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Over-expression of Wilms' tumor 1-associating Protein Promotes Tumorigenesis and Predicts Poor Prognosis in Endometrial Cancer

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Abstract

Background. Wilms' tumor 1-associating protein (WTAP) was previously reported to play critical roles in the tumorigenesis of different malignancies, and its expression level has been linked to a poor prognosis of several cancers. We evaluated the role of WTAP in endometrial carcinoma (EC) using publicly available data from The Cancer Genome Atlas (TCGA).

Methods. The relationship between WTAP expression and clinical characteristics was assessed with the Wilcoxon signed-rank test and logistic regression. Clinical factors associated with prognosis were evaluated using Cox regression analysis and Kaplan-Meier method. Gene set enrichment analysis (GSEA) was conducted using TCGA dataset.

Results. Our results revealed that WTAP was significantly up-regulated in EC. Increased WTAP expression in EC was significantly associated with high-grade tumor (odds ratio [OR] = 2.126) and high-risk histology (OR = 1.915) (all $P < 0.05$). Patients with high WTAP expression had a worse overall survival (OS) (hazard ratio [HR] = 2.868, 95% confidence interval [CI] = 1.259-6.532; P = 0.0087) and recurrence-free survival (RFS) (HR =3.148, 95% CI = 1.372-7.220; P = 0.004) than those with low WTAP expression. Multivariate analysis showed that WTAP expression remained an independent predictor of OS and RFS. Furthermore, GSEA showed that wnt/β-catenin signaling pathway was differentially enriched in WTAP high expression phenotype.

Conclusions. WTAP was over-expressed in EC. WTAP expression was an independent predictor of poor OS and RFS in EC, and it was associated with advanced grade, high-risk EC. Moreover, the wnt/β-catenin signaling pathway might be the key process regulated by WTAP in EC.

Introduction

Endometrial carcinomas (EC) is the second most commonly diagnosed gynecological malignancy worldwide, and its mortality rate is globally increasing [1, 2]. Despite a favorable prognosis for type I (known as endometrioid endometrial carcinomas [EEC]) and early-stage EC, the survival rate for type II (characterized by clear cell or serous histology) and advanced-stage EC still remains poor [3–5]. Therefore, it is urgently necessary to identify novel and reliable molecular markers involved in the progression and prognosis of the disease in order to provide guidance in the management of EC patients in the clinical practice.

Mammalian Wilms' tumor 1-associating protein (WTAP) is widely expressed in adult tissues at different developmental stages, and it can specifically interact with a transcriptional regulatory factor, the Wilms' tumor 1 suppressor gene WT1 [6–8]. WTAP has been proposed to be a critical mediator in regulating cell proliferation, cell cycle and apoptosis in human umbilical vein endothelial cells (HUVECs) and vascular smooth muscle cells (VSMCs) [9–11]. Besides its psychological functions, literatures have reported that WTAP is over-expressed and functions as an oncogene in tumorigenesis and progression of malignancies, and its over-expression is linked with an unfavorable prognosis of several cancers,

including renal cell carcinoma, glioma, colorectal cancer, cholangiocarcinoma, pancreatic ductal adenocarcinoma, and acute myelogenous leukemia [12–18]. However, the expression status of WTAP and its underlying mechanisms in EC as well as its association with the prognosis of EC patients remain largely unexplored.

Therefore, we aimed to investigate the WTAP expression and assess its prognostic value in EC patients in the present study. We performed Gene set enrichment analysis (GSEA) to gain more insights into the underlying molecular procedures and biological pathways involved in EC pathogenesis-associated WTAP molecular network. We found that WTAP was over-expressed in EC. WTAP expression was linked with an inferior recurrence-free survival (RFS) and overall survival (OS) and associated with advanced grade, high-risk EC. Moreover, GSEA showed that wnt/β-catenin signaling pathway was correlated with the high expression phenotype of WTAP.

