian cancer

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Abstract: Objective: The goal of this study was to explore the prognostic role of STC1 in patients with ovarian cancer (OC). Methods: A retrospective study was performed. In total, there were 136 OC patients undergoing surgical treatment in Renmin Hospital of Wuhan University during January 2013 to September 2016. The tissue expression of STC1 was measured by immunohistochemistry staining. Serum level of STC1 and CA125 were tested by ELISA kits. Results: STC1 expression was elevated in OC patients compared with non-OC patients and it correlated with OC patients' clinical characteristics and overall survival. Additionally, higher serum STC1 level was positively correlated with CA125 and both serum STC1 and CA125 were independent prognostic markers in OC. Furthermore, the combination of STC1 and CA125 showed better predictive power than the two indicators operated alone. Conclusion: Therefore, STC1 acted as an oncogene in OC development and progression. Detection of either STC1 expression in tissue or serum could be a useful prognosis marker.

Keywords: STC1, ovarian cancer, biomarkers, ROC, prognosis

Introduction

Ovarian cancer (OC) is one of the most malignant gynecological cancers and its annual incidence is 11.4 per 100,000 [1]. The prognosis of ovarian cancer is extremely poor. About 60% of patients with OC are diagnosed with metastases and it has been estimated that 14080 OC patients died in 2017 in the USA [2]. Even though most OC patients underwent surgery and standard chemotherapy, about half of the patients still had recurrence within 16 months after surgery. In UK, about 9 in 10 women with ovarian cancer would survive for 5 years or more when they were diagnosed at its earliest stage, while less than 5 in 100 of women would survive for 5 years or more when diagnosed at the latest stage [3]. Heterogeneity between tumors makes it less reliable in predicting the clinical prognosis of OC patients solely relying on histopathological results or extent of tumor resection [4]. Accumulated evidence has shown that several serum biomarkers are associated with clinical severity of OC patients, which might have important role in early assessment of the clinical outcome of OC patients. Accordingly, exploring non-invasive and more reliable markers remains one of the main issues in current areas of research.

CA125, a serum biomarker, is one of the most important prognostic parameters of OC. While accumulating evidence shows that serum CA125 alone still has many deficiencies in predicting clinical outcome of OC patients [5, 6]. The combination of CA125 and other serum biomarkers improved the prognostic value, especially for patients with advanced OC [7-9]. Stanniocalcin 1 (STC1) is a glycoprotein first described in 1996 from human brain and reported to mediate many physiological and pathological functions, including hypoxia, tumorigenesis, angiogenesis, cell proliferation, apoptosis, and microenvironment regulation [10-12]. STC1 can be secreted into the patient's peripheral blood or body fluid by tumor cells or stromal cells. Several studies have found that circulation STC1 could be used as a promising serum candidate biomarker for tracking tumor progression [13]. Ma X et al. found that STC1 expression could be induced under hypoxic conditions and the expression of STC1 was significantly correlated with TNM stage of patients with clear cell renal cell carcinoma [14]. Furthermore, studies revealed that STC1 was overexpressed in ovarian cancer tissue than in normal ovarian tissue and STC1 might play a crucial role in ovarian tumorigenesis [15]. However, little is currently known about the role

of STC1 in predicting the prognosis of OC patients.

This is a retrospective study to investigate the prognostic role of STC1 in OC. As a result, either tissue or serum level of STC1 was found to be elevated in OC patients when compared with non-OC patients. Additionally, both serum CA-125 and STC1 were independent risk factors for poor outcome of OC patients. When CA125 was combined with STC1 to predict the poor outcome of OC, the combination of these two markers was found to improve the predictable value. These results indicate that STC1 acts as an oncogene in OC development and progression. Serum STC1 can be used as a complementary marker for CA125 in predicting clinical prognosis of OC.

Methods

Study population

This study included 136 OC patients who underwent surgical treatment in Renmin Hospital of Wuhan University during January 2013 to September 2016. Another 32 patients with benign pelvic tumor are also included. Data collection included age, Menopausal status, tumor stage, tumor size, metastasis state, pathological type. Tumor stage was determined according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system. All patients or their relatives were informed and consents were written for all patients. The study was approved by the Ethical Committee at Renmin Hospital of Wuhan University.

