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Tumor necrosis factor receptor modulator spermatogenesis-associated protein 2 is a novel predictor of outcome in ovarian cancer

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Inflammation plays a crucial role in the pathogenesis of cancer with tumor necrosis factor-α (TNF-α) as a key mediator. Recently, spermatogenesis-associated protein 2 (SPATA2) was identified as a TNF receptor modulator which is required for TNF-induced inflammation and apoptosis. The available data on TNF-α in ovarian cancer (OC) are inconsistent, and SPATA2 is completely uncharacterized in tumorigenesis. We analyzed expression of *SPATA2* and *TNFA* by quantitative real-time polymerase chain reaction in tissues of 171 patients with low-grade serous (LGSOC), high-grade serous (HGSOC), endometrioid and clear cell OC compared with 28 non-malignant control tissues. We stimulated OC cells (OVCAR3) with pro-inflammatory (TNF-α, interleukin [IL]-1β) and mitogenic stimuli (IL-6, lysophosphatidic acid) to establish a direct effect between inflammatory signaling and *SPATA2*. Pro-inflammatory, but not mitogenic stimuli, potently induced *SPATA2* expression in OC cells. Expression of *TNFA* and *SPATA2* was higher in OC compared with control tissues ($P = 0.010$ and $P = 0.001$, respectively) and correlated with each other ($P = 0.034$, $r_s = 0.198$). When compared with grade 1 cancers, *SPATA2* was expressed higher in grade 2 and 3 tumors ($P = 0.011$) as well as in HGSOC compared with LGSOC ($P = 0.024$). Multivariate survival analyses revealed that OC with high *SPATA2* expression were associated with reduced progression-free survival ($P = 0.048$) and overall survival ($P < 0.001$). In conclusion, *SPATA2* expression is regulated by TNF-α and IL-1β and is found to independently affect clinical outcome in OC patients. These data implicate a role of SPATA2 in tumorigenesis which warrants further investigation in gynecological malignancies.

Abbreviations: CI, confidence interval; CYLD, cylindromatosis; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; FSH, follicle-stimulating hormone; HGSOC, high-grade serous ovarian cancer; HOIP, HOIL-1L interacting protein; HR, hazard ratio; IFN, interferon; IL, interleukin; LGSOC, low-grade serous ovarian cancer; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; NF, nuclear factor; OC, ovarian cancer; OS, overall survival; PARP, poly (ADP-ribose) polymerase; PCR, polymerase chain reaction; PFS, progression-free survival; SPATA2, spermatogenesis-associated protein 2; TBP, TATA box-binding protein; TNF-R, TNF receptor; TNFR1, TNF receptor 1; TNF, tumor necrosis factor.

KEYWORDS
inflammation, ovarian cancer, spermatogenesis-associated protein 2, tumor necrosis factor-α, tumorigenesis

1 | INTRODUCTION

Ovarian cancer is one of the most common cancers amongst women in Europe and the striking cause of death in gynecological cancer entities.[1] In recent years, multiple treatment modalities have emerged including surgical therapy, chemotherapy, antiangiogenic agents and PARP inhibitors. Compared with other tumor entities, immunotherapy has not been established and prognosis remains devastating.[2] These observations highlight the necessity for a better understanding of disease pathogenesis.

The link between inflammation and cancer, which is termed "cancer-related inflammation", has been increasingly emerging over the last decade.[3,4] It is conceived that malignant processes are fueled by a "smoldering" inflammation in the tumor microenvironment that has many tumor initiating and promoting effects.[5] Cancer-related inflammatory events have been shown to play a crucial role in the pathogenesis of OC.[4,6] More specifically, OC is characterized by a pro-inflammatory network that acts on the tumor microenvironment thereby affecting not only tumor growth but also leukocyte infiltration and neoangiogenesis in peritoneal tumor deposits.[7] Urinary neopterin, a marker for IFN-induced macrophage activation, overwhelming reflects inflammation and has been shown to be a potent prognostic factor in OC.[8] The use of non-steroidal anti-inflammatory drugs was recently associated with improved survival among 4117 patients with serous tumor histology corroborating the overwhelming inflammatory conditions affecting OC biology and potentially highlighting anti-inflammatory therapies as treatment options especially for HGSOC patients.[9] TNF-α, a key mediator in acute and chronic inflammation, is expressed in the OC microenvironment and seems to promote tumor progression by the induction of cytokines, pro-angiogenic factors and metalloproteinases.[6] Moreover, TNF-α may be implicated in the control of key disease features including cachexia, depression and fatigue, alters energy metabolism and aggravates tumor anemia[10] and the TNF-α receptor repertoire may play a role in cancer immune-editing through modulation of immune responses.[11]

Recently, SPATA2 was identified as a novel component of the TNFR1 complex and is required for TNFR1 signaling.[12] More specifically, SPATA2 links two subunits of the TNFR1 pathway, namely CYLD and HOIP, to allow recruitment of CYLD to the TNFR1 receptor upon TNF-α ligation.[13,14] Moreover, SPATA2 acts as an allosteric activator for CYLD attenuating TNF-induced NF-κB and MAPK signaling[15] suggesting that SPATA2 is required for TNF-induced apoptosis and necroptosis.[13-15] However, loss of SPATA2 had different effects on the pro-inflammatory TNF signaling,[13-16] indicating heterogeneous effects on NF-κB activation by TNF-α.[12]

