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Original Article

TTLL12 expression in ovarian cancer correlates with a poor outcome

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Abstract: The tubulin-tyrosine ligase (TTLL) family is involved in the progression of many cancers. Tubulin-tyrosine ligase-like protein 12 (TTLL12), a member of the TTLL family, has functions of histone methylation and affects the activities of tubulin tyrosine ligase, which are often observed abnormally in many cancers. Recently, a TTLL12 isoform was reported as abnormal in many cancer cells, but the potential role of TTLL12 in ovarian cancer (OC) is still unknown. In this study, we used quantitative real-time RT-PCR and western blot to determine the expressions of TTLL12 in ovarian cancer cells and tissues and also performed immunohistochemical staining to examine the TTLL12 expression levels in 72 OC tissues and their matched adjacent normal ovarian tissues (ANOTs), to further explore the potential clinical features. The results showed that the TTLL12 expression level in OC tissues was significantly increased when compared to the ANOTs. In addition, TTLL12 expression was also remarkably upregulated in OC cell lines compared to the normal ovarian cell line. Furthermore, we found that the TTLL12 level was significantly associated with the clinical features of the FIGO stage (P=0.001) and peritoneal cytology (P=0.042). Moreover, TTLL12 is thought to be an independent risk factor for the overall survival (OS, P=0.022) and disease-free survival (DFS, P=0.040) of OC patients. In conclusion, this study identified TTLL12 as a potential molecular marker for predicting the invasion and progression of OC.

Keywords: Biomarker, TTLL12, ovarian cancer, prognosis

Introduction

Accounting for the primary common causes of relevant cancer deaths in women, ovarian cancer (OC) has caused an estimated 125,000 deaths worldwide each year [1]. Until now, OC has been credited for a high mortality rate, with the 5-year survival rate being 30% in the advanced stages [2]. Although huge modern clinical improvements have been made in OC treatment including the development of iconography, the introduction of chemotherapy, and refined surgical skills, the prognosis of OC is still unsatisfactory, and the percentage of OC patients in the progressing period (stage III or stage IV) in their first visit in hospital is over 60% [3], which is primarily blamed for the deficiency of early detection and the improvement against recurrence and metastasis. It is clear that novel biomarkers and biotargets will be appreciated for enabling early diagnosis and more precise therapies for OC and for preventing the recurrence and metastasis of OC.

Recently, there have been more and more studies about the functions of tubulins in tumor progression since evidence has revealed that tubulin genes become more abnormal during cancer progression [4, 5]. The distinctive expression of tubulin-related genes has been found in lots of tumors [6]. βIII-tubulin was found highly expressed in non-small cell lung cancer (NSCLC) and is related to poor prognosis after lesion resection [7]. Experiments showed that groups with high expression levels of βIII-tubulin displayed poor responses to chemotherapy in many different cancers [8]. Further, other related tubulin genes, like βIVa-tubulin [9] and βV-tubulin [10], that promote insensitivity to chemotherapy have been discovered in experiments using low expression control groups in many cancers. The data suggest that the abnormal activities of tubulins might be involved in cancer progression and in promoting resistance to chemotherapy. Moreover, microtubules, assessed by tubulins, recently have been determined to be important in tumor therapy [11, 12]. Reports

Table 1. Correlations between TTLL12 and the clinicopathologic variables of OC

Variable	Number	TTLL12		*P* value
		Low	High	
Age (years)				
<50	54	22	32	0.790
≥50	18	8	10	
Tumor size (cm)				
<10	32	12	20	0.632
≥10	40	18	22	
FIGO stage				
I-II	26	18	8	0.001
III-IV	46	12	34	
CA125 (before surgery/ml)				
<1000	26	9	17	0.458
≥1000	46	21	25	
Peritoneal cytology				
negative	21	5	16	0.042
positive	51	25	26	

showed that the abnormal activities of microtubules might cause function and structure errors in chromosomes which could cause chromosome instability (CIN) [13]. Studies have shown that CIN can lead to intracellular variations that can promote the transition of cell phenotypes into malignant features, such as enhanced proliferation ability and insusceptibility to chemotherapy [14]. Therefore, the activities of tubulins are remarkably important for tumor progression in terms of their functions related to regulating microtubules, so targeting tubulin-related genes will serve as a potential cancer therapy and a good way to improve susceptibility to chemotherapy.