Materials And Methods

RNA-sequencing patient data

Original RNA expression data as well as the corresponding clinic information for patients with EC were downloaded from the official website of The Cancer Genome Atlas (TCGA). Both cancerous samples and normal endometrial samples were included, and the EC patients without sufficient survival data were excluded from the survival analysis. Finally, 24 normal endometrial samples and 177 EC samples with both WTAP expression data and clinical information were included in the present research. A total of 167 EC patients with both WTAP expression values and OS data were available, while 144 EC patients with both WTAP expression values and RFS data were retained for further survival analysis. The clinic information was examined, including age, tumor grade, tumor stage, histological type, surgical approach and peritoneal cytology. The basic clinic characteristics were listed in Table 1.

Table 1
Basic characteristics of endometrial cancer patients

Characteristics	Number of cases	%
Age at diagnosis, y	65 (33–87)	
Age, y		
≤ 65	89	50.3
> 65	85	48.0
unknown	3	1.7
Histology		
Endometrioid	107	60.5
Serous	59	33.3
Mixed endometrioid and serous	11	6.2
Grade		
Well	14	7.9
Moderate	21	11.9
Poor	142	80.2
Tumor stage		
I	98	55.4
II	24	13.6
III	45	25.4
IV	10	5.6
Peritoneal cytology		
Positive	20	11.3
Negative	101	57.1
unknown	56	31.6
Surgical approach		
Open	78	44.1
Minimally invasive	82	46.3
unknown	17	9.6

GSEA

GSEA was performed to determine whether an identified gene sets showed dramatically significant molecular functions between two biological states [19]. In the present report, based on the association of an ordered list of all genes with WTAP expression, GSEA was firstly carried out. Then, significant differences in survival observed between “high” and “low” WTAP groups was elucidated by GSEA. In this study, GSEA was performed in the pathways and biological processes of gene sets using the GSEA software 3.0 to clarify the biological roles of WTAP expression on EC. Gene set permutations were carried out 1,000 times for each analysis. The WTAP expression values served as a phenotype label. The false discovery rate (FDR) q value and normalized p value were used to find the biological processes and pathways enriched in each phenotype.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software, USA) and SPSS 22.0 software (IBM, Ehningen, Germany). The correlation between WTAP expression and the clinic pathologic characteristics was tested with the Wilcoxon signed rank tests and logistic regression analysis. ROC curves were used to examine the pooled diagnostic power of WTAP in EC. Differences in RFS and OS between WTAP “high” and “low” expression groups were compared with Kaplan-Meier method, and P values were examined via log-rank test. Univariate and multivariate Cox regression analyses were utilized to assess the effects of WTAP expression along with other clinical features (age, tumor grade, histological type, tumor stage, surgical approach, and peritoneal cytology) on survival. Variables with $P < 0.1$ were selected for multivariate analysis. The cut-off value of WTAP expression was defined by its median value.

Results

Patient characteristics

From the TCGA EC data, 177 primary tumor samples and 24 normal endometrial samples with both clinic information and WTAP expression data were obtained. Median age of EC patients at diagnosis was 65 years old, ranging from 33 to 87 years. In our study cohort, the histological types included EEC, serous endometrial carcinoma (SEC), and mixed serous and endometrioid carcinoma (MSE). The majority of tumors (60.5%, n = 107) were of EEC, 33.3% (n = 59) were SEC, and the remaining 6.2% (n = 11) were MSE. Most tumors (80.2%, n = 142) were poorly differentiated (G3), 7.9% (n = 14) of the tumors were well differentiated (G1), while 11.9% (n = 21) were moderately differentiated (G2). Stage I, II, III, and IV accounted for 55.4% (n = 98), 13.6% (n = 24), 25.4% (n = 45) and 5.6% (n = 10), respectively. Positive peritoneal cytology was found in 11.3% (n = 20) patients, while it was negative in 57.1% (n = 101) patients. Slightly less than half of all patients (44.1%, n = 78) underwent open surgery, and 46.3% (n = 82) patients received minimally invasive surgery. Median follow-up period was 22.6 months for patients who survived at the last contact, ranging between 0 and 138.5 months.

