High SPAG5 Expression Is Associated With Oncological Features in Endometrial Carcinoma

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Abstract

Sperm-associated Antigen 5 (SPAG5, also called astrin) is a mitotic spindle protein. SPAG5 has emerged as a promising biomarker and therapeutic target in a variety of cancers. However, its expression and role in endometrial cancer (EC) remain to be studied. Using multi-omic authoritative datasets from the TCGA and CPTAC studies, we characterized theexpression, regulation of SPAG5 and its association with clinicopathological and molecular features in EC. SPAG5 was observed to be overexpressed in tumor tissues compared to controls and receiver operating characteristic analysis suggested that its mRNA levels are an excellent predictor of tumor presence (AUC>0.98). SPAG5 overexpression was associated with serous histology and The Cancer Genome Atlas (TCGA) defined molecular subtypes. Analysis of DNA methylation levels at SPAG5 genomic regions exhibiting negative correlation to SPAG5 expression. Further, SPAG5 expression was associated with copy number gain in EC. Univariate and multivariate survival analysis revealed that higher SPAG5 expression was independently associated with poor patient outcomes in EC. Additionally, gene set enrichment analysis of SPAG5 correlated genes revealed its association with numerous oncological pathways which suggest its critical involvement in this malignancy.

Introduction

Endometrial carcinoma (EC) is the sixth most commonly diagnosed cancer in women with an estimated 417,367 new cases worldwide in 2020 [1]. EC is commonly treated using surgery, radiotherapy, chemotherapy, and hormone therapy. Based on the histopathological classification, there are two common types of EC. Type I includes endometrioid histology with a better prognosis and most patients present with the early-stage disease compared to Type II, which commonly includes serous histology and exhibits a poorer outcome. Early detection of EC is associated with better patient outcomes, but a sensitive and specific screening test for endometrial cancer is not yet available for clinical use so far. Integrated genomic characterization of endometrial cancer by the TCGA study group identified PTEN mutation to be frequent in Type I tumors, along with mutations in *FGFR2*, *CTNNB1*, *ARID1A*, *PIK3CA*, *PIK3R1*, and *KRAS* genes [2]. Type II tumors frequently exhibit mutations in *TP53*, *PIK3CA*, and *PPP2R1A* genes. TCGA study further defined four molecular subgroups of endometrial cancer: POLE ultramutated, microsatellite instability hypermutated, copy number low, and copy number high. This molecular subgrouping also correlates with clinical features with copy number high tumors exhibiting the worst prognosis However, as this molecular characterization is time and labor-intensive, therefore, studies are being done to provide surrogate methods for the identification of these molecular subtypes in the clinics and to further define their prognostic utility [3]. Furthermore, histological identification remains difficult with interobserver variabilities. It was recently reported that 40% of cases initially diagnosed as high-grade endometrial endometrioid adenocarcinomas were misidentified as uterine serous carcinomas as revealed by immunostaining pattern of p53, p16, estrogen receptor (ER), and mammaglobin [4]. Therefore, utilizing the molecular biomarkers or histopathological classification alone possesses a considerable degree of limitation. This necessitates the identification of novel diagnostic biomarkers for early detection along with predictive biomarkers for drug response and patient prognosis.

Abnormal cell division is a hallmark of cancer [5]. Therefore regulation of cell division remains a critical target for cancer therapies. The expression pattern and functions of cell cycle regulatory proteins are frequently altered in cancers. The oncogenic role of some cell cycle regulatory genes, such as cyclin-dependent kinases (CDKs) has been well identified in selective cancers and targeted therapies have been approved [6]. After the success of CDK inhibitors, it is widely accepted that individual cell cycle components can serve as useful targets for anticancer therapies [7]. Sperm-associated Antigen 5 (SPAG5, also called astrin) is a mitotic spindle protein. During normal cell division, it ensures the correct separation of sister chromatids into daughter cells. The SPAG5 gene is located on chromosome 17(17q11.2). The SPAG5 protein consists of 1193 amino acids and is present as a homodimer that binds to microtubules in the mitotic spindle and regulates its functioning during cell division. The essential functions of SPAG5 include chromosome alignment, sister chromatid segregation, and spindle pole formation. Its expression is dynamically regulated during cell cycle with the highest levels in telophase.

A pro-oncogenic role for SPAG5 has been demonstrated in breast [8–11], cervical cancer[12,13], prostate cancer, hepatocellular carcinoma[14–17], bladder carcinoma, non-small cell lung carcinoma [18–24]. At the functional level, SPAG5 has been shown to regulate numerous cancer-associated pathways such as PI3K/AKT and WNT/β-catenin pathways [16,25]. Considering its evident role in gynecological malignancies, we hypothesized that SPAG5 expression might also function as a novel oncogene in endometrial cancer. Therefore, we assessed the expression pattern of SPAG5 using multi-omic datasets and determined its associations with different molecular and clinical features in this malignancy.