Immunohistochemistry staining

The OC tissues were used for IHC analysis. Samples were dewaxed, hydrated, and incubated with 3% hydrogen peroxide after being baked at 60°C for 2 hours. A microwave was used for antigen retrieval in 0.01 M sodium citrate buffer (pH 6.0) for 30 minutes and then 10% normal goat serum was taken to prevent non-specific staining for 30 minutes. The sections were then incubated with STC1 rabbit monoclonal antibody overnight at 4°C. The next day, sections were incubated with biotinylated goat anti-rabbit secondary antibodies for 30 minutes at room temperature. The stained sections were independently evaluated by two experienced pathologists. The score was based on the positive rate of cell staining (number of positive cells stained per 100 cells) and staining intensity. The positive rate of staining less than 5% scored 0, 5%-25% scored 1, 26%-50% scored 2, 51%-75% scored 3 and >75% scored 4. Additionally, 0 points for non-staining, 1 for light yellow, 2 points for brown, and 3 for brown. Final quantification was obtained by multiplying two scores. Scores were defined as 0 score for negative, 1-4 score for weak, 5-8 score for positive and 9-12 score for strong. Finally, those with an IHC score <4 were grouped as low expression and those with IHC score was 4 or more were grouped as high expression.

Serum markers measurement

Blood samples of patients were collected only on admission before receiving any medical treatments. All samples were centrifuged at 2000 rpm for 10 minutes at 4°C and the supernatant fluid was stored at -80°C for further measurement. ELISA kits (ELISA Kit for CA125 (SEA154 Mu), STC1 (SEC874Hu), Wuhan USCN business Co., Ltd) were used. All operations were performed according to the manufactures' instruction.

Bioinformatics analysis

Online bioinformatics website is utilized. GEPIA (http://gepia.cancer-pku.cn/index.html) is a TCGA-based tumor database. This database was used to analyze the expression of STC1 in OC patients and non-OC patients and the relationship between STC1 expression and overall survival of OC patients.

Statistical analysis

The descriptive analysis (mean, standard deviation, median) was used for continuous variables and percentage for categorical variables. t test was used for comparisons between two groups and One-Way ANOVA analysis for three or more groups comparisons. Box-plot was used to present levels of Nesfastin-1 between different groups, which were conducted by using Graphpad Prism 5. Univariate and multivariate regression analyses were used to analysis the independent risk factors of poor outcome and Receiver operating characteristic (ROC) curves was used to access the variables' prediction ability. Regression analyses and ROC curves were conducted using SPSS 21.

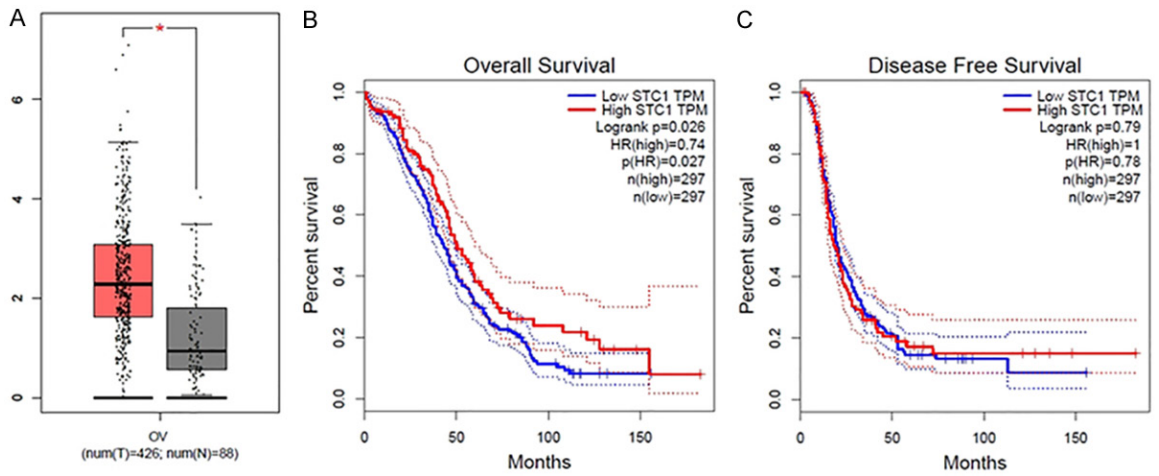

Figure 1. Bioinformatics Analysis of STC1 expression in OC STC1 was overexpressed in OC tissues than normal ovary tissues (A). OC patients with higher expression of STC1 had poorer overall survival than patients with lower STC1 expression (B). While there was no difference in disease free survival between two groups (C).