WTAP is significantly up-regulated and associated with more aggressive clinicopathological features in EC

We first investigated the expression of WTAP at the mRNA level in 177 EC samples and 24 normal endometrial samples from TCGA database. Figure 1A shows that in EC tissues, WTAP was significantly up-regulated compared with the normal endometrial samples ($P < 0.0001$). The area under the curve (AUC) was 0.8425 (95% confidence interval [CI] = 0.7755–0.9096) with a specificity of 95.8% and a sensitivity of 59.3% (Fig. 1F), indicating that WTAP could be an underlying indicator to assist the diagnosis of EC. Moreover, the expression of WTAP was significantly increased in no matter well (G1), moderately (G2) and poorly differentiated (G3) tumors ($P = 0.0121$, 0.0004 and $P < 0.0001$, respectively, Fig. 1D) compared with the normal endometrial samples, showing an up-regulation of 1.17-, 1.35- and 1.46-fold in G1, G2 and G3 groups, respectively, compared with the control group (normal endometrium). A higher expression of WTAP at the mRNA level in G1 and G2 groups was also observed compared with the G3 group ($P = 0.0437$, Fig. 1C). Considering the stage of tumors, a significantly increased WTAP expression was found at any tumor stages (stage I, II, III and IV) compared with the normal endometrial samples (all P values < 0.0001, Fig. 1E). The WTAP expression at the mRNA level in both EEC and non-EEC groups was higher compared with the control group (both P values < 0.0001). Moreover, the WTAP expression was also higher in the non-EEC groups (MSE or SEC) than that in EEC patients ($P = 0.0155$, Fig. 1B).

Univariate logistic regression analysis (WTAP expression as a categorical variable according to the median value of 0.93) showed that high WTAP expression was linked with poor prognostic clinical factors (Table 2). Increased WTAP expression was obviously correlated with high-grade tumor (odds ratio [OR] = 2.126 for poor vs. well or moderate, 95%CI = 1.019–4.433, $P = 0.044$) and high-risk histology (OR = 1.915 for non-EEC vs. EEC, 95%CI = 1.038–3.531, $P = 0.037$) in EC. These findings suggested that EC patients with high WTAP expression were prone to develop to a more advanced stage in comparision with those with low WTAP expression.

Table 2

WTAP expression [a] associated with clinical pathological characteristics (logistic regression).

Clinical characteristics	Total (N)	Odds ratio in WTAP expression	P values
Age (> 65 vs. ≤65)	174	1.741 (0.955–3.173)	0.07
Grade (grade 3 vs. grade1/2)	177	2.126 (1.019–4.433)	**0.044**
Stage (III -IV vs. I-II)	177	1.425 (0.751–2.704)	0.278
Histology (non-endometrioid vs. endometrioid)	177	1.915 (1.038–3.531)	**0.037**
Peritoneal cytology (positive vs. negative)	126	1.152 (0.439–3.018)	0.774
Surgical approach (open vs. minimally invasive)	160	0.902 (0.485–1.678)	0.746

[a] Categorical dependent variable, greater or less than the median expression level.

High expression of WTAP predicts poor survival of EC patients

We carried out Kaplan-Meier survival analysis to assess the effects of WTAP expression on survival in EC patients. Figure 2 shows that EC subjects with high WTAP expression had a more inferior RFS and OS compared with those with low WTAP expression (P = 0.0044 and 0.0087, respectively). The univariate Cox regression model showed that high WTAP expression was obviously correlated with a poor OS (hazard ratio [HR] = 2.868; 95% CI = 1.259–6.532; P = 0.009) and RFS (HR = 3.148; 95% CI = 1.372–7.220; P = 0.004). Other clinical features correlated with poor OS included positive peritoneal cytology, advanced stage, and non-endometrioid histology, while advanced stage was also a factor associated with poor RFS (Table 3).