Results

SPAG5 expression pattern in endometrial cancer

To analyze the expression pattern of the SPAG5 gene in endometrial carcinoma, we assessed The Cancer Genome Atlas (TCGA) and Clinical Proteomic Tumor Analysis Consortium (CPTAC) study datasets (Table 1). For the former, mRNA expression data was available while for the latter, both mRNA and protein estimation data were available. In the TCGA dataset, expression of SPAG5 was significantly higher in tumor tissues compared with adjacent normal tissues (Figure 1A). Further, data for additional tumor tissues was also available in TCGA dataset without paired normal tissue data. Analysis of all available samples also revealed upregulation of SPAG5 expression in EC tissues compared to a combined group of all normal tissues (Figure 1B). Furthermore, Receiver operating characteristic analysis indicated that SPAG5 mRNA expression exhibited a high diagnostic value to differentiate tumor tissues from normal tissues with an area under the curve of 0.9853, with a sensitivity and specificity of 93.60 and 97.14, respectively. Similarly, analysis of CPTAC mRNA and protein estimation data also suggested higher expression of SPAG5 in tumor tissues compared to normal tissues with AUC of 0.9940 and 0.8047 for mRNA and protein levels, respectively. Analysis of paired mRNA and protein levels of SPAG5 further revealed a high correlation (Spearman's r=0.8952, p<0.0001, Figure 1J).

To gain insight into the subcellular localization of SPAG5 protein in endometrial carcinoma, we extracted the immunohistochemical staining performed with two different antibodies for SPAG5 in endometrial cancer from the Human Protein Atlas (HPA) database (Figure 2A). The immunostaining patterns in normal

and tumor tissues have been summarized in Supplementary Tables 1 and 2, respectively. The data of normal endometrium stained using HPA022008 suggested that that SPAG5 expression is weak (4/6) to moderate (2/6 in stromal tissues while its expression was moderate (4/6) to strong (2/6) in glandular cells with cytoplasmic and membranous staining patterns (Supplementary Table 1). The staining pattern of the second antibody HPA022479 in normal endometrium suggested moderate staining (5/5) in stromal tissues and moderate (3/5) to strong (2/5) staining in glandular cells. This suggested the predominant expression of SPAG5 in glandular cells of the endometrium. In tumor tissues using the first antibody (HPA022008), 2/12 patients exhibited high staining of SPAG5 in tumor cells, while 10/12 patients exhibited medium staining. The staining pattern of the second antibody (HPA022479), suggested staining pattern as high (4/12), medium (3/12), and low (3/12) patients, while 1 patient did not exhibit positive staining. Both the antibodies confirmed consistent cytoplasmic/membranous localization of SPAG5 in normal and tumor tissues.

Association of SPAG5 with clinicopathological and molecular features in endometrial cancer

We utilized the MEXPRESS webserver to analyze the association of SPAG5 mRNA expression with clinicopathological features in the TCGA dataset. SPAG5 expression was observed to be higher in serous histology compared to endometrioid histology tumors in both TCGA and CPTAC data (Figure 2B-D). We also determined the association of SPAG5 expression with TCGA defined molecular subgroups of EC. This revealed that in both the datasets, the CNV-low subtype exhibited the least expression of SPAG5 whereas the difference among the other three groups (POLE, MSI-high, and CNV high) were inconsistent in these datasets (Figure 2E-G). Comparison of SPAG5 expression among different stages and grades revealed that in the TCGA dataset, higher stage and grade tumors exhibited higher SPAG5 mRNA levels (Figure 3A-B). Contrary to this, CPTAC data did not show such association of SPAG5 expression with tumor stage and grade (Figure 3C-F). The least expression of SPAG5 mRNA and protein was seen in the lowest grade of EC across datasets.

Genetic and epigenetic regulation of SPAG5 in EC

To explore the potential regulatory mechanisms of SPAG5 expression in UCEC, we utilized DNA methylation and copy number variation data (CNV) data of SPAG5 from the TCGA study. Firstly, we compared DNA methylation across different CpG sites in the SPAG5 gene. This revealed that mean methylation was higher at several CpG sites at the 3’ end of the SPAG5 gene in the case of tumors, whereas there was no clear difference at the CpG sites present at 5’end (Figure 4A). A detailed analysis of the correlation between DNA methylation levels and mRNA expression revealed that in tumor tissues, DNA methylation and SPAG5 mRNA expression was highly negatively correlated at several CpG sites, including most sites present at 3’ end (Figure 4B). Contrary to this, normal tissues exhibited a positive correlation between DNA methylation and SPAG5 mRNA expression at these sites (Figure 4C). This highlights that SPAG5 expression might be differentially regulated in the case of tumor tissues. Furthermore, analysis of CNV data revealed frequent copy number variations in the SPAG5 gene in UCEC. Most notably, out of 509 patients with CNV data, 67 patients (13.16%) exhibited shallow deletion and 377 patients (74.07%) were diploid for SPAG5. Shallow deletion and diploid status did not exhibit a difference in SPAG5 expression (Figure 4D). Further, 53 patients (10.41%) exhibited copy number gain and 12 patients (2.36%) exhibited amplification of SPAG5; and both copy number gain and amplification were associated with higher SPAG5 expression compared to shallow deletion and diploid status (Figure 4D).