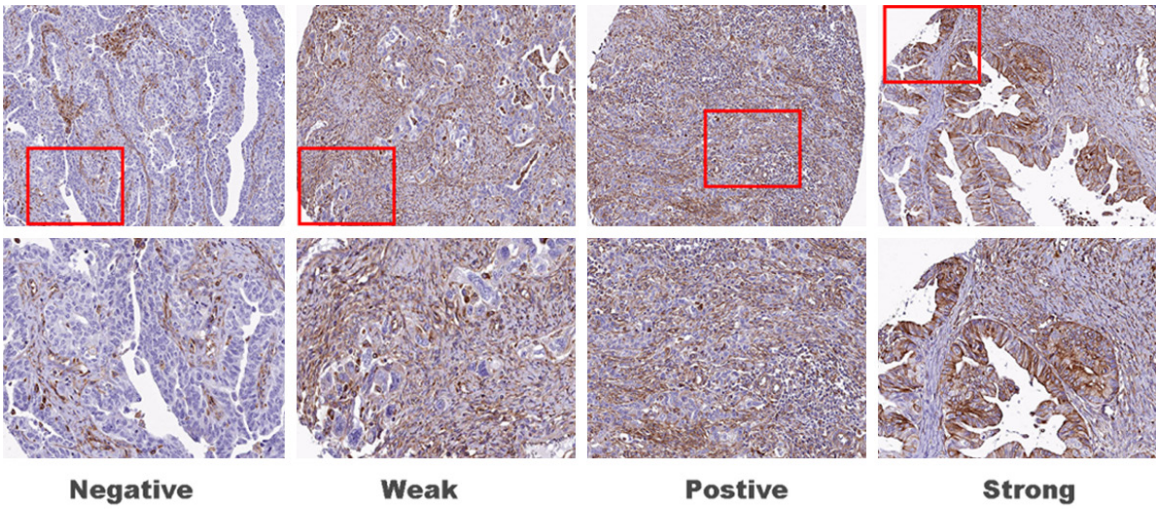

Figure 2. STC1 was detected in OC tissues by using immune-histochemical staining. The stained sections were independently evaluated by two experienced pathologists. Final quantification was obtained by multiplying positive rate of staining and staining intensity. Negative and weak staining were grouped as low expression, while positive and strong staining were grouped as high expression.

Results

Bioinformatics analysis

First, bioinformatics were used to find out the expression of STC1 in ovarian cancer. The results showed that STC1 was significantly elevated in ovarian cancer tissues compared with normal ovarian tissues. Furthermore, patients with high expression of STC1 had shorter overall survival time than patients with low expression of STC1. But no significant difference was observed in disease free survival between two groups. See **Figure 1**.

STC1 expression elevated in OC tissues and correlated with clinical characteristics

Next, immune-histochemical staining was used to detect expression of STC1 in the tumor tissues of 136 patients with OC. The results showed that 74 tissues (54.41%) were identified as high expression and another 62 tissues (45.59%) were staining lower expression (**Figure 2**). The comparison of clinic-pathologic

Table 1. STC1 expression and clinicopathologic parameters

Groups	Total	Expression of STC1		
		Low	High	*P* value
Age >50 y	72	38	44	0.77
Histological type				
Serous cystadenocarcinoma	73	32	41	
Endometrioid adenocarcinoma	24	11	13	
Mucinous cystadenocarcinoma	21	10	11	
Others	18	9	9	
Grading				0.017
G1+G2	43	26	17	
G3	93	36	57	
FIGO stage				0.039
Early stages (I+II)	54	25	29	
Late stages (III+IV)	82	21	51	
Lymph node metastasis				0.878
Yes	58	26	32	
No	78	36	42	
CA125				0.002
≤500	96	52	44	
>500	40	10	30	

characters between the high expression group and low group is presented in **Table 1**.