Table 3
Univariate and multivariate overall survival and recurrence-free survival analyses of prognostic factors

Variables	OS		RFS	
	HR (95% CI)	P-value	HR (95% CI)	P-value
A. Univariate analysis				
WTAP expression (high vs low)	2.868 (1.259–6.532)	**0.0087**	3.148 (1.372–7.220)	**0.004**
Age (> 65 vs ≤ 65)	1.062 (0.516–2.183)	0.872	1.404 (0.657–3.003)	0.38
Grade (grade 3 vs grade 1/2)	5.128 (0.697–38.462)	**0.073**	1.185 (0.446–3.148)	0.73
Stage (III-IV vs I-II)	4.167 (1.852–9.346)	**< 0.001**	3.650(1.704–7.813)	**< 0.001**
Histology				
non-endometrioid vs endometrioid	2.370 (1.130–4.975)	**0.019**	1.595 (0.757–3.357)	0.22
Peritoneal cytology				
positive vs negative	2.887 (1.160–7.182)	**0.018**	2.031 (0.589–7.002)	0.25
Surgical approach				
open vs minimally invasive	1.287 (0.604–2.742)	0.513	1.571 (0.699–3.533)	0.27
B. Multivariate analysis				
WTAP expression(high vs low)	2.999 (1.189–7.563)	**0.02**	2.640 (1.138–6.122)	**0.024**
Grade (grade 3 vs grade 1/2)	2.824 (0.337–23.689)	0.339		
Stage (III-IV vs I-II)	4.499 (1.606–12.603)	**0.004**	3.087 (1.431–6.659)	**0.004**
Histology				
non-endometrioid vs endometrioid	1.693 (0.588–4.875)	0.329		
Peritoneal cytology				
positive vs negative	1.309 (0.433–3.956)	0.633		

HR: hazard ratio; CI: confidence interval; OS: overall survival; RFS: recurrence free survival.

High expression of WTAP was independently correlated with an inferior RFS (HR = 2.640, 95% CI = 1.138–6.122, P = 0.004) and OS (HR = 2.999, 95% CI = 1.189–7.563, P = 0.02) along with tumor stage in EC patients in a multivariate analysis.

WTAP regulates wnt/β-catenin signaling pathway in EC via GSEA

To identify potential signaling pathways of WTAP implicated in the EC tumorgenesis, we conducted GSEA between WTAP “high” and “low” expression groups. GSEA reveals statistical differences (FDR < 0.25 and normalized P value < 0.05) in enrichment of MSigDB Collection (h.all.v6.2.symbols.gmt) for a certain gene set. The results showed that among the first eight enriched gene sets (wnt/β-catenin, UV-response, Hedgehog signaling, TGF-β signaling, KRAS signaling, mitotic spindle, angiogenesis, spermatogenesis), wnt/β-catenin pathway was significantly enriched in the group with high WTAP expression (Table 4, Fig. 3).

Table 4
Gene sets enriched in phenotype high

Gene set name	NES	Nomalized p-value	FDR
HALLMARK_WNT_BETA_CATENIN	1.730	**0.001**	**0.174**
HALLMARK_UV_RESPONSE_DN	1.42764	0.009	0.611
HALLMARK_HEDGEHOG_SIGNALING	1.35473	0.015	0.560
HALLMARK_TGF_BETA_SIGNALING	0.96982	0.023	1
HALLMARK_KRAS_SIGNALING_DN	0.96436	0.035	1
HALLMARK_MITOTIC _SPINDLE	0.8545	0.038	1
HALLMARK_ANGIOGENESIS	0.83975	0.045	1
HALLMARK_SPERMATOGENESIS	0.83931	0.048	1
NES: normalized enrichment score; FDR: false discovery rate; Gene sets with normalized p-value < 0.05 and FDR < 0.25 are considered as significant.			