Prognostic value of SPAG5 expression in UCEC

To determine the prognostic values of SPAG5 mRNA expression in UCEC, we utilized clinical data for overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) from the TCGA-UCEC study. Kaplan- Meier survival analysis using TCGA data revealed that high SPAG5 expression in endometrial cancer is associated with poor OS (HR=1.97, 95% CI=1.28 to 3.05, p<0.01, Figure 5A), DSS (HR=2.33, 95% CI=1.35 to 4.0, p<0.01, Figure 5C) and PFI (HR=1.94, 95% CI=1.34 to 2.79, p<0.001, Figure 5D), while having marginal association with poor DFI (HR=1.68, 95% CI=0.98 to 2.88, p=0.054, Figure 5B). As SPAG5 expression was observed to be higher in tumors with serous histology, we performed separate Kaplan-Meier survival analyses for the prognostic value of SPAG5 in both endometrial and serous histology. Interestingly, for endometrioid histology, higher SPAG5 was associated with poor OS (HR=1.8, 95% CI=1.03 to 3.14, p=0.035, Figure 5E) and PFI (HR=1.71, 95% CI=1.08 to 2.68, p=0.019, Figure 5H). Furthermore, in serous/mixed histology, high SPAG5 expression was associated with poor DSS (HR=2.61, 95% CI=0.99 to 6.89, p=0.044, Figure 5K) and PFI (HR=2.19, 95% CI=1.04 to 4.59, p=0.034, Figure 5L).

To further validate the prognostic utility of SPAG5 expression, we performed univariate and multivariate COX regression analysis in the TCGA dataset using SPAG5 expression as a continuous variable (Table 2). Univariate analysis revealed that higher SPAG5 expression is associated with poor OS (HR=1.42, 95% CI= 1.11 to 1.82, p=0.01), DFI (HR=1.52, 95% CI=1.12 to 2.07, p=0.01), DSS (HR= 1.50, 95% CI=1.11 to 2.03, p=0.01), and PFI (HR=1.46, 95%CI=1.18 to 1.81, p=0.00). Further, Cox Multivariate hazard model was utilized to determine whether the observed association of SPAG5 with patient prognosis is independent of other covariates such as age, stage, grade, and histology (Table 3). This revealed that among the four survival parameters analyzed, high SPAG5 expression was independently associated with poor DFI (HR=1.46, 95% CI=1.01 to 2.10, p=0.04).

SPAG5 associated pathways in UCEC

To elucidate the cellular pathways associated with SPAG5 expression in endometrial cancer, we determined the whole transcriptome correlations of SPAG5 mRNA expression in endometrial cancer and performed gene set enrichment analysis using cancer hallmarks gene sets in GSEA software (Figure 6A). SPAG5 mRNA expression exhibited a positive correlation with E2F targets, G2M checkpoint, MYC targets, mitotic spindle proteins, mTORC1 signaling, DNA repair, oxidative phosphorylation, spermatogenesis, unfolded protein responses. Further, it was negatively associated with estrogen response genes, coagulation, TNF- alpha signaling, and inflammatory response, KRAS signaling, epithelial to mesenchymal transition, xenobiotic metabolism, complement pathway, hedgehog signaling, and IL2-STAT5 signaling. We further determined the association of SPAG5 expression with cellular pathways, listed in KEGG (Figure 6B). This revealed the expected positive association of SPAG5 expression with DNA replication and DNA repair pathways such as mismatch repair, Fanconi anemia pathway, homologous recombination, nucleotide excision repair. Further, cellular pathways which were negatively correlated to SPAG5 expression included several immunity-associated pathways such as complement pathway, autoimmune thyroid disease, allograft rejection, graft-versus-host disease, and

drug metabolism associated pathways such as cytochrome P450 pathway, tyrosine metabolism, lysosomes. Keeping in view the observed association of SPAG5 expression with a reduced level of immunity-associated signaling, we utilized the TIMER tool to determine the association of SPAG5 mRNA expression with immune cell composition in UCEC (Figure 6C). This analysis revealed that high SPAG5 expression was negatively correlated with intratumoral B cell ($r=-0.146$, $p<0.05$), macrophages ($r=-0.129$, $p<0.05$), and dendritic cells ($r=-0.129$, $p<0.05$), while it was positively correlated with neutrophils ($r=0.229$, $p<0.0001$).

Materials And Methods

RNA sequencing data of the GDC-TCGA-UCEC study were extracted from the UCSC XENA browser (http://xena.ucsc.edu/) in the available data normalization format of HTSeq-FPKM-UQ [26]. The clinical and molecular features along with copy number variation data for SPAG5 were extracted from the TCGA-UCEC study (PanCancer Atlas Dataset) from cBioPortal web server [27,28]. Survival data was extracted from the TCGA-UCEC dataset of the UCSC XENA browser. MEXPRESS webserver was used to access DNA methylation data of TCGA-UCEC study (https://mexpress.be/) [29], where normal and tumor tissues were analyzed separately. Multi-omics data of Clinical Proteomic Tumor Analysis Consortium (CPTAC) study (https://proteomic.datacommons.cancer.gov/pdc/) [30] for uterine cancer was retrieved using LinkedOmics tool [31]. Immunostaining images of SPAG5 were extracted from Human Protein Atlas (HPA) database [32].ROC analysis for TCGA and CPTAC data was performed by comparing mRNA or protein expression data of SPAG5 between all available normal tissues and tumor tissues.