Tumor necrosis factor-α and downstream-mediated functions in tumorigenesis are context-dependent and incompletely understood for OC.[17-20] TNF-α inhibitors were shown to improve tolerability of dose-intensive chemotherapy in cancer patients and stabilization of progressing OC.[21] Controversially, TNF-α may reduce tumor size of OC.[22] Whether pro- or antitumor, TNF-α seems to be highly relevant in cancer biology but we may have to better understand its downstream cascade to dissect the role of TNF-α signaling in various conditions.[21]

Here, we investigate the expression of *TNFA* and the TNF receptor modulator *SPATA2* in OC and found that TNF-α and IL-1β induced *SPATA2* in OC cells and that increased *SPATA2* expression was associated with reduced PFS and OS of OC patients. Our data implicate a role for SPATA2 in the pathogenesis of OC.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

Ovarian tissue samples from 170 patients with OC obtained at primary debulking (patients were 24-90 years old; median age at diagnosis was 60 years) and control tissues from 28 patients obtained by elective salpingo-oophorectomy for benign conditions (14 non-neoplastic tubal tissues [30-73 years old, median 50 years], 14 non-neoplastic ovaries [33-74 years old, median 57 years]) were collected and processed at the Department of Obstetrics and Gynecology of the Medical University of Innsbruck, Austria, between 1989 and 2010 as described recently.[23] Written informed consent was obtained from all patients before enrolment. The study was reviewed and approved by the ethics committee of the Medical University of Innsbruck (reference no. 1263/2017) and conducted in accordance with the Declaration of Helsinki. All samples were anonymized before the commencement of the analysis. All patients were monitored within the outpatient follow-up program of our department. The median observation period was 5.5 years (range, 0.1-26.1). All patients were of Caucasian race. Clinicopathological features are shown in Table 1.

2.2 | RNA isolation and reverse transcription

Total cellular RNA extraction from tissue samples and in vitro experiments and reverse transcription were performed as previously described.[23]

TABLE 1 Association of *SPATA2* and *TNFA* mRNA expression with clinicopathological features in ovarian cancer patients

		SPATA2 mRNA expression (rel. to *TBP*)				*TNFA* mRNA expression (rel. to *TBP*)		
Variable	**n**	**Median**	**IQR**	***P***	**n**	**Median**	**IQR**	***P***
Total	170				105			
Age								
≤50.0 y	31	0.97	0.72-1.25	n.s.	22	0.22	0.08-0.44	n.s.
>50.0 y	139	0.98	0.78-1.33		83	0.22	0.10-0.44	
FIGO stage								
I	38	0.94	0.80-1.29	n.s.	19	0.22	0.10-0.44	n.s.
II	13	0.94	0.68-1.36		9	0.16	0.05-0.90	
III	102	0.98	0.75-1.27		67	0.22	0.13-0.37	
IV	17	1.19	0.90-1.99		10	0.30	0.08-0.69	
Tumor grade								
1	12	0.77	0.70-0.91	**0.011**	8	0.23	0.18-0.31	n.s.
2	81	1.02	0.77-1.42		42	0.15	0.08-0.40	
3	77	0.98	0.82-1.29		54	0.27	0.13-0.91	
Residual disease after surgery								
No	78	0.98	0.77-1.31	n.s.	42	0.22	0.10-1.86	n.s.
Yes	87	0.97	0.78-1.33		59	0.21	0.10-0.65	
Unknown	5							
Histology								
HGSOC	106	1.00	0.78-1.41	**0.020**	61	0.19	0.08-0.47	n.s.
LGSOC	11	0.73	0.70-0.91		8	0.23	0.18-0.31	
Endometroid	43	0.97	0.81-1.29		28	0.22	0.10-0.47	
Clear cell	10	0.96	0.89-1.08		8	0.23	0.16-0.40	

Bold values have a significance level of $P < 0.05$.
The significance level (*P*) was determined by Mann-Whitney *U*-test or Kruskal-Wallis test, respectively.
FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; HGSOC, high-grade serous ovarian cancer; IQR, interquartile range; LGSOC, low-grade serous ovarian cancer; n.s., not significant; rel., relative.

2.3 | Quantitative real-time PCR

Primers and probes for *TNFA*, *CYLD* and *RNF31* were purchased from Applied Biosystems (Hs00174128_m1, Hs01031576_m1, Hs00215938_m1). Primers and probes for *SPATA2* (GenBank no. NM_001135773.1) were determined with the assistance of the computer program Primer Express (Life Technologies, Carlsbad, CA, USA): *SPATA2* forward primer, 5′-CCG TGG AAG AAG GAA TTC AGA A-3′; *SPATA2* reverse primer, 5′-CCA GTA ATG TCG ACT TGA CAT AAT AAA CA-3′; and *SPATA2* TaqMan probe, 5′-FAM-CAT CAA GAC CTA CAC GGG CCC TT-3′-TAMRA. *TBP* was used as the reference gene. PCR reactions were performed as previously described.[23]

2.4 | Immunohistochemistry

Immunohistochemistry was performed using an automated immunostainer (BenchMark ULTRA; Ventana Medical Systems, Tucson, AZ, USA). In short, formalin-fixed, paraffin-embedded tissue sections were prepared with cell conditioning reagent for antigen retrieval. Anti-SPATA2 antibody (HPA048581; Sigma-Aldrich, St Louis, MO, USA) was incubated for 30 minutes at 37°C and for visualization the Ultra View DAB Detection Kit (Ventana Medical Systems) was used as recommended. Slides were counterstained with hematoxylin and bluing reagent. Images were acquired with a Zeiss AxioCam.