Tubulin-tyrosine ligase-like 12 (TTLL12) is a member of tubulin tyrosine ligase-like (TTLL) family [4] and located at 22q13 with a protein size of 644-amino acids [15]. The TTLL family was primarily discovered as tubulin related proteins that could modulate the activities of tubulin or microtubules [16], including TTLL12. More experiments and tests have found that the TTLL family is closely related to abnormal expressions in tumors and cancer progression [17]. In recent studies, increased copy numbers of TTLL1 have been found in neuroblastoma [18, 19], and the expression of TTLL4 in pancreatic ductal adenocarcinoma (PDAC) was also found to be upregulated. The knockdown of TTLL4 in PDAC cells can inhibit the proliferation ability in PDAC cells, and upregulated TTLL4 can promote PDAC growth [20]. Further, additional studies have found that TTLL12 could modulate intracellular changes for promoting histone methylation, modifying chromosome structures and the activities of tubulins in larynx cancer cells [17]. Further, the expression level of TTLL12 has also been found to be upregulated in prostate cancer [17, 21]. Based on these findings and the functions of TTLL12, there is no study of TTLL12 in ovarian cancer, yet TTLL12 could be related to ovarian cancer and play a vital role in ovarian cancer progression.

In this study, we examined the expressions of TTLL12 in ovarian cancer tissues and cell lines in messenger RNA (mRNA) and the protein levels. The correlations between TTLL12 expression and the clinical features of our cohort were also studied. The clinicopathologic characteristics and prognosis of ovarian cancer were further analyzed. We suggest that TTLL12 upregulation in ovarian cancer could contribute to tumorigenesis through its effects on microtubule dynamics.

Materials and methods

Bioinformatics analysis

We collected three ovarian statistical datasets from the Oncomine database (https://www.oncomine.org/) to analyze the differences between tumor tissues and normal tissues.

Patients and specimens

Our research was approved by the ethical boards of Yangpu Hospital of Tongji University, Shanghai, China. The cohort was made up of 72 cases of OC and their tissues and matched adjacent normal ovarian tissues (ANOTs) collected at Department of Obstetrics and Gynecology, Yangpu Hospital of Tongji University, from June 2006 to July 2016. Authorizations from all the OC participants were acquired. None of these patients had been treated using any medical therapy. The clinical features, including age, CA125, peritoneal cytology, tumor size, and FIGO stage are documented and analyzed in **Table 1**. Fresh samples were collected and kept in an -80°C refrigerator and other tissues were fixed using a 10% formalin solution and embedded by paraffin.

Cell culture

Human epithelial ovarian cell lines SK-OV-3 and TOV21G and OV90 were donated by the Medical Department of Shanghai Tongji University (Shanghai, China). The normal ovarian cell line (HOEpiC) was donated by the Cancer Institute of Fudan University (Shanghai, China). These cells were cultured in DMEM media containing 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA) at 37°C with 5% CO_2.

RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted using Trizol reagent (Life Technologies, Carlsbad, CA, USA) and transformed into cDNA using a reverse transcription kit following the manufacturer's instructions. qRT-PCR was performed using a PCR kit (Roche Applied Science). The primers used in these experiments are as follows: TTLL12 (forward: 5'-GACATCCGCTACATCGTGCT-3 and reverse: 5'-TCTTCACAGTGCACCTGCTT-3'). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed as an internal control. The expression levels of TTLL12 and GAPDH were calculated using the comparative 2-ΔΔCt algorithm.

Western blot analysis

Cultured cells were collected and decomposed using a radioimmunoprecipitation assay (Beyotime, Jiangsu, China). The concentration of proteins was determined, and different proteins were isolated and moved into a polyvinylidene difluoride (PVDF) membrane (Beyotime, Jiangsu, China). The bands with targeted proteins were incubated with a TTLL12 antibody (1:1000, Thermo Scientific, Waltham, MA, USA) at 4°C overnight and incubated with a secondary antibody (1:5000, Proteintech, USA). The target protein membranes were visualized using an ECL kit (Ebio, Shanghai, China) to enhance their detection, which was exposed on X-ray film. β-actin was used as a quality control. The expression measurements of the different proteins were calculated using Image J software.

Immunohistochemical analysis

The expression of the TTLL12 protein (1:50, Proteintech, USA) in the OC tissues was measured using immunohistochemistry. The expression levels of TTLL12 were scored according to the percentage of positive OC cells: 0 (0-10%); 1 (11-25%); 2 (26-50%); and 3 (51-100%) [22]. The TTLL12 expression was defined as low if the score was 0 or 1; 2 and 3 were described as high expression. Based on the TTLL12 expression, the OC patients were divided into two groups: the low-expression group and the high-expression group.