Statistical analysis

Statistical analyses were performed using Graphpad Prism (version 6). Mann-Whitney U-test and Wilcoxon paired t-test was used for comparison between unpaired and paired groups, respectively. For expression graphs, level of significance was denoted as *p-value <0.05, **p-value <0.01, ***p value<0.001 and ****p value<0.0001. For Kaplan-Meier survival analysis based on median gene expression value was performed using the KM-plotter tool (https://kmplot.com/) [33]. Stata version 11 was used for univariate and multivariate survival analysis. For gene set enrichment analysis, whole transcriptome correlations of SPAG5 expression were extracted from TCGA-UCEC study using cBioPortal server and analyzed using Hallmarks gene sets in GSEA tool (GSEA, Broad Institute) [34,35]. The same gene list was used in the String web tool (https://string-db.org/)[36]. Immunological associations of SPAG5 expression were assessed by the TIMER tool using default parameters [37].

Discussion

A recent attempt to characterize EC into TCGA defined molecular subgroups by only using targeted sequencing panels provided limited utility and the implementation and practical usage of molecular classification in the clinical setting remains difficult [38,39]. Although histopathological characterization has been widely used for risk stratification in endometrial cancer, especially in endometrioid subtype, the frequent overlap between histological subtypes and inter-observer diagnostic variability remains a major clinical problem in this malignancy. Therefore, a combination of approaches is necessary till more useful methods are identified. Nevertheless, this also highlights that more studies are required to determine critical regulators of endometrial cancer progression and high throughput sequencing data has allowed identifying some novel biomarkers and therapeutic targets [40–43].

In this study, we assessed the diagnostic potential of SPAG5 mRNA and total protein measured by high throughput techniques in UC. The data convincingly revealed the potential of SPAG5 expression as a diagnostic marker as both its mRNA and total protein levels were observed to be higher in tumor tissues compared to normal tissues, with high AUC in ROC analysis. To our interest, SPAG5 expression exhibited positive immunohistochemical staining mainly in glandular tissues, with weak to absent expression in endometrial stroma. Further, a variable staining pattern of SPAG5 was observed in tumor tissues. This suggests that a detailed study for comparison of SPAG5 immunohistochemical staining is further required. Nevertheless, its variable expression in tumors suggests its potential implication in patient prognosis, for which we determined the detailed molecular and clinicopathological associations of SPAG5 in endometrial cancer.

Comparison of SPAG5 expression among different stages and grades of EC in TCGA but not CPTAC data suggested increasing SPAG5 expression in a stage and grade-wise manner. The inconsistency between the two datasets can be due to the difference in the distribution of higher-grade tumors as TCGA data contained 57.81% of grade III tumors while CPTAC contained only 8.42%. This also suggests SPAG5 upregulation in EC is an early event during EC tumorigenesis. Furthermore, SPAG5 expression may help to differentiate between two histologies, but this requires a detailed comparison of immunohistological staining patterns. A previous study suggested that women with breast cancer who subsequently developed endometrial cancer exhibited a 2.6 fold higher risk of developing uterine serous endometrial cancer as compared to an endometrioid carcinoma[44]. The observation that SPAG5 expression is upregulated in both these cancer types further strengthens the possibility of a link between these two cancer types. This is consistent with the previous observations that serous endometrioid carcinoma possesses molecular similarities to breast cancer and serous ovarian carcinoma, where overexpression of SPAG5 has been previously reported [2].

In the context of the mechanism behind the upregulation of SPAG5 expression in endometrial cancer, we observed a high correlation between its mRNA and protein levels in the CPTAC dataset, suggesting that post-transcriptional modification is less likely to regulate total SPAG5 protein levels in UCEC. This is in contrast to the previous observations in other cancers where recently, several studies have demonstrated miRNA-mediated regulation of SPAG5 expression. miR-1179, miR-539, miR-367-3p, miR-539-5p, and miR-363-3p have been shown to target SPAG5 expression in hepatocellular cancer, prostate cancer, lung cancer, and cervical cancer [12,16,20]. We also observed that tumors with a high copy number of SPAG5 exhibited higher SPAG5 expression. This was apparent as we observed that among the four molecular subtypes defined by TCGA, the copy number low subtype, which also represents mostly endometrioid tumors exhibited the least expression of SPAG5. Further, analysis of DNA methylation suggested that promoter methylation is less likely to regulate SPAG5

expression in endometrial cancer, while some CpG sites present at the 3' end of this gene exhibited a negative correlation to its expression and might be involved in its regulation.