2.5 | Culture and stimulation of OC cells

OVCAR3, HOC7, SKOV6 and HTB77 human OC cells were purchased from ATCC (Middlesex, UK) and cultured in RPMI supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were stimulated with recombinant human IL-1β (10 ng/mL; Invitrogen, San Diego, CA, USA), TNF-α (25 ng/mL; Peprotech, Rocky Hill, NJ, USA), IL-6 (10 ng/mL; Peprotech), LPA (20 μmol/L; Sigma-Aldrich) and FSH (50 mIE/mL; Fostimon®) for indicated time points.

2.6 | Statistical analysis

The non-parametric Mann-Whitney *U*-test or Kruskal-Wallis test were applied to test for statistical significance between two groups or more than two groups, respectively. The correlations between *SPATA2* and *TNFA* mRNA expression were assessed by Spearman's rank correlation coefficient analyses. PFS was defined as the time

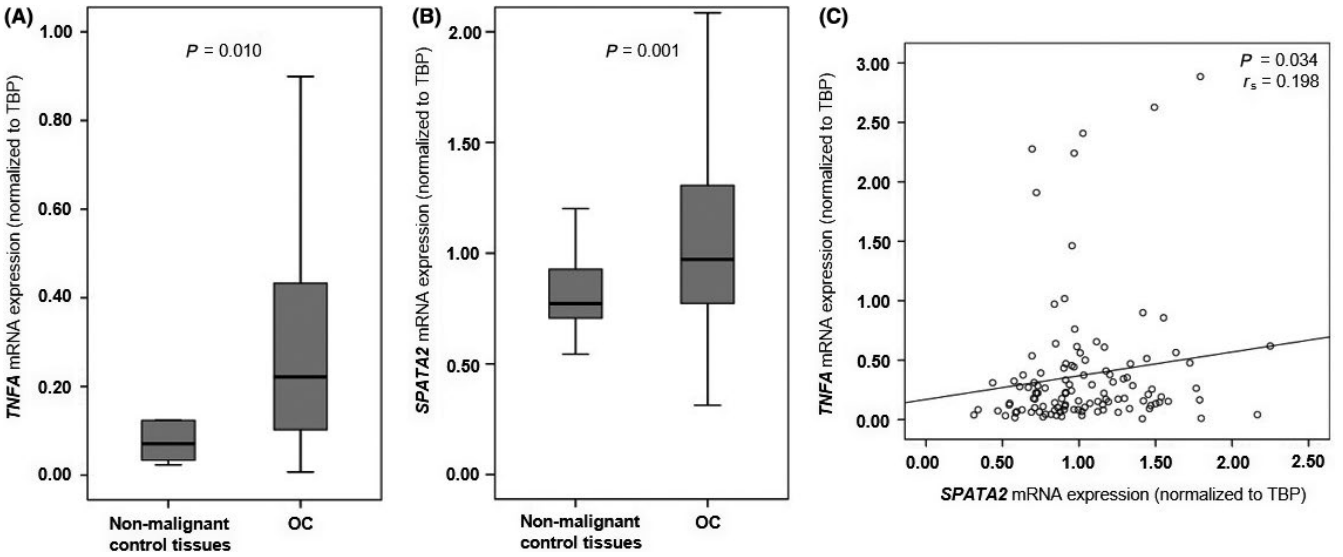

FIGURE 1 *TNFA and SPATA2* expression is elevated in ovarian cancer (OC) tissue compared with non-neoplastic fallopian tubes. A, *TNFA* expression in non-neoplastic control tissues (fallopian tubes, n = 7; ovaries, n = 3) and OC (n = 105). B, *SPATA2* expression in non-neoplastic control tissues (fallopian tubes, n = 14; ovaries, n = 14) and OC (n = 170). C, Linear regression analysis of *TNFA* (n = 115) and *SPATA2* (n = 198) in non-malignant control tissues and OC. *TNFA* and *SPATA2* mRNA expression values were normalized to *TBP* expression

from diagnosis of the primary tumor to the histopathological confirmation of recurrence or metastases, and OS as the time from diagnosis of the primary tumor to death from any cause or to the last clinical inspection. Univariate Kaplan-Meier analyses and multivariable Cox survival analyses were used to explore the association of *TNFA* and *SPATA2* expression with PFS and OS (the *P*-value cut-off for inclusion to the multivariable Cox analysis was 0.2). For survival analyses, patients were dichotomized into low and high mRNA expression level groups by the optimal cut-off expression value calculated by Youden's index.[24] Experiments with more than two comparisons were tested for statistical significance by one-way ANOVA. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS statistical software (version 20.0.0; SPSS, Chicago, IL, USA).