Statistical analysis

SPSS 19.0 was used for the data analysis. Statistical comparisons of the data were performed using a *t*-test or a non-parametric test. Associations between TTLL12 expression and the clinicopathologic characteristics were analyzed using an ANOVA test. Using a Kaplan-Meier analysis, we depicted the survival curves. and a log-rank test was used to check the significance. A Cox regression analysis was applied to decide whether it would be an independent factor for the survival rate. The differences were considered significant when the *P* value was less than 0.05.

Results

TTLL12 is frequently upregulated in OC tissues and OC cell lines

To determine the expression levels of TTLL12 in ovarian cancer tissues, we first checked the TTLL12 expressions in the Oncomine database (https://www.oncomine.org/resource/login.html). As shown in **Figure 1**, we found that the TTLL12 expression levels were remarkably upregulated in ovarian cancer tissues in data from different databases, which indicated that TTLL12 might participate in ovarian cancer progression. We continued to test the TTLL12 expression levels in the 30 OC tissues and their matched normal tissues using qRT-PCR. The mRNA levels of TTLL12 in the OC tissues were significantly higher than they were in normal tissues ($P<0.05$, **Figure 2A**). Further, to check whether the TTLL12 mRNA expression was in accordance with the TTLL12 protein expression, we randomly took four paired OC tissues from among the 30 OC paired tissues to perform this test, As shown in **Figure 2C**, the protein expressions of TTLL12 were significantly unregulated in the OC tissues ($P<0.05$). Furthermore, we tested the TTLL12 expression in mRNA and the protein levels of one normal

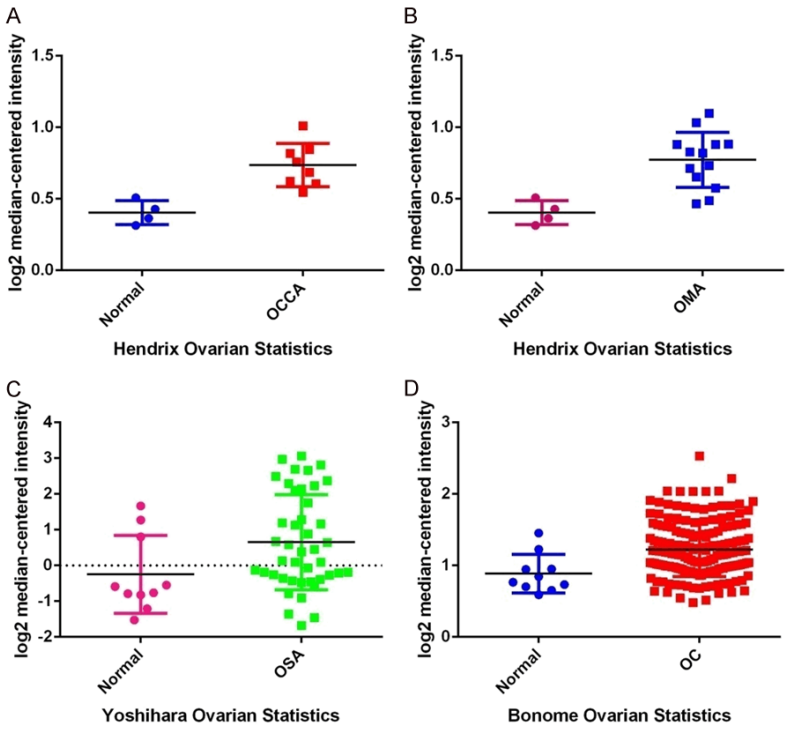

Figure 1. The overexpression of TTLL12 in ovarian cancer. A, B. RNA-Seq analysis of TTLL12 mRNA expression in ovarian cancer and normal tissues in Hendrix Ovarian Statistics. The RNA-Seq analysis used a data download from Oncomine, *P* value<0.001. C. RNA-Seq analysis of TTLL12 mRNA expression in ovarian cancer and normal tissues in the Yoshihara Ovarian Statistics. The RNA-Seq analysis used data download from Oncomine, *P* value =0.02. D. RNA-Seq analysis of TTLL12 mRNA expression in ovarian cancer and normal tissues in Bonome Ovarian Statistics. The RNA-Seq analysis used data download from Oncomine. *P* value =0.002.

ovarian cell line (HOEpiC) and three OC cell lines (SK-OV-3, T OV21G, OV90). As shown in **Figure 2B** and **2D**, our results indicated that the expression levels of TTLL12 in the OC cell lines also had been increased (P<0.001). In short, we found that TTLL12 expression was remarkably upregulated in OC and could play a positive role in the malignant features of OC.