Toward functional associations, we performed pathway analysis of mRNA expression profile concerning correlation to SPAG5 expression. Interestingly we observed multiple DNA repair pathways to be associated with SPAG5 expression besides its expected association with cell cycle checkpoint. Previous studies have reported that SPAG5 inhibits p53 mediated DNA damage response in cancer cells[45]. Therefore, in the majority of p53 deficient cancers such as ovarian cancer and breast cancer, SPAG5 overexpression further augments cell survival pathways in response to DNA damage. Interestingly, SPAG5 overexpression also leads to resistance to DNA damage-inducing agents in a variety of cancers and SPAG5 overexpression in BRCA1 deficient tumors may also confer resistance to PARP1 inhibitors [46,47]. Other observed pathways positively correlated to SPAG5 expression include MYC targets, mTOR signaling, and oxidative phosphorylation. This is in agreement with previous reports where SPAG5 has been shown to regulate MYC transcription and thereby SPAG5 upregulation may lead to the expression of MYC regulation genes [46]. During oxidative stress, the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) is activated and regulates the expression of various stress-responsive genes. SPAG5 has also been shown to regulate levels of mTORC1 during oxidative stress, thereby preventing mTORC1 hyperactivation induced apoptosis in cancer cells[48]. Further, the interaction of SPAG5 with centrosomal protein CEP55 has been shown to promote activating phosphorylation of AKT at Ser473, thereby activating mTOR signaling.

Serous type endometrial cancer has a poorer prognosis compared to endometrioid histology. As we observed that tumors with serous histology exhibited higher SPAG5 expression compared to endometrioid histology but the survival analysis performed in this study highlighted that SPAG5 expression was associated with poor outcome of the disease independent of the tumor histology. Nevertheless, we also observed that higher SPAG5 expression was indeed associated with aggressive disease measures in endometrial cancer such as tumor invasion. Conclusively, these results highlight an important role of SPAG5 in endometrial cancer. However, there were several limitations in this study. The immunohistochemical staining data in endometrial cancer tissues was limited by a small sample size. While comparison of protein levels conclusively suggests a higher level of SPAG5 in tumors compared to normal tissues, however, the utility of assessing SPAG5 by immunohistochemistry will require further validation after inclusion of paired normal tumor tissues. Furthermore, it will be interesting to determine SPAG5 expression levels in clear cell carcinoma and whether SPAG5 immunohistochemical staining can differentiate high-grade endometrioid tumors from serous histology.

Declarations

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Tables

Table 1: Patient characteristics in the TCGA and CPTAC study for uterine corpus endometrial carcinoma.

Table 2: Univariate and multivariate analysis of SPAG5 mRNA expression in TCGA-UCEC study.

	TCGA				CPTAC			
	Data Available	Characteristic	Total	%	Data Available	Characteristic	Total	%
mRNA expression Data	**547**	**-**	**547**	**-**	**mRNA/Protein data**	**-**	**95**	**-**
Age (years)	510	⩽60	198	38.82	95	⩽60	35	36.84
		>60	311	61.18		>60	60	63.18
Stage	512	I	321	62.70	95	I	68	71.58
		II	46	8.98		II	10	10.53
		III	118	23.05		III	13	13.68
		IV	27	5.27		IV	4	4.21
Grade	512	I	94	18.36	95	I	37	38.95
		II	115	22.46		II	38	40.00
		III	296	57.81		III	8	8.42
		High grade	7	1.37		Not available	12	12.63
Histology	512	Endometrioid	395	77.15	95	Endometrioid	83	87.37
		Serous	95	18.55		Serous	12	12.63
		Mixed	22	4.30		Mixed	-	-
SPAG5 CNV status	509	Deletion	67	13.16		-	-	-
		Diploid	377	74.07		-	-	-
		Gain	53	10.41		-	-	-
		Amplification	12	2.36		-	-	-