3 | RESULTS

3.1 | *TNFA* expression correlates with *SPATA2* expression in OC tissue

To investigate a potential role of TNF-α and SPATA2 in the biology of OC, we measured *SPATA2* and *TNFA* mRNA levels in tumor tissues of 170 OC patients by quantitative PCR and compared it with 24 non-neoplastic tissues of healthy controls. *TNFA* expression was elevated in OC tissue compared with non-malignant tubes or ovaries ($P = 0.010$; Figure 1A). We further observed higher levels of *SPATA2* in OC tissue compared with non-neoplastic control tissues ($P = 0.001$; Figure 1B). Performing Spearman's rank correlation coefficient analyses in malignant and non-malignant samples, we noted a significant correlation between *SPATA2* and *TNFA* expression ($P = 0.034$, $r_S = 0.198$; Figure 1C). Immunohistochemical analyses identified tumor epithelial cells as the main source of SPATA2 (Figure S1) which was approximately 90% positive (range, 10-99%). In contrast, tumor stromal cells were negative for SPATA2. In control tissue, non-malignant ovaries and stromal cells of the fallopian tubes were negative for SPATA2. The epithelium of fallopian tubes was slightly positive for SPATA2 expression which appeared to a lesser extent when compared to OC epithelium (Figure S1).

3.2 | *SPATA2* mRNA expression is induced by TNF-α, IL-6 and IL-1 in OC cell lines

Immunohistochemical analyses (Figure S1) identified tumor epithelial cells as the predominant cellular source of SPATA2 in ovarian tumors. We therefore determined the impact of inflammatory or mitogenic signaling on *SPATA2* expression in human OC cell lines and stimulated OVCAR3, HOC7, SKOV6 and HTB77 cells with TNF-α, IL-1β, IL-6 and LPA. FSH, which was shown to induce SPATA2,[25] served as a positive control. Baseline *SPATA2* expression was expressed in all cell lines with the highest levels detected in OVCAR3 cells ($P < 0.001$; Figure 2A). Notably, *RNF31* (HOIP, a member of the LUBAC complex that interacts with SPATA2)[26] transcript levels directly correlate with SPATA2 levels in OVCAR3, HOC7, SKOV6 and HTB77 cells ($r_S = 0.995$, $P = 0.005$; Figure 2B). In OVCAR3 cells, TNF-α and IL-1β induced *SPATA2* expression more than FSH with the maximal effect after 3 hours of treatment (Figure 2C). LPA and IL-6 (known to induce OC proliferation)[27,28] did not have an impact on *SPATA2* expression (data not shown). Despite directly correlating with *SPATA2* levels at baseline, *RNF31* could not be induced by TNF-α and IL-1β, LPA or IL-6. In contrast, CYLD, which is also component of the TNF-R signaling pathway, exhibited similar induction patterns compared with SPATA2. In