The relationship between TTLL12 expression and the patients' clinical characteristics

Next, we determined the relationship between TTLL12 expression and the clinicopathologic features of OC. Based on the TTLL12 expression levels, the OC patients (n=72) were assigned to two different groups: the low TTLL12 expression group (n=30, **Figure 3A** and **3B**) and the high TTLL12 expression group (n=42, **Figure 3C** and **3D**). To determine whether the TTLL12 expression was related to the clinicopathologic features of OC, we analyzed the potential relationships and found that TTLL12 expression was remarkably related to the FIGO stage (P=0.001) and the peritoneal cytology stage (P=0.042, **Table 1**). As these characteristics are related to the malignant features of OC progression, we concluded that TTLL12 might become a prognostic factor for OC patients.

The association between TTLL12 expression and the prognosis of OC

Further, we performed a Kaplan-Meier analysis to determine whether the survival rate was related to the TTLL12 expression levels. The results showed that patients in the high TTLL12 expression group had a lower overall survival rate than the patients in the low TTLL12 expression group (P=0.018) (**Figure 3E**). Further, the disease-free survival rate was much lower in the high TTLL12 expression group than it was in the low TTLL12 expression group (P=0.035) (**Figure 3F**). The results indicated that the TTLL12 expression level could affect the OC patients' survival rate. Subsequently, a Cox regression was also performed to analyze whether TTLL12 is an independent risk factor of OC prognosis. We examined overall survival (OS) and disease-free survival (DFS), FIGO stage (P=0.025, P=0.007), the peritoneal cytology stage (P=0.038, P=0.025), and TTLL12 expression (P=0.021, P=0.040), and they all had notable significance respectively (**Tables 2** and **3**) according to our univariate survival analysis. In addition, FIGO stage (P=0.008, P=0.033) and TTLL12 expression (P=0.022, P=0.040) reached distinctive significance for OS and DFS, respectively (**Tables 2** and **3**) according to our multivariate survival analysis. According to these findings, we demonstrated that increased TTLL12 could promote the malignant progression of OC and indicate a poor prognosis for OC patients.