Discussion

More recent studies have shown the expression and biological roles of WTAP in human malignancies [12–18]. WTAP functions as an oncogene in tumorigenesis of several malignant tumors, and its enhanced expression is associated with poor prognosis as well [12–18]. As far as we known, the expression of WTAP and its potential effect on regulation of biological behaviors involved in EC pathogenesis, as well as its prognostic value in EC have not yet been investigated. Our present research provided more insights into the functions of WTAP in carcinogenesis, development, prognostic evaluation as well as therapeutic regimen of EC.

In this research, bioinformatic analysis utilizing RNA sequencing data as reported by the TCGA revealed an increased expression of WTAP in EC, and its over-expression was associated with unfavorable clinical features, such as high-grade tumor and high-risk histology. This finding suggested that EC patients with high WTAP expression were more prone to contributing to progression compared with those with low WTAP expression. Furthermore, WTAP high expression was correlated with short survival time, poor OS and RFS. To further explore the biological roles of WTAP in EC, we carried out GSEA using data from TCGA. GSEA showed that wnt/β-catenin signaling pathway was differentially enriched in the WTAP high expression phenotype. This finding implicated that WTAP might act as a novel diagnostic and prognostic biomarker for EC and offer more treatment choices.

Studies have reported that embryonic stem cells and embryos with mutant WTAP fail to differentiate into mesoderm and endoderm during embryonic development, revealing the indispensable role of WTAP in keeping normal growth and differentiation in mouse embryo [20, 21]. We demonstrated that WTAP was correlated with differentiation in EC tumor tissues in our present analysis. A similar association between WTAP and tumor differentiation has also recently been reported, including malignant glioma, pancreatic ductal adenocarcinoma and acute myelogenous leukemia [14, 15, 17]. However, the molecular mechanisms underlying differentiation still remain largely unexplored.

It is widely believed that the majority of EC patients are classified as type I EEC linked with unopposed estrogen stimulation and favorable prognosis or type II non-endometrioid tumor with poor outcomes [5, 22, 23]. In this study, we demonstrated that WTAP was significantly over-expressed in EC tissues, especially in type II non-endometrioid tumors, suggesting that WTAP, like p53, acted as a useful biomarker to distinguish non-EEC from EEC [24–26]. This finding indicated that WTAP might serve as an underlying marker for pre-operative identification of EC patients needing aggressive treatment.

EEC represents a range of carcinomas, from poorly to well differentiated tumors (such as high to low grade), whereas non-EEC is a high-grade tumor by definition. This may be explained to some extent by the enhanced expression of WTAP in non-EEC groups compared with EEC groups considering the association of high WTAP expression with poor differentiation in EC tissues as demonstrated in our present study. Additionally, high expression of WTAP in EEC might also contribute to the poor differentiation in EEC tissues. However, this need to be further studied in future research.

In this report, we observed that WTAP high expression phenotype was associated with wnt/β-catenin signaling pathway by GSEA. A few reports have proposed that wnt/β-catenin signaling pathway is involved in multiple cellular functions. Previous studies have revealed a critical role of wnt/β-catenin signaling pathway in cell proliferation in liver regeneration [27, 28]. Aberrant activation of wnt/β-catenin signaling pathway is correlated with tumorigenesis of several cancers as well, such as EC and prostate cancer [29–31]. Furthermore, wnt/β-catenin signaling pathway has been shown to be crucial in the process of invasion and migration in laryngeal squamous cell carcinoma and non-small cell lung cancer [32, 33]. Additionally, literature has reported that WTAP can enhance malignant potential of tumor cells in several cancers [12–18], including inhibiting apoptosis, promoting proliferation, and accelerating invasion

and migration of cancer cells. Zhang et al. have reported that WTAP exerts its cancer-promoting role by regulating wnt/β-catenin signaling pathway via WTAP– WT1–TBL1 axis in colorectal cancer [18]. However, whether WTAP exerts its effects on EC through wnt/β-catenin signaling pathway as demonstrated by GSEA in this current study needs to be further elucidated.