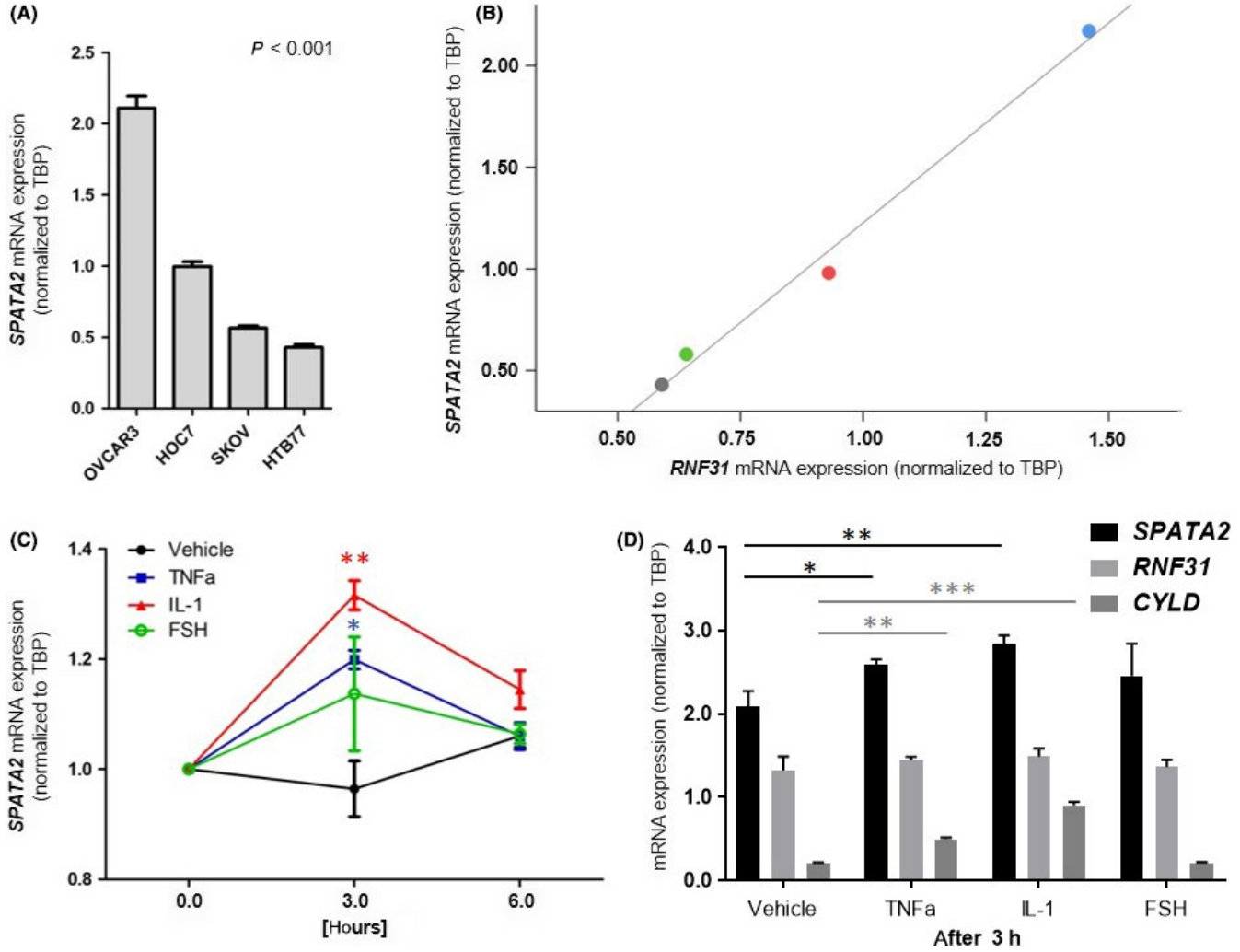

FIGURE 2 *SPATA2* expression is induced by tumor necrosis factor (TNF)-α and interleukin (IL)-1β in ovarian cancer cell lines. A, Baseline *SPATA2* expression in the human ovarian cancer (OC) cell lines OVCAR3, HOC7, SKOV6 and HTB77. B, Linear regression analysis of *RNF31* and *SPATA2* in HTB77 (grey point), SKOV6 (green point), HOC7 (red point) and OVCAR3 (blue point) cells. C, OVCAR3 cells were stimulated with TNF-α, IL-1β and follicle-stimulating hormone (FSH) for indicated time points (n = 3). Stars indicate significance levels between vehicle and TNF-α or IL-1β, respectively. D, *SPATA2*, *RNF31* and *CYLD* expression in OVCAR3 cells after stimulation with TNF-α, IL-1β and FSH for 3 hours (n = 3). *SPATA2*, *RNF31* and *CYLD* mRNA expression values were normalized to *TBP* expression

detail, both *SPATA2* and *CYLD* were induced by TNF-α and IL-1β (but not FSH) after 3 hours (Figure 2D). Our data establish a direct effect of pro-inflammatory cytokines such as TNF-α and IL-1β on *SPATA2* expression in human OC cells.

3.3 | Increased *SPATA2* expression occurred in higher tumor grades

Next, we explored the association between *SPATA2* and *TNFA* expression with clinicopathological features. As demonstrated in Table 1 and Figure 3, we found that increased *SPATA2* expression was associated with higher tumor grade. Specifically, we found higher *SPATA2* mRNA levels in tumor grade 2 and 3 compared with tumor grade 1 (Figure 3A) which was in line with higher *SPATA2* expression in HGSOC compared with LGSOC ($P = 0.024$; Figure 3B). In contrast, *TNFA* expression was not associated with tumor grade and did not differ between histological subtypes (Table 1). *SPATA2* and *TNFA* expression was independent from FIGO stage.

3.4 | High *SPATA2* mRNA expression is associated with a poor prognosis

To evaluate *SPATA2* and *TNFA* levels regarding clinical outcome of OC patients, we first determined Youden's index[24] which grouped the OC cohort into patients with "high" and "low" *SPATA2* and *TNFA* expression. Univariate survival analyses (Table 2) demonstrated that patients with low *SPATA2* expression exhibited a median PFS of 50.5 months (CI, 0.0-105.1) whereas patients with high *SPATA2* expression exhibited a median PFS of only 22.9 months (CI, 14.2-31.6) ($P = 0.073$; Figure 4A). This difference in PFS was even