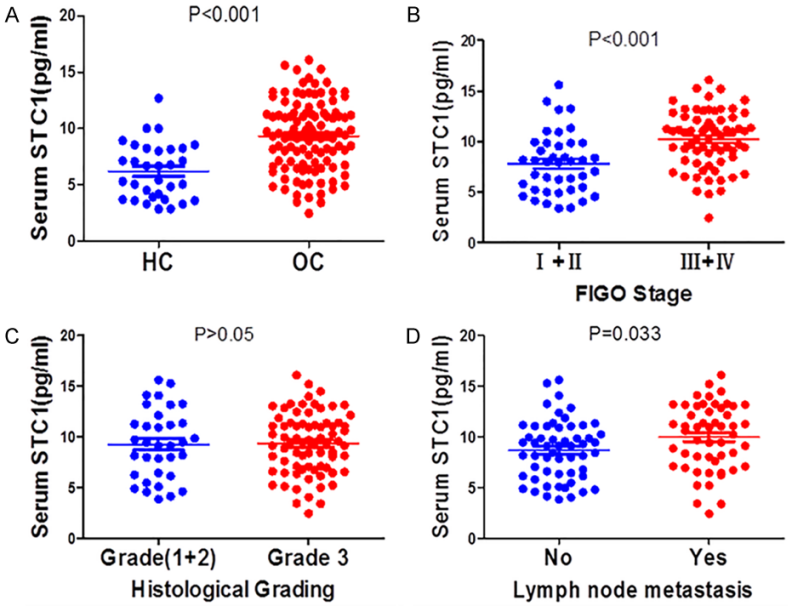

Figure 3. Serum STC1 correlated with clinical indicators. ELISA kits were used to detect the level of serum STC1 and CA125. The results show that OC patients had higher level of STC1 than non-OC patients and serum STC1 level was elevated in patients with higher FIGO stage, positive lymph node metastasis compared with patients with lower FIGO stage and negative lymph node metastasis.

High expression of STC1 predicts poor overall survival OS of OC patients

Kaplan-Meier survival curve was used to evaluate the prognostic role of tissue STC1 in OC patients. The median survival time of patients in the high expression STC1 group and was 19.00 months, while the median survival time of patients in the low expression STC1 group was 26.5 months (OR=0.72, 95% CI [0.003 to 1.43], P<0.0001). These results indicate that OC patients with higher level of STC1 might have poor clinical prognosis.

Serum STC1 correlated with clinical indicators

Blood samples were routinely collected from OC patients. In total, 112 patients were included, and retrospectively analyzed blood samples from these 112 OC patients and 32 non-OC patients were used. The supernatants were kept and stored in a -80 degree refrigerator for further use. ELISA kits were used to detect the level of STC1 and CA125. Level of serum STC1 was positively correlated with CA125 (r=0.425, P<0.01). Additionally, the results showed that serum level of STC1 was elevated in patients with OC compared with non-OC patients (9.59±2.93 vs 6.07±1.32, P<0.001, **Figure 3A**). Furthermore, the patients with higher FIGO stage (III+IV) or positive lymph node metastasis had higher level of serum STC1 when compared with patients with lower FIGO stage

Table 2. Univariate analyses of factors associated with overall survival

Variables	Overall survival; HR 95% CI	*P* value
Age (>50 y vs ≤50)	1.34 (0.41-2.16)	0.264
Menopause (Pre vs post)	0.79 (0.46-1.05)	0.361
Serous cystadenocarcinoma	2.81 (1.30-5.94)	0.032*
Tumor size (>5 cm vs ≤5 cm)	1.13 (1.01-1.87)	0.218
Histological Grading (3 vs 1-2)	2.07 (1.27-4.31)	0.180
FIGO stage (III+IV vs I+II)	4.70 (2.71-8.96)	0.007*
Lymph node metastasis (yes vs no)	2.16 (1.38-9.87)	0.016*
Serum CA125 (high vs low)	2.02 (1.16-3.47)	0.0013*
Serum STC1 (high vs low)	1.72 (1.23-3.74)	0.002*

*, statistical significance.

Table 3. Multivariate analyses of factors associated with overall survival

Variables	Overall survival; HR 95% CI	*P* value
FIGO stage (III+IV vs I+II)	4.24 (2.41-10.07)	0.0014
Serum CA125 (high vs low)	1.67 (1.16-2.87)	0.04
Serum STC1 (high vs low)	1.90 (1.21-3.27)	0.02

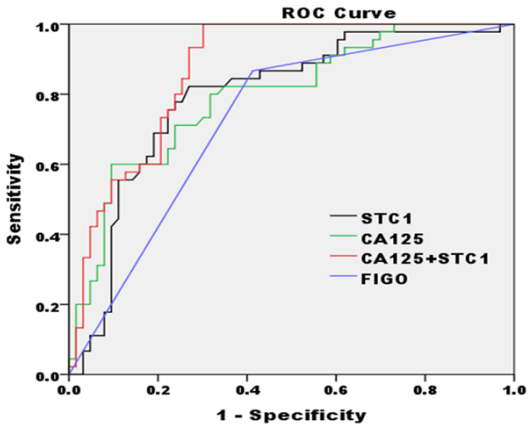

Figure 4. ROC of serum STC1 in predicting overall survival of OC patients.