There are limitations in the present study. On the one hand, the association between the WTAP expression at the mRNA and protein levels could not be clearly evaluated in this report due to the limitations in our research design. On the other hand, only small amount of data regarding recurrence sites and distant metastasis were available, which were not enough for further analysis, therefore limiting the clinical outcome evaluation of this study. Consequently, further study is required.

Collectively, over-expression of WTAP in EC tumor tissues suggested its potential role as a diagnostic biomarker for EC. In addition, WTAP could function as a promising target for therapeutic utility due to its potential effects on EC. Besides, high expression of WTAP might be an underlying prognostic biomarker of poor OS and RFS in EC. Moreover, the wnt/β-catenin signaling pathway could be the critical signaling pathway regulated by WTAP in EC. However, further experimental validation should be conducted to elucidate the biological effects of WTAP.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the TCGA (http://cancergenome.nih.gov/).

Competing interests

The authors declare that there are no conflicts of interest.

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Author' contributions

Wenli Xie contributed to the study design and wrote the paper. Naifu Liu participated in the study design. Xiangyu Wang and Wenyan Xie contributed to data collection and statistical analysis. Dapeng Li and Xiugui Sheng supervised the research. All authors read and approved the final version of this manuscript.

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Figures

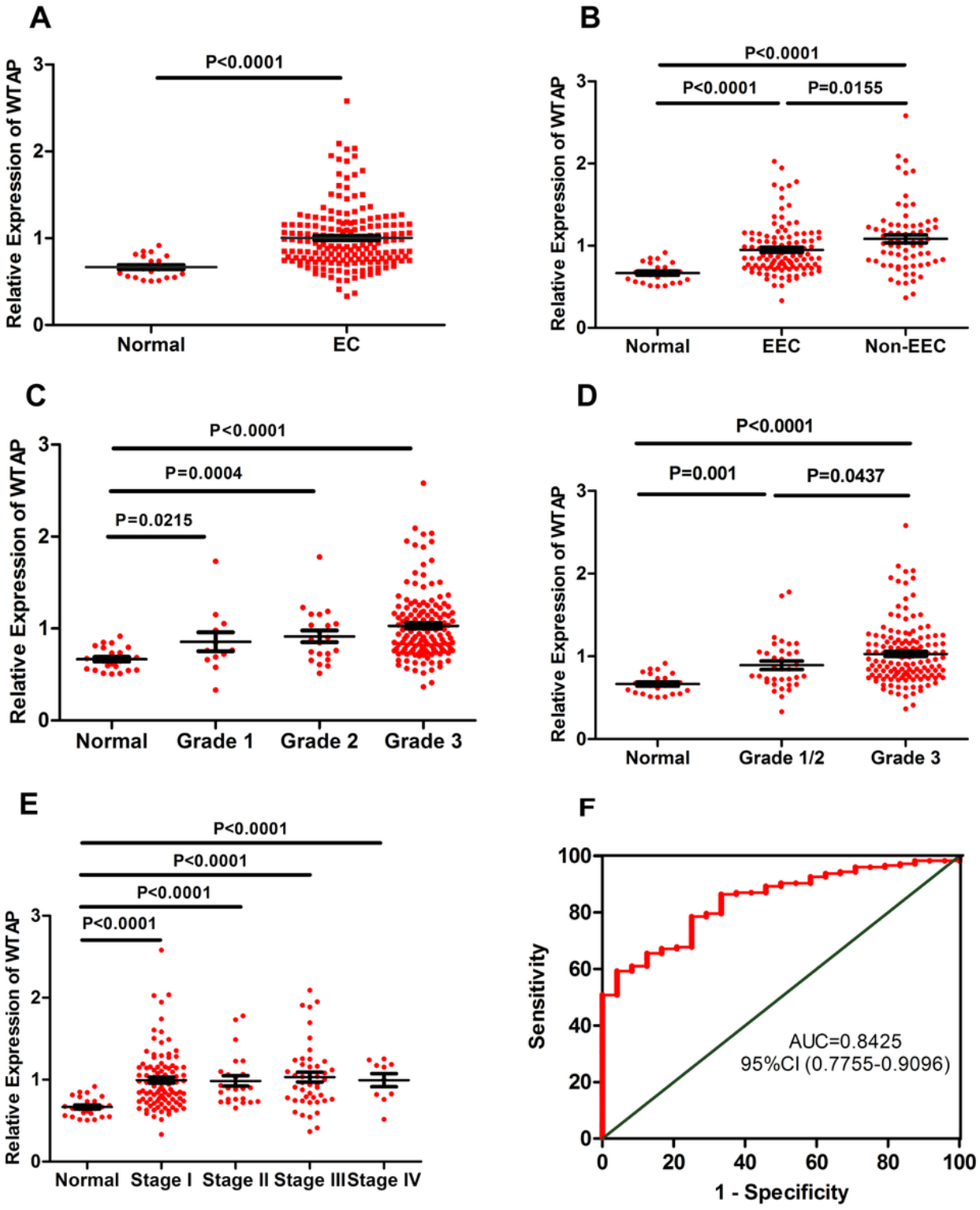

Figure 1

WTAP expression in EC tissues compared with normal endometrium (A), and its association with clinicopathologic features, including (B): Histological type (normal vs. EEC vs. non-EEC), (C): Grade (normal vs. grade 1 or grade 2 vs. grade 3), (D): Grade (normal vs. grade 1 vs. grade 2 vs. grade 3), (E): Stage (normal vs. Stage I vs. Stage II vs. Stage III vs. Stage IV), (F): ROC curves for evaluating the diagnostic power of WTAP in EC.