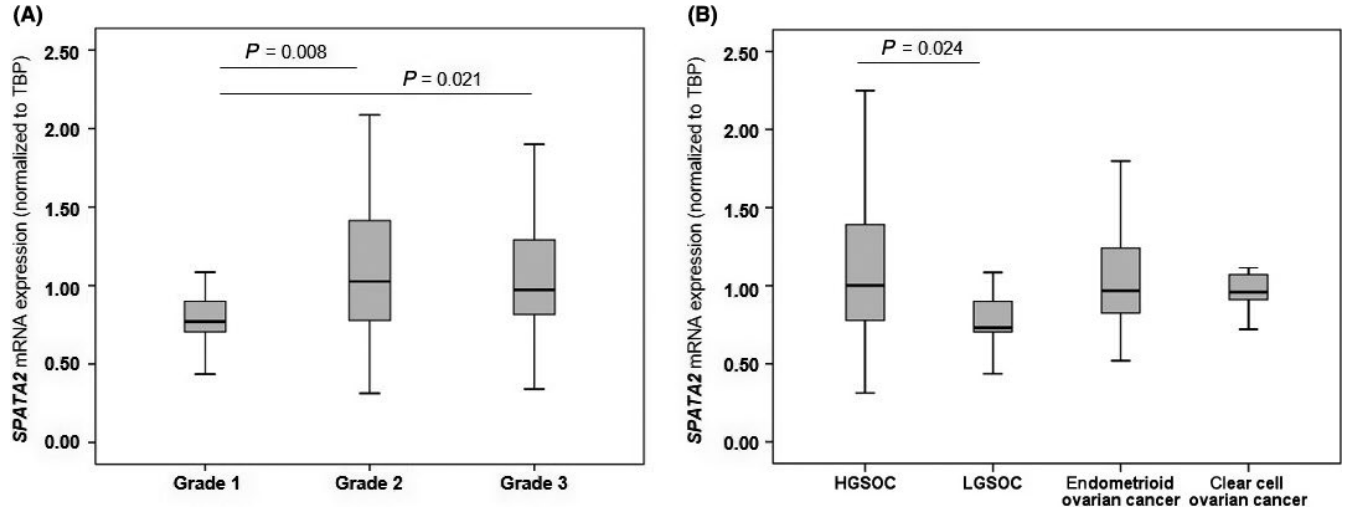

FIGURE 3 *SPATA2* expression according to tumor grades (A) and histological subtypes (B). Expression values were normalized to *TBP* expression

TABLE 2 Univariate survival analysis in ovarian cancer patients

Variable	No. patients (relapsed/total)	Progression-free survival		No. patients (died/total)	Overall survival	
		Median, months, 95% CI	*P*		Median, months, 95% CI	*P*
Age						
<50 y	18/31	50.3 (2.2-98.5)	0.466	15/31	151.065.6-236.5)	**0.015**
≥50 y	78/139	24.2 (5.8-42.6)		94/139	49.6 (28.2-71.0)	
FIGO stage						
I/II	11/51	n.r.	**<0.001**	20/51	n.r.	**0.000**
III/IV	85/119	20.0 (14.7-25.3)		89/119	47.3 (26.6-68.0)	
Tumor grade						
1/2	47/93	48.8 (0.0-101.2)	0.110	53/93	100.0 (70.1-129.9)	**0.012**
3	49/77	23.6 (12.6-34.7)		56/77	44.4 (30.4-58.5)	
Residual disease after surgery						
No	24/79	n.r.	**<0.001**	30/78	n.r.	**<0.001**
Yes	68/87	15.7 (13.2-18.3)		76/87	35.2 (24.4-46.1)	
Histology						
HGSOC	69/106	23.4 (16.0-30.9)	**0.008**	80/106	47.1 (27.5-66.7)	**0.003**
Others	27/64	n.r.		29/64	132.7 (n.r.)	
SPATA2 mRNA expression						
Low	35/71	50.5 (0.0-105.1)	0.073	67/116	105.1 (71.4-138.9)	**0.001**
High	61/99	22.9 (14.2-31.6)		42/54	43.4 (29.9-56.9)	
Subgroup: HGSOC						
Low	47/78	28.8 (7.1-50.5)	**0.008**	52/76	58.7 (23.4-93.9)	**0.002**
High	22/28	13.5 (6.9-20.2)		28/30	35.7 (21.0-50.4)	
TNFA mRNA expression						
Low	12/27	n.r.	0.222	68/117	68.8 (37.0-100.7)	0.434
High	50/78	22.9 (13.9-31.8)		42/54	69.6 (21.3-118.0)	
Subgroup: HGSOC						
Low	11/20	30.0 (13.8-46.1)	0.283	10/16	49.0 (0.0-120.4)	0.171
High	31/41	22.8 (18.7-27.0)		38/45	41.1 (30.8-51.5)	

Bold values have a significance level of $P < 0.05$.
The optimal cut-off points for *SPATA2* and *TNFA* were calculated by Youden's index. The significance level (*P*) was determined by log-rank test.
CI, confidence interval; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; HGSOC, high grade serous ovarian cancer; n.r., not reached.