Univariate Analysis													
	OS				DFI				DSS				PF
	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Ha Ra
Age	1.04	0.00	1.01	1.06	1.01	0.40	0.99	1.04	1.01	0.52	0.98	1.03	1.(
Histology (Endometrioid)													
Serous/ Mixed	2.68	0.00	1.74	4.13	2.20	0.01	1.26	3.85	3.76	0.00	2.22	6.36	1.9
Stage I (Ref)													
1.00													
II	2.03	0.06	0.96	4.26	0.48	0.31	0.11	1.99	2.30	0.15	0.74	7.13	1.(
III	3.07	0.00	1.85	5.10	2.39	0.00	1.38	4.14	6.90	0.00	3.45	13.77	2.5
IV	8.35	0.00	4.55	15.33					19.82	0.00	9.23	42.57	7.2
Grade I													
II	7.09	0.01	1.61	31.21	2.20	0.09	0.89	5.46	3260000000.00	0.00	868000000.00	12300000000.00	2.4
III	12.41	0.00	3.04	50.72	2.04	0.09	0.90	4.64	11800000000.00	0.00	4220000000.00	33100000000.00	3.3
High grade	51.41	0.00	9.90	266.92	5.79	0.03	1.20	28.05	51800000000.00	.	.	.	7.8
SPAG5 expression	**1.42**	**0.01**	1.11	1.82	**1.52**	**0.01**	1.12	2.07	**1.50**	**0.01**	1.11	2.03	**1.4**
Multivariate Analysis													
	OS				DFI				DSS				PF
	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Haz. Ratio	p value	95% CI (UL)	95% CI (LL)	Ha Ra
Age	1.03	0.01	1.01	1.05	1.01	0.51	0.98	1.04	0.99	0.67	0.97	1.02	1.(
Histology (Endometrioid)													
Serous/ Mixed	1.12	0.67	0.67	1.88	1.58	0.25	0.73	3.41	1.42	0.28	0.75	2.69	1.1
Stage I (Ref)													
II	1.57	0.24	0.74	3.32	0.36	0.17	0.09	1.53	1.60	0.42	0.51	4.98	0.8
III	2.48	0.00	1.45	4.26	1.93	0.03	1.05	3.53	4.47	0.00	2.18	9.16	2.1
IV	4.94	0.00	2.61	9.34					10.56	0.00	4.81	23.18	5.5
Grade I (Ref)													
II	6.04	0.02	1.36	26.76	2.10	0.11	0.84	5.24	1260000000.00	0.00	289000000.00	5500000000.00	2.1
III	6.79	0.01	1.59	28.92	1.09	0.86	0.42	2.83	2750000000.00	0.00	920000000.00	8200000000.00	2.(
High grade	17.73	0.00	3.11	101.00	2.19	0.38	0.38	12.45	5960000000.00	.	.	.	3.3
SPAG5 expression	1.12	0.44	0.84	1.49	**1.46**	**0.04**	1.01	2.10	1.03	0.85	0.72	1.48	1.1

Figures

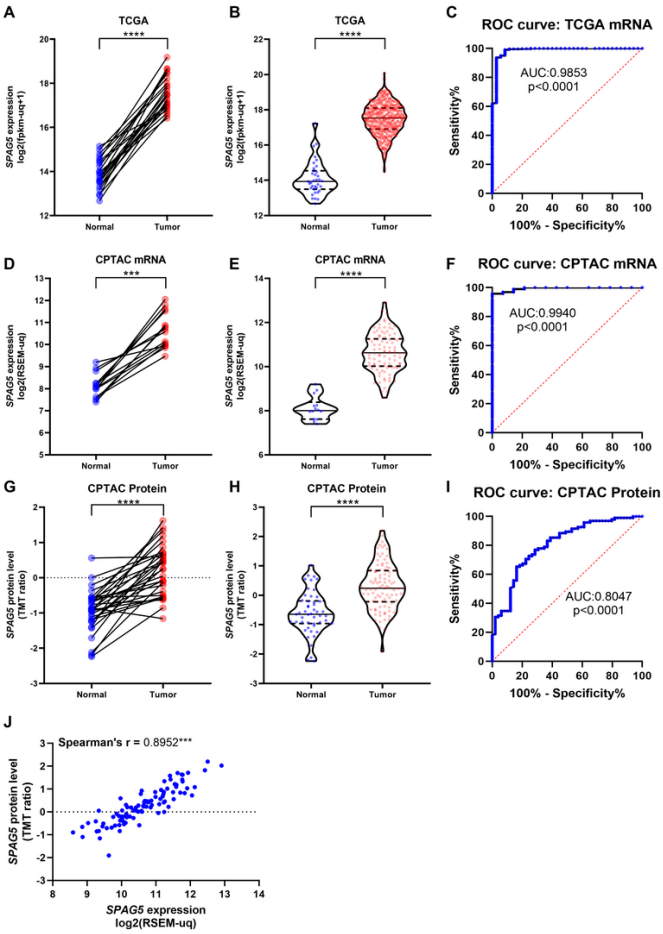

Figure 1

Comparison of the expression pattern of SPAG5 between tumor and normal tissues from TCGA and CPTAC study datasets of endometrial carcinoma. (A,D,G) comparison of SPAG5 expression between paired normal and tumor tissues; (B, E, H) comparison of SPAG5 expression between all available normal and tumor tissues; (C, F, I) AUC-ROC analysis of the diagnostic performance of SPAG5 expression in TCGA and CPTAC datasets.