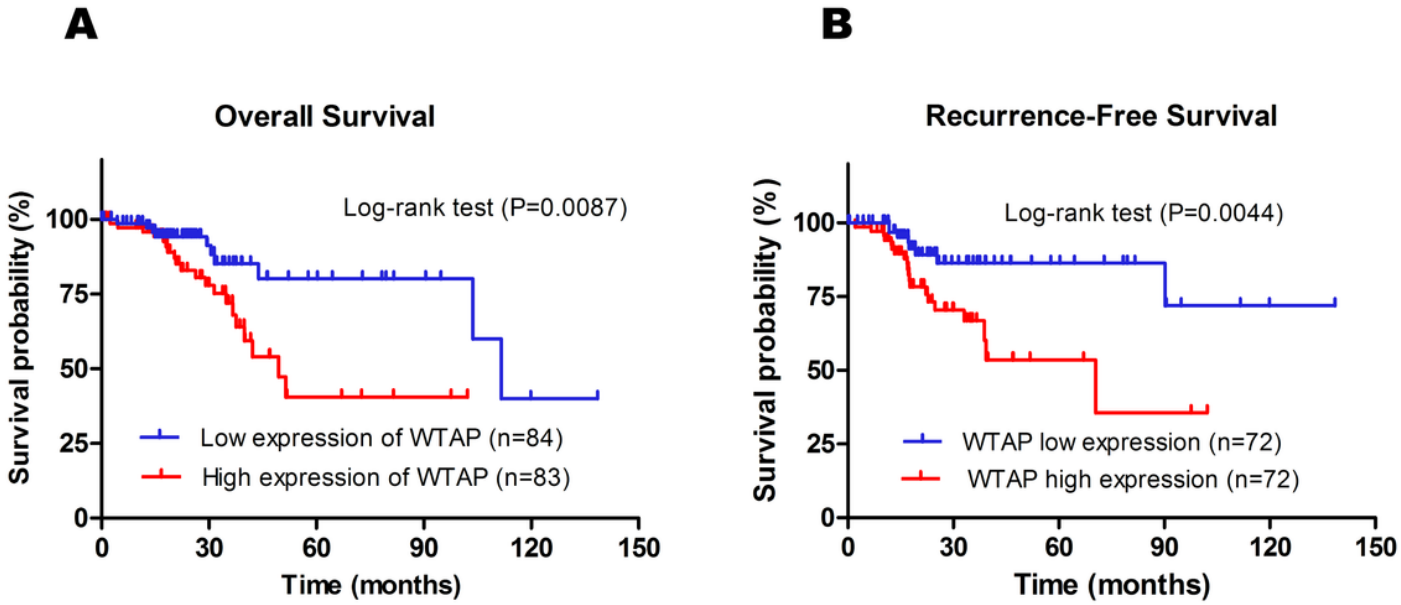

Figure 2

Kaplan-Meier survival curve between high expression WTAP and OS or RFS in EC.

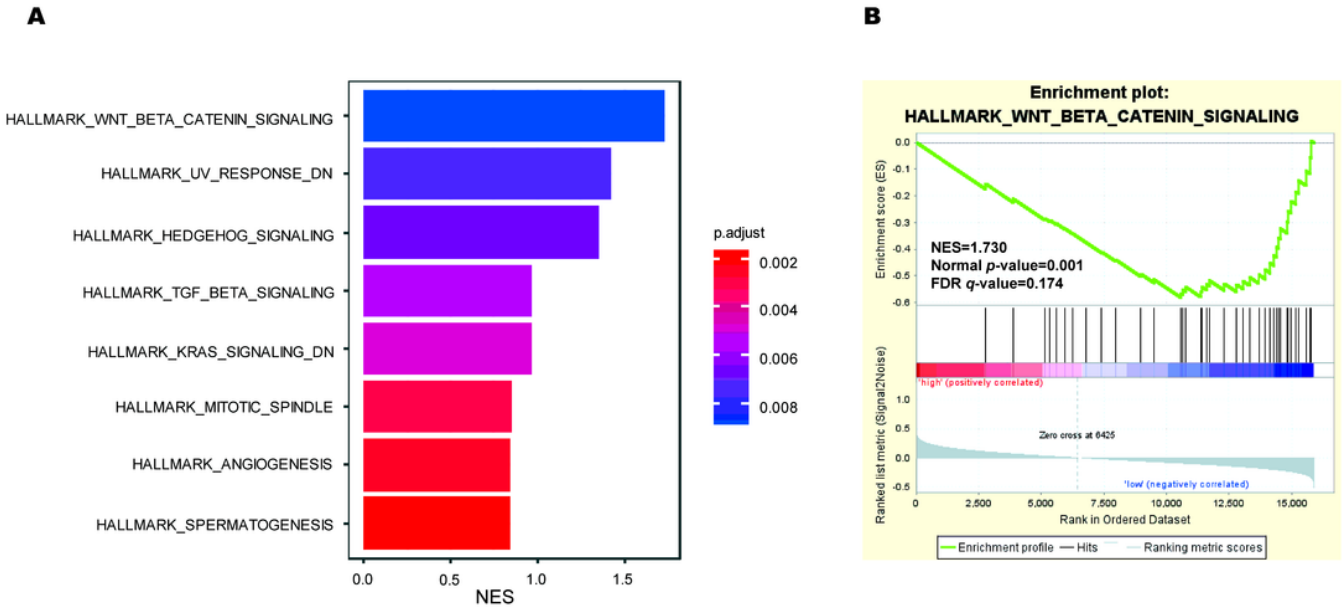

Figure 3

Enrichment plots from GSEA. A, Gene sets enriched in WTAP-high group. B. Wnt/β-catenin signaling pathway is significantly enriched (normalized P<0.05 and FDR <0.25) in WTAP-related EC.