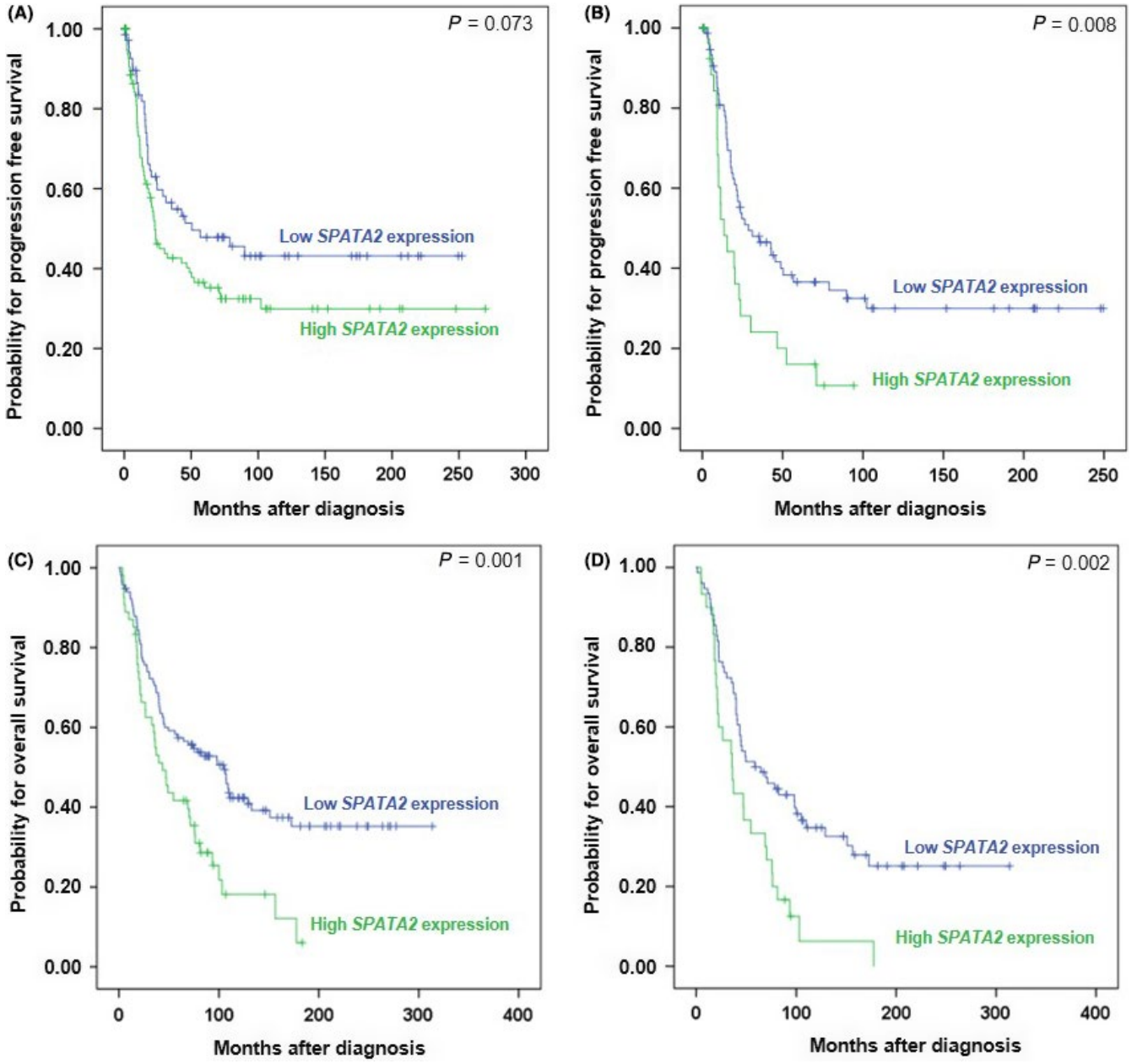

FIGURE 4 Kaplan-Meier survival analyses and *SPATA2* expression in ovarian cancer (OC) patients. Progression-free survival according to low and high *SPATA2* mRNA expression in (A) OC patients (n = 170) and (B) the subgroup of patients with HGSOC (n = 61). Overall survival according to low and high *SPATA2* mRNA expression in (C) OC patients (n = 170) and (D) the subgroup of patients with HGSOC (n = 61)

more prominent in the subgroup of HGSOC patients ($P = 0.008$; Figure 4B). A clear association between high *SPATA2* expression and impaired OS was revealed. Patients with low *SPATA2* expression exhibited a median OS of 105.1 months (CI, 71.4-138.9) while patients with high *SPATA2* expression exhibited a median OS only of 43.4 months (CI, 29.9-56.9), ($P = 0.001$; Figure 4C). This was also true for the subgroup analysis of HGSOC ($P = 0.002$; Figure 4D). Importantly, multivariate analyses identified *SPATA2* as an independent prognostic factor for PFS (HR, 1.55; $P = 0.048$; Table 3) and OS (HR, 2.13; $P < 0.001$; Table 3). *TNFA* expression, however, failed to be of prognostic significance either regarding PFS or OS in OC (Table 2, Figure S2A,B).

4 | DISCUSSION

In this study, we investigated the regulation of *TNFA* and *SPATA2* and the impact on clinical outcome in a Caucasian OC cohort. We found that *TNFA* and *SPATA2* are significantly higher expressed in OC compared with non-malignant control tissues. Immunohistochemical staining of our cohort and an OC database (human protein atlas) identified OC cells as the main cellular source of SPATA2 expression. Using the OC cell line OVCAR3, we demonstrate that *SPATA2* expression was markedly induced by TNF-α and IL-1β, indicating that pro-inflammatory signals induced expression of *SPATA2* in OC. Increased *SPATA2* expression, which correlated with *TNFA*

TABLE 3 Multivariate survival analysis in ovarian cancer patients

Variable		Progression-free survival		Overall survival	
		HR of progression (95% CI)	*P*	HR of death (95% CI)	*P*
Age	<50 y ≥	1.46 (0.86-2.47)	**0.162**	2.26 (1.29-3.95)	**0.004**
FIGO stage	I/II vs III/IV	2.68 (1.33-5.42)	**0.006**	1.33 (0.75-2.33)	0.327
Tumor grade	1/2 vs 3	1.21 (0.79-1.84)	0.378	1.39 (0.94-2.05)	0.102
Residual disease after surgery	No vs yes	2.92 (1.71-4.98)	**<0.001**	3.02 (1.96-5.24)	**<0.001**
Histology	HGSOC vs others	1.03 (0.62-1.68)	0.922	0.86 (0.54-1.37)	0.519
SPATA2 mRNA expression	Low vs high (< or > optimal cut-off)	1.55 (1.00-2.40)	**0.048**	2.13 (1.41-3.24)	**<0.001**

Bold values have a significance level of $P < 0.05$.
The optimal cut-off points for *SPATA2* were calculated by Youden's index. The significance level (*P*) was determined by Cox regression.
CI, confidence interval; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; HGSOC, high grade serous ovarian cancer; HR, hazard ratio; n.r., not reached.

expression, was associated with increasing tumor grade, and consequently was higher in HGSOC compared with LGSOC. High *SPATA2* (but not *TNFA*) expression independently reflected poor clinical outcome with regard to PFS and OS in OC patients. This was especially true for the subgroup of HGSOC.