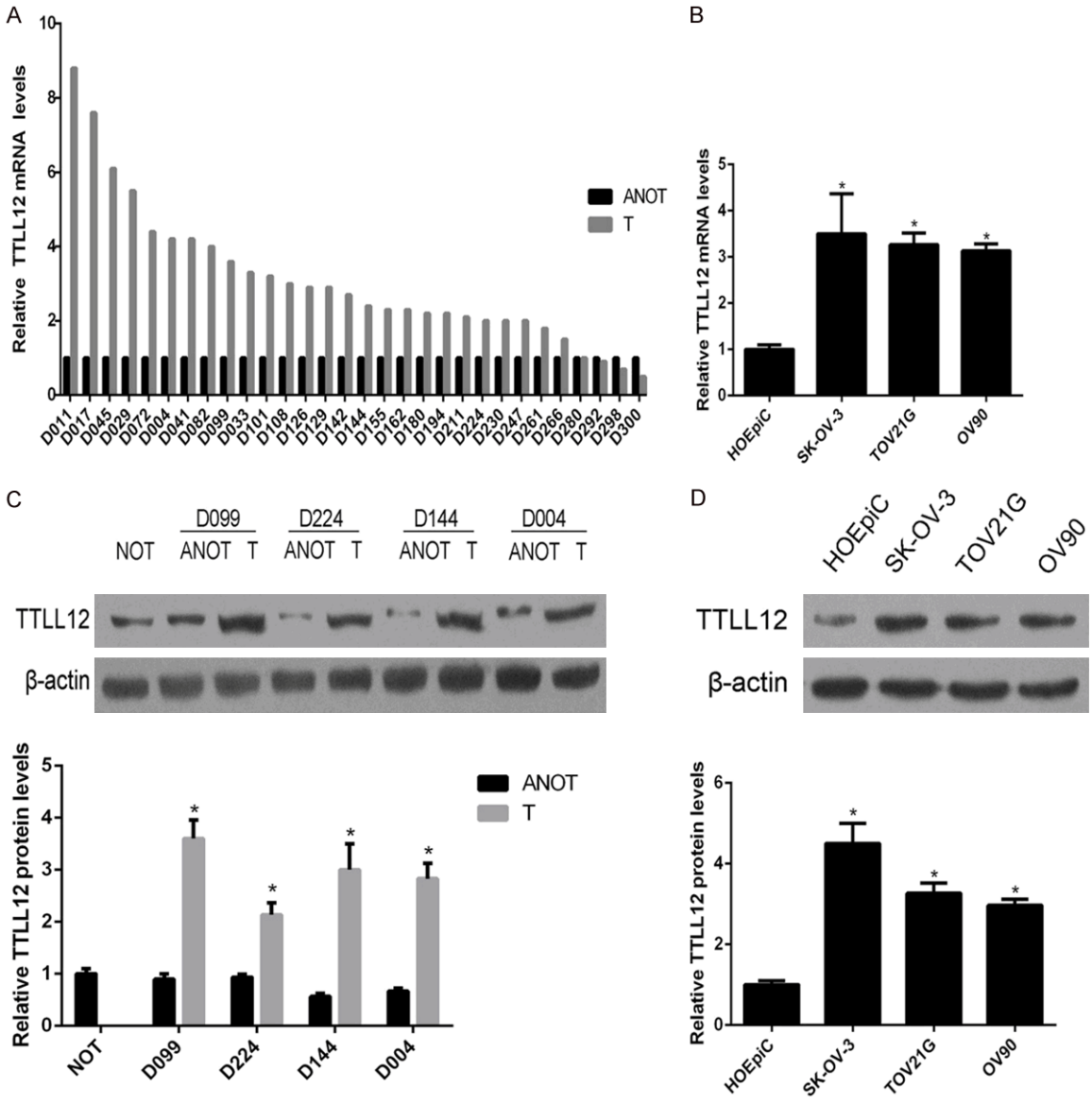

Figure 2. A. The mRNA expression of TTLL12 in 30 pairs of OC tissues and in adjacent non-tumorous ovarian tissues (ANOTS) was examined by real-time RT-PCR. GAPDH was used as an internal reference. B. The relative mRNA expressions of TTLL12 in one normal ovarian cell line (HOEpiC) and three OC cell lines (SK-OV-3, TOV21G, OV90) were analyzed by real-time RTPCR. C. The protein expression of TTLL12 in four matched OCs and ANOTs was analyzed by western blot. β-Actin was used as internal reference. NOT was used as the normal ovarian tissue. *P<0.05 vs. ANOTs. D. The protein expression of TTLL12 in the cell lines was analyzed by western blot. β-Actin was used as an internal reference. *P<0.05 vs. HOEpiC.

Discussion

Ovarian cancer is one of the most common gynecologic malignancies and has to a high mortality rate due to the lack of of specific symptoms in its early stages [23]. In addition, recurrence and metastasis are the main obstacles of OC treatment [24]. Therefore, reliable biomarkers for detecting tumor progression or cancer target therapy are important and much needed. In consideration of the role of microtubules in cell division, a lot of compounds and biomarkers have been discovered, and some of them are extensively used in the clinic to fight against cancer [10, 25]. At present, the antitubulin medicines with promising outcomes in clinical trials need more work in order to focus on safety and efficacy in cancer treatment.