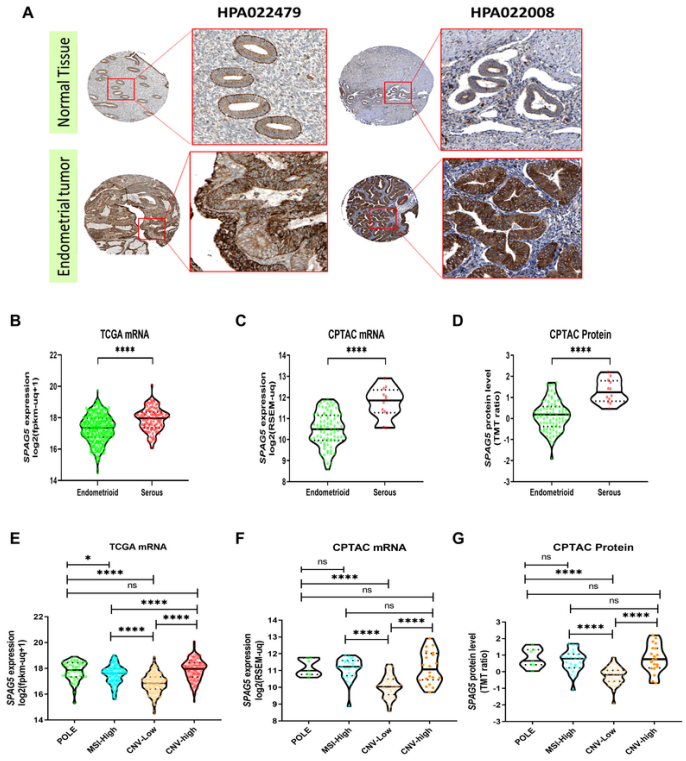

Figure 2

(A) Immunohistochemical staining of SPAG5 in normal endometrium and endometrial cancer tissues. (B-D) Association of SPAG5 expression with tumor histology. (E-G) Association of SPAG5 expression with molecular subtypes defined by TCGA.

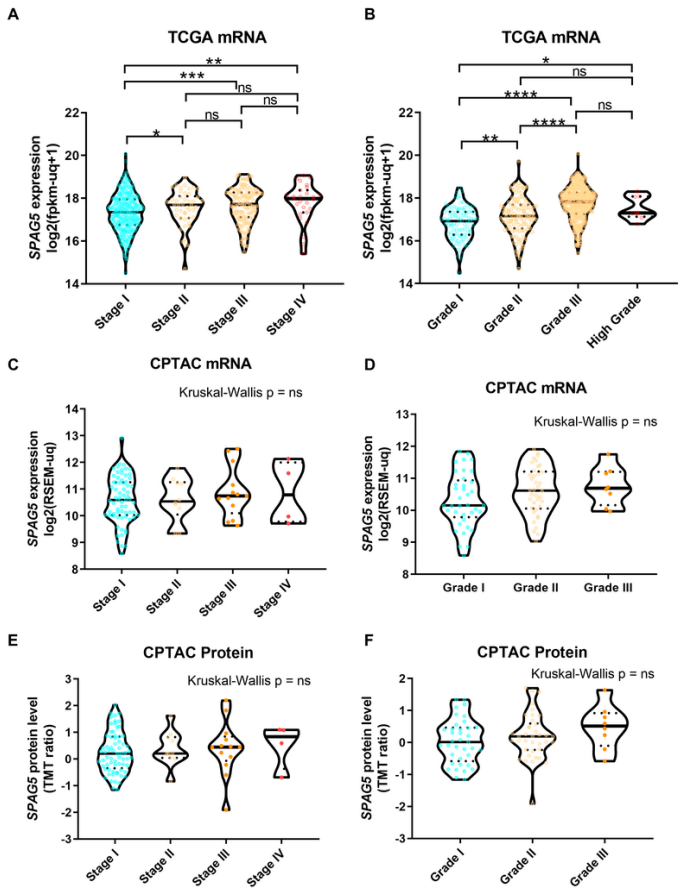

Figure 3

Association of SPAG5 expression with tumor stage (A, C, E) and grade (B, D, F) in TCGA and CPTAC dataset.