Tumor necrosis factor-α has been initially discovered as initiator of tumor cell necrosis[21] and is now known as a potent pro-inflammatory cytokine which exerts deleterious effects in chronic inflammation, antimicrobial immunity and autoimmune diseases.[29] Contrary to its discovery, TNF-α failed as an anticancer agent as various studies have clearly demonstrated a tumor-promoting role for TNF-α in experimental cancers.[21] In OC, TNF-α and its potential role in disease progression has been described earlier.[30] OC cells secrete TNF-α protein[31] which stimulates a constitutive network of other cytokines, angiogenic factors and chemokines that may act in an autocrine/paracrine manner to promote colonization of the peritoneum and neovascularization of developing tumor deposits.[32] Furthermore, Charles et al[20] demonstrated that chronic production of TNF-α in the tumor microenvironment increases myeloid cell recruitment and consequently tumor growth in vivo. Previous studies demonstrated an increase of TNF-α protein and gene expression in human OC compared with non-malignant controls.[18,30,33] High ascitic TNF-α protein levels have previously been found to be associated with poor survival in univariate analyses,[34] however, data concerning intra-tumor TNF-α expression and clinical outcome are not available. In line with previous data, we demonstrate high levels of *TNFA* in human OC compared with non-malignant control tissues.[18,30,33] However, we were unable to determine a significant prognostic effect of *TNFA* expression in OC patients. Clinical studies investigating TNF-α inhibitors (etanercept, infliximab) as a therapeutic option or as supportive treatment to improve chemotherapy tolerability demonstrated biologic activity and safety of TNF blockade in recurrent OC.[21] However, the same was true when TNF-α itself was used in high pharmacological doses combined with chemotherapy to refine the necrotic activity of TNF-α and to boost antitumor activity.[22] Thus, it appears that TNF-α represents a "double-dealer" with regard to cancer biology.[35] On one hand, TNF-α through its pro-inflammatory properties could be an endogenous tumor promoter stimulating cancer cell growth, proliferation, metastasis, angiogenesis and leukocyte infiltration; and on the other hand, TNF-α could also act as a killer of cancer cells. These divergent observations may be one reason why more recent studies focused on downstream TNF-α signaling.

A number of investigations have disclosed complex and diverging TNF-R signaling pathways described as a "double-edged" sword.[36] We found that SPATA2, a novel component of the TNFR1 signaling complex, is an independent predictor for adverse PFS and OS in OC patients. SPATA2 is an adaptor for the recruitment of CYLD to the TNF-R signaling cascade and an activator of CYLD which controls TNF-induced apoptosis and necroptosis.[13-15] Cells lacking SPATA2 exhibited reduced TNF-induced cell death due to reduced caspase-3 suggesting that SPATA2 is required for TNF-induced cell death. Currently, there are no data on the role of SPATA2 in tumorigenesis and cancer progression. However, CYLD, the co-factor for SPATA2 is a known tumor suppressor[37] shown to inhibit NF-κB, MAPK and Wnt signaling. On the other hand, CYLD also acts as a mediator of immune activation and inflammation.[38,39] We found that both SPATA2 and CYLD are induced by TNF-α and IL-1β in vitro, indicating a similar regulation in OC. CYLD and SPATA2 were shown to synergistically promote TNF-induced NF-κB signaling, caspase activation and apoptosis.[15] Considering that, OC with high SPATA2 expression levels may also show high rates of apoptosis. In other cancer entities, such as colon carcinoma and breast cancer, high apoptotic rates were associated with increased cellular proliferation and poor prognosis.[40-42] Nonetheless, in various tumor types such as malignant melanoma or breast cancer, downregulation of CYLD is associated with tumor progression.[43] However, our data illustrate that in OC high *SPATA2* expression is independently associated with worse PFS and OS. As no data on *SPATA2* expression and clinical outcome in cancer are currently available, it remains speculative whether CYLD and SPATA2 have always identical biologic functions or whether both could be endowed with additional mutually independent properties.

Nonetheless, it should be emphasized that cancer-related inflammation plays an exceptional role in OC, and especially in HGSOC, due to its semi-solid dissemination throughout the abdominal cavity. In this context, OC can hardly be compared with other solid tumors. Our in vitro results showing an induction of *SPATA2* expression selectively by pro-inflammatory cytokines argue for a tight involvement of SPATA2 in the pro-inflammatory cytokine network within the microenvironment of OC. In this regard, the herein presented clinical results may reflect the tumor-promoting activity of the tumor-associated inflammation with its detrimental effect on patients' prognoses. Thus, SPATA2 fits well into the large row of other pro-inflammatory factors (such as urinary neopterin,[8] 90K,[44] ascitic TNF-α and IL-12),[34] which proved to predict adverse clinical outcome in OC.

In conclusion, our study suggests a potential biologic role of *SPATA2*, possibly as a downstream regulator of TNF-mediated actions in the pathogenesis and dissemination of OC. Further studies are needed to investigate the exact functional role of SPATA2 in the biology of OC.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

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