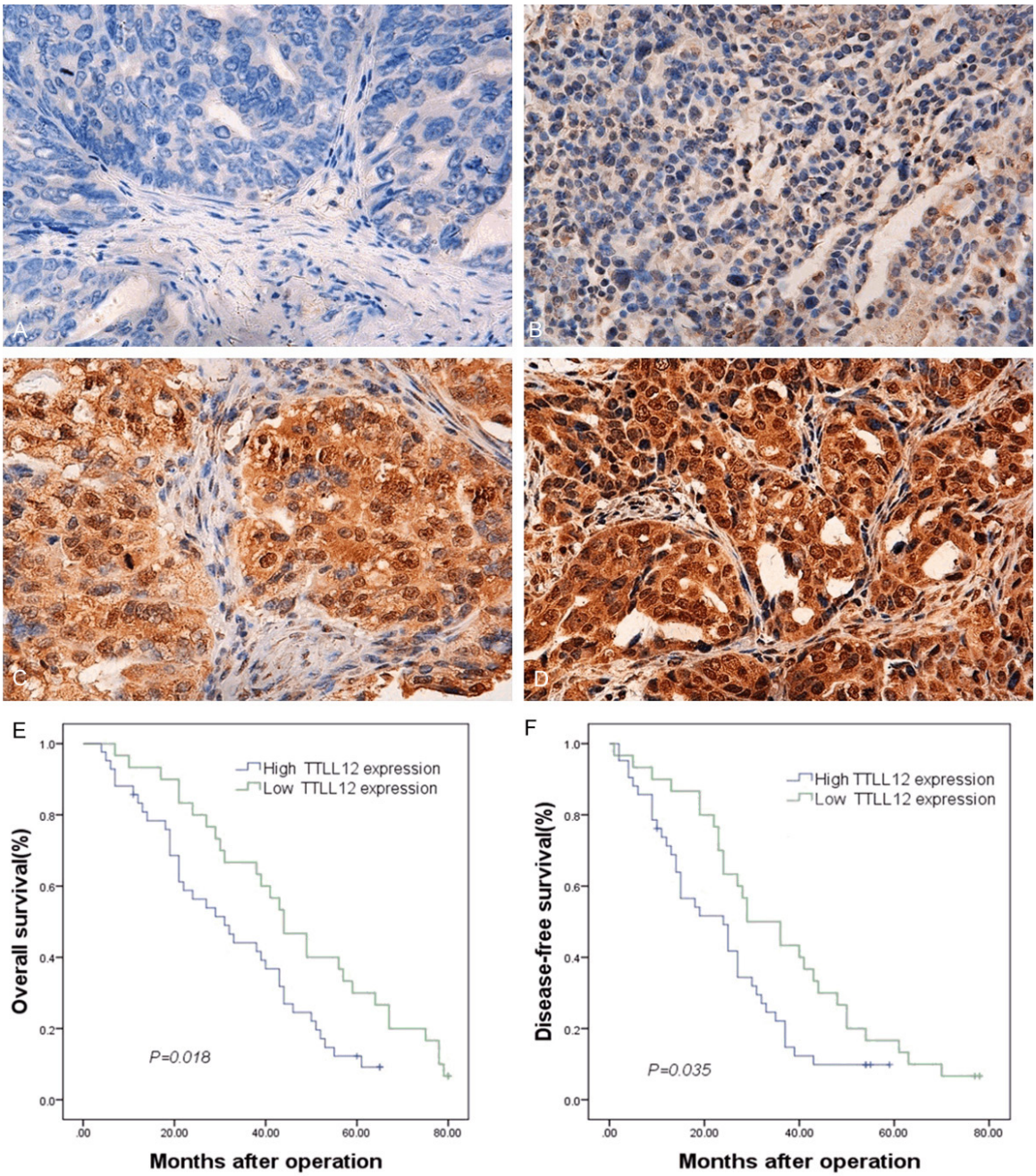

Figure 3. Immunohistochemistry was used to examine the TTLL12 expression in 72 cases of OC tissues. A. TTLL12 is negative (scored as 0). B. TTLL12 is observed in 10-24 % of HCC cells (scored as 1+). C. TTLL12 is observed in 25-49% of HCC cells (scored as 2+). D. TTLL12 is observed in more than 50% of HCC cells (scored as 3+). E, F. Overall survival and disease-free survival were analyzed according to the TTLL12 expression in 72 cases of OCs (using the Kaplan-Meier and log-rank test).

Thus, it wouldn't be too early to develop more precise tubulin inhibitors or biomarkers as cancer therapeutics. Since the tubulin-tyrosine ligase (TTLL) family plays an important role in tubulin modification, its role in human cancers and its close connection with the poor prognosis of cancers cannot be ignored

Recently, it was found that TTLL12, a member of the TTLL family, could modulate intracellular changes of histone structures and tubulin activities when its expression levels were altered. It was also reported that the TTLL family is related to caner progression, including TTLL12. The overexpression of the TTLL12 protein is

Table 2. Univariate and multivariate analysis of overall survival (OS) and TTLL12 using the Cox proportional hazards regression mode

Variable	Number	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years): <50 vs. ≥50	54 vs. 18	0.746 (0.424-1.315)	0.311		
Tumor size (cm): <10 vs. ≥10	32 vs. 40	0.916 (0.558-1.503)	0.729		
FIGO stage: I-II vs. III-IV	26 vs. 46	0.550 (0.326-0.928)	0.025	0.479 (0.279-0.823)	0.008
CA125 (before surgery U/ml): <1000 vs. ≥1000	26 vs. 46	0.965(0.525-1.773)	0.908		
Peritoneal cytology: negative vs. positive	21 vs. 51	0.569 (0.334-0.969)	0.038	0.651 (0.384-1.101)	0.109
TTLL12: low vs. high	30 vs. 42	0.533 (0.313-0.910)	0.021	0.500 (0.277-0.904)	0.022

Table 3. Univariate and multivariate analysis of BCR-free survival and TTLL12 using the Cox proportional hazards regression mode