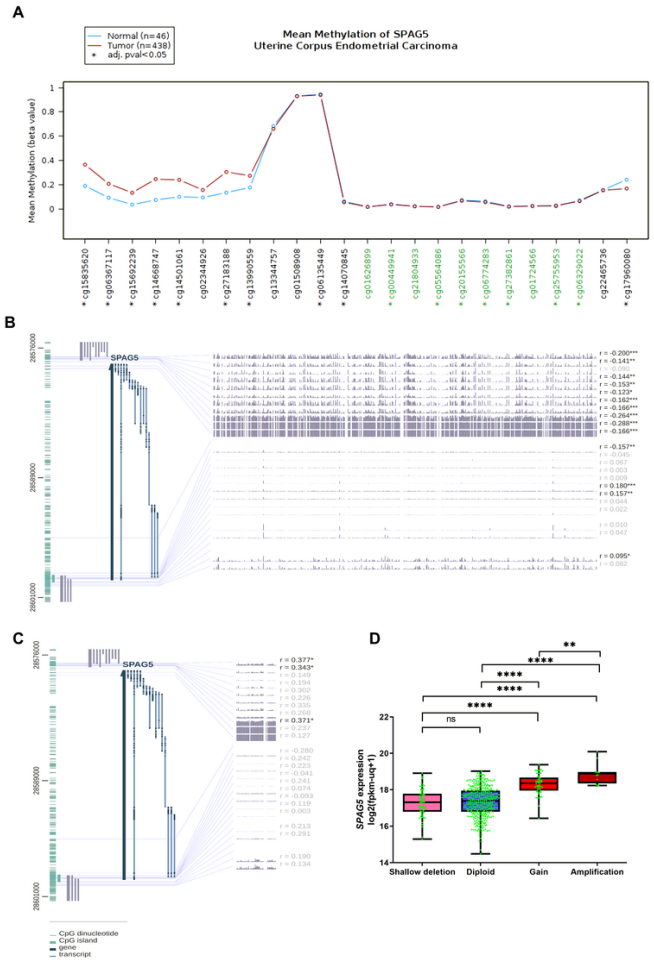

Figure 4

Potential regulation of SPAG5 expression by alterations in DNA methylation in UCEC. (A) Comparison of DNA methylation levels along SPAG5 gene between normal and tumor tissues in TCGA-UCEC dataset. (B-C) Correlation of SPAG5 expression and DNA methylation in tumor and normal tissues, respectively in TCGA-UCEC dataset. (D) Association of SPAG5 expression with its copy number alterations in TCGA-UCEC dataset.

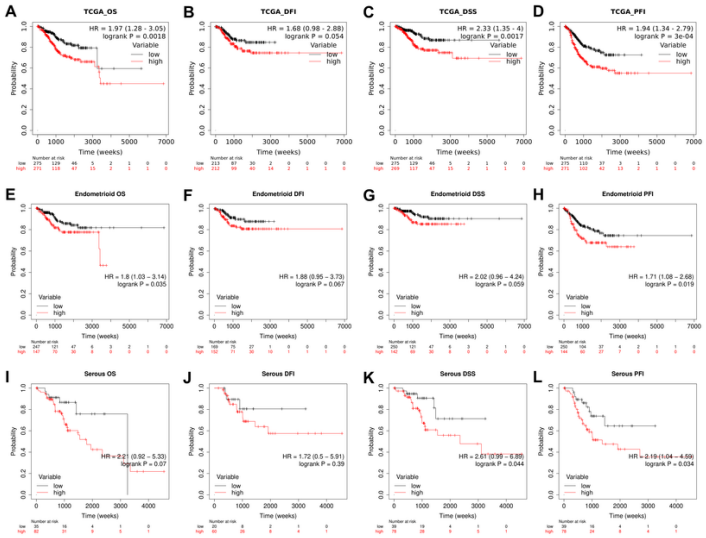

Figure 5

Kaplan Meier survival analysis for the prognostic potential of SPAG5 expression in TCGA-UCEC dataset. (A-D) All samples, (E-H) endometrioid histology, and (I-L) serous histology. HR, hazard ratio; OS, overall survival; DFI, disease-free interval; DSS, disease-specific survival; PFI, progression-free interval.

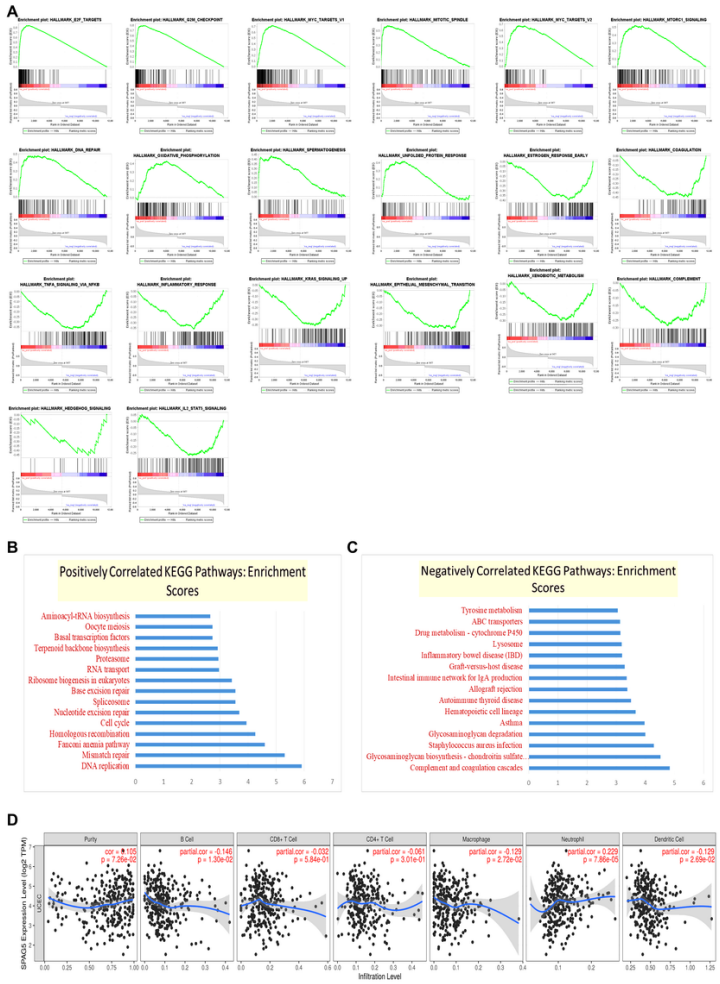

Figure 6

Gene set enrichment analysis for correlation profile of SPAG5 expression in TCGA-UCEC dataset (A). KEGG pathway analysis for SPAG5 correlated genes in TCGA-UCEC dataset; (B) positively correlated pathways; (C) negatively correlated pathways.