or negative lymph node metastasis (10.24±2.84 vs 7.84±3.02, P<0.0001, 10.00±3.23 vs 8.71±2.91, P=0.033, respectively, **Figure 3B** and **3D**). But there was no difference of serum STC1 level between patients with higher histological grading and lower grading (9.35±3.01 vs 9.28±3.39, P>0.05, **Figure 3C**).

Risk factors associated with poor overall survival

Next, univariate and multivariate analysis were used to find out the prognostic factors of OC patients. In univariate analysis, the following prognostic markers associated with poor overall survival were identified: higher CA125 (HR, 1.72, 95% CI: 1.23-3.74, P=0.0013), higher serum STC1 expression (HR, 1.72, 95% CI: 1.23-3.74, P=0.002), serious cystadenocarcinoma (HR, 2.81, 95% CI: 1.30-5.94; P=0.032), higher FIGO stage (HR, 4.70, 95% CI: 2.71-8.96; P=0.007), positive lymph nodes (HR, 2.16, 95% CI: 1.38-9.87; P=0.016) as shown in **Table 2**. Next multivariate analysis was used to find independent risk factors associated with poor overall survival of OC patients (**Table 3**). In multivariate analysis, FIGO stage III+IV (HR, 4.24, 95% CI: 2.41-10.07; P=0.0014), higher serum CA-125 level (HR, 1.67, 95% CI: 1.16-2.87; P=0.04), and higher serum STC1 level (HR, 1.90, 95% CI: 1.21-3.27; P=0.02) were significantly associated with poor overall survival.

ROC of serum STC1 in predicting overall survival of OC patients

ROC was used to access the predictive power of serum STC1 and CA125. When STC1 and CA125 predicted the overall survival of OC patients independently, the area under the curve (AUC) were 0.793, 0.813, respectively (**Figure 4**). But when the two indicators were combined to predict the overall survival of OC patients, the AUC could be as high as 0.872. These results indicated that serum STC1 might be a good complement for CA125 in predicting overall survival of OC patient.

Discussion

In this study, STC1 was found to be elevated in OC tissue compared to non-OC tissues both in bioinformatics analysis and our tissue specimens verification. Further analysis revealed that patients with higher tissue STC1 expression had shorter overall survival. Additionally, univariate and multivariate analysis found that serum STC1 was an independent risk factor for poor overall survival. The combination of STC1 and CA125 to predict poor overall survival of OC patients was superior to CA125, which indicated serum STC1 could be used as a useful prognostic biomarker.

STC-1 gene could directly regulate the proliferation and differentiation of bone cells, and it also participated in the regulation of several subcellular functions [16]. STC1 was also found to be elevated in many solid tumors, such as glioma, breast cancer and renal cell carcinoma [17-19]. Circulation STC1 could be used as a promising serum candidate biomarker for tracking tumor progression. Early diagnosis of OC and prognosis prediction have been difficult for clinicians [20]. CA125 had been widely used as a prognostic biomarker of OC. But CA125 still had a lot of deficiencies [21, 22]. Recent literature had reported that combined with CA125 and other serum markers, such as HE4, CA199 and CEA [6, 8], could improve the ability of early diagnosis and prediction of the overall survival of OC patients. In this study, STC1 was highly expressed in OC patients and OC patients with high expression of STC1 had a poor clinical prognosis. Moreover, it was more accurate of combining serum STC1 and CA125 together to predict poor overall survival of OC patients. These results suggest that serum STC1 might be used as a supplemental marker for CA125 in the prediction of overall survival of OC patients. Therefore, STC1 can be used as a new good biomarker for predicting the overall survival of OC patients.

There are however some limitations in this study. This was a retrospective study and the number of patients included was relatively small. In addition, OCs of different pathological types were included, which may cause bias in subsequent survival analysis. Furthermore, the treatments received after surgery in different patients did not appear complete, and the treatments after tumor surgery were particularly important for the prognosis of patients.

Acknowledgements

This study was approved by the Ethics Committee of Renmin Hospital of Wuhan University and informed consents of patients included were signed by themselves or relatives.

Disclosure of conflict of interest

None.

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