Variable	Number	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years): <50 vs. ≥50	54 vs. 18	0.798 (0.453-1.406)	0.435		
Tumor size (cm): <10 vs. ≥10	32 vs. 40	0.981 (0.599-1.609)	0.941		
FIGO stage: I-II vs. III-IV	26 vs. 46	0.487 (0.289-0.821)	0.007	0.532 (0.297-0.951)	0.033
CA125 (before surgery U/ml): <1000 vs. ≥1000	26 vs. 46	0.945 (0.514-1.739)	0.856		
Peritoneal cytology: negative vs. positive	21 vs. 51	0.542 (0.317-0.928)	0.025	0.746 (0.428-1.299)	0.300
TTLL12: low vs. high	30 vs. 42	0.583 (0.348-0.976)	0.040	0.561 (0.323-0.975)	0.040

involved in cancer progression and may act as a oncogene. What's more, additional evidence has suggested that TTLL12 expression has been associated with metastasis and poor prognosis in cancer. So far, there has been no study investigating whether the TTLL12 gene is involved in ovarian cancer and or whether it has a prognostic value.

Therefore, we determined for the first time that TTLL12 is remarkably upregulated in OC tissues and cell lines. Furthermore, we revealed that TTLL12 expression was significantly related to lymph node metastasis, FIGO stage, pathological differentiation, and peritoneal cytology stage. These results suggest that an increased expression of TTLL12 correlates with the malignant features of OC. We further confirmed that the patients in the high TTLL12 expression group had worse OS and DFS than the patients in the low TTLL12 expression group, which might make TTLL12 a potential target for the targeted therapy of OC.

In this research, some limitations are inevitable due to the small cohort and the lack of multicenter cohorts, but our research discovered that the overexpression of TTLL12 is involved in OC for the first time. Our work found that TTLL12 is a potential target for the targeted therapy of OC. In our research, a high TTLL12 expression was associated with a poor prognosis. Further, not only functional experiments but also mechanistic experiments are needed to clear the TTLL12-mediated progression of OC.

In conclusion, our work not only displayed the relationship between TTLL12 and OC but also discovered the value of TTLL12 for the prognosis of OC.

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Disclosure of conflict of interest

None.

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References

[1] Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Kurzeder C, du Bois A,

Sehouli J, Kimmig R, Stahle A, Collinson F, Essapen S, Gourley C, Lortholary A, Selle F, Mirza MR, Leminen A, Plante M, Stark D, Qian W, Parmar MK, Oza AM and Investigators I. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 2011; 365: 2484-2496.

[2] Zhang L, Han J, Jackson AL, Clark LN, Kilgore J, Guo H, Livingston N, Batchelor K, Yin Y, Gilliam TP, Gehrig PA, Sheng X, Zhou C and Bae-Jump VL. NT1014, a novel biguanide, inhibits ovarian cancer growth in vitro and in vivo. J Hematol Oncol 2016; 9: 91.

[3] Barnholtz-Sloan JS, Schwartz AG, Qureshi F, Jacques S, Malone J and Munkarah AR. Ovarian cancer: changes in patterns at diagnosis and relative survival over the last three decades. Am J Obstet Gynecol 2003; 189: 1120-1127.

[4] Janke C, Rogowski K, Wloga D, Regnard C, Kajava AV, Strub JM, Temurak N, van Dijk J, Boucher D, van Dorsselaer A, Suryavanshi S, Gaertig J and Edde B. Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science 2005; 308: 1758-1762.

[5] Chen H, Lin Z, Arnst KE, Miller DD and Li W. Tubulin inhibitor-based antibody-drug conjugates for cancer therapy. Molecules 2017; 22.

[6] Visconti R and Grieco D. Fighting tubulin-targeting anticancer drug toxicity and resistance. Endocr Relat Cancer 2017; 24: T107-T117.

[7] Levallet G, Bergot E, Antoine M, Creveuil C, Santos AO, Beau-Faller M, de Fraipont F, Brambilla E, Levallet J, Morin F, Westeel V, Wislez M, Quoix E, Debieuvre D, Dubois F, Rouquette I, Pujol JL, Moro-Sibilot D, Camonis J and Zalcman G; Intergroupe Francophone de Cancérologie Thoracique (IFCT). High TUBB3 expression, an independent prognostic marker in patients with early non-small cell lung cancer treated by preoperative chemotherapy, is regulated by K-Ras signaling pathway. Mol Cancer Ther 2012; 11: 1203-1213.

[8] Seve P, Isaac S, Tredan O, Souquet PJ, Pacheco Y, Perol M, Lafanechere L, Penet A, Peiller EL and Dumontet C. Expression of class III {beta}-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. Clin Cancer Res 2005; 11: 5481-5486.

[9] Christoph DC, Kasper S, Gauler TC, Loesch C, Engelhard M, Theegarten D, Poettgen C, Hepp R, Peglow A, Loewendick H, Welter S, Stamatis G, Hirsch FR, Schuler M, Eberhardt WE and Wohlschlaeger J. betaV-tubulin expression is associated with outcome following taxane-based chemotherapy in non-small cell lung cancer. Br J Cancer 2012; 107: 823-830.

[10] Kavallaris M, Kuo DY, Burkhart CA, Regl DL, Norris MD, Haber M and Horwitz SB. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes. J Clin Invest 1997; 100: 1282-1293.

[11] Gyoeva FK and Gelfand VI. Coalignment of vimentin intermediate filaments with microtubules depends on kinesin. Nature 1991; 353: 445-448.

[12] Dunn S, Morrison EE, Liverpool TB, Molina-Paris C, Cross RA, Alonso MC and Peckham M. Differential trafficking of Kif5c on tyrosinated and detyrosinated microtubules in live cells. J Cell Sci 2008; 121: 1085-1095.

[13] Parker AL, Teo WS, McCarroll JA and Kavallaris M. An emerging role for tubulin isotypes in modulating cancer biology and chemotherapy resistance. Int J Mol Sci 2017; 18.

[14] Cirillo L, Gotta M and Meraldi P. The elephant in the room: the role of microtubules in cancer. Adv Exp Med Biol 2017; 1002: 93-124.

[15] Ju LG, Zhu Y, Lei PJ, Yan D, Zhu K, Wang X, Li QL, Li XJ, Chen JW, Li LY and Wu M. TTLL12 inhibits the activation of cellular antiviral signaling through interaction with VISA/MAVS. J Immunol 2017; 198: 1274-1284.

[16] Ersfeld K, Wehland J, Plessmann U, Dodemont H, Gerke V and Weber K. Characterization of the tubulin-tyrosine ligase. J Cell Biol 1993; 120: 725-732.

[17] Wen R, Xiao Y, Zhang Y, Yang M, Lin Y and Tang J. Identification of a novel transcript isoform of the TTLL12 gene in human cancers. Oncol Rep 2016; 36: 3172-3180.

[18] Trichet V, Ruault M, Roizes G and De Sario A. Characterization of the human tubulin tyrosine ligase-like 1 gene (TTLL1) mapping to 22q13.1. Gene 2000; 257: 109-117.

[19] Khan FH, Pandian V, Ramraj S, Natarajan M, Aravindan S, Herman TS and Aravindan N. Acquired genetic alterations in tumor cells dictate the development of high-risk neuroblastoma and clinical outcomes. BMC Cancer 2015; 15: 514.

[20] Kashiwaya K, Nakagawa H, Hosokawa M, Mochizuki Y, Ueda K, Piao L, Chung S, Hamamoto R, Eguchi H, Ohigashi H, Ishikawa O, Janke C, Shinomura Y and Nakamura Y. Involvement of the tubulin tyrosine ligase-like family member 4 polyglutamylase in PELP1 polyglutamylation and chromatin remodeling in pancreatic cancer cells. Cancer Res 2010; 70: 4024-4033.

[21] Wasylyk C, Zambrano A, Zhao C, Brants J, Abecassis J, Schalken JA, Rogatsch H, Schaefer G, Pycha A, Klocker H and Wasylyk B. Tubulin tyrosine ligase like 12 links to prostate cancer through tubulin posttranslational modification and chromosome ploidy. Int J Cancer 2010; 127: 2542-2553.

[22] Liu T, Chen J, Xiao S and Lei X. H2.0-like homeobox 1 acts as a tumor suppressor in hepatocellular carcinoma. Tumour Biol 2016; 37: 6419-6428.
[23] Ma Z, Wang X, He J, Xia J and Li Y. Increased expression of protein kinase CK2alpha correlates with poor patient prognosis in epithelial ovarian cancer. PLoS One 2017; 12: e0174037.
[24] Li J, Li Q, Huang H, Li Y, Li L, Hou W and You Z. Overexpression of miRNA-221 promotes cell proliferation by targeting the apoptotic protease activating factor-1 and indicates a poor prognosis in ovarian cancer. Int J Oncol 2017; [Epub ahead of print].
[25] Nebbioso A, Tambaro FP, Dell'Aversana C and Altucci L. Cancer epigenetics: Moving forward. PLoS Genet 2018; 14: e1007362.