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High prevalence of FAP+ cancer-associated fibroblasts predicts poor outcome in patients with high-grade serous ovarian cancer with high CD8 T-cell density

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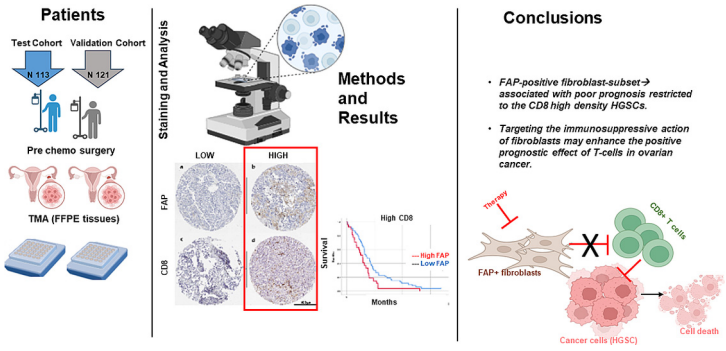
HIGHLIGHTS

- CD8+ T-cells improve clinical outcome in HGSC.
- FAP+ fibroblasts are associated with poor prognosis restricted to a CD8+ high density group.
- Therapy targeting the fibroblasts may enhance the known positive prognostic effect of CD8+ cells in HGSC.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. Studies have implied that fibroblasts may act as regulators of immune cells in the tumor microenvironment (TME). We investigated the clinical relevance of fibroblast activation protein (FAP) positive stroma in high-grade serous ovarian cancer (HGSC) in relation to CD8+ lymphocyte's infiltration.

Methods. In a discovery cohort ($N = 113$) of HGSC, expression of FAP and CD8 in the TME was analyzed with immunohistochemistry. Results were correlated with overall survival (OS) and progression-free survival (PFS). The findings were validated in an independent cohort of HGSC ($N = 121$) and in public available datasets.

Results. High infiltration of CD8+ cells in the TME of HGSC was found to be associated with longer OS, as previously known. Increased expression of FAP was associated with shorter median PFS (11.4 vs. 18.6 months) in tumors with high density of CD8+ cells (HR 4.03, CI 95 % 1.38–11.72, $p = 0.01$). Similarly, in the validation

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FAP
T cell
Fibroblast

cohort, high intensity of FAP in cases with high density of CD8+ cells was associated with shorter OS, 31.5 vs 76.9 months (HR 2.83; 95 % CI 1.17–6.86, $p = 0.02$). The results were consistent in multivariable analyses. The association between high FAP expression and poor outcome in high density CD8 HGSC was also confirmed in publicly available datasets.

Conclusions. The TME infiltration of FAP-positive fibroblasts is associated with poor prognosis in HGSC with high CD8+ cells density. Targeting the FAP+ subset of fibroblasts may unlock the local immune-activation in the TME thus enhance the positive prognostic effect of T-cells in ovarian cancer.

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1. Introduction

A challenge in the field of ovarian cancer is the discovery of new predictors of response to treatment to tailor more effective therapeutic approaches. Ovarian cancer is the fifth leading cause of cancer related death for women [1]. The high-grade serous (HGS) subtype represents the most common epithelial subtype and is mostly diagnosed at advanced stage. Recent advances and challenges in T cell mediated immunotherapies such as checkpoint inhibitors and chimeric antigen receptor (CAR) T cells, has highlighted the importance of the cells in the tumor microenvironment in determining outcomes of these treatment approaches [2].

There is a growing body of evidence supporting the role of cancer-associated fibroblasts (CAFs) and immune cells in tumorigenesis and tumor progression [3]. CAFs are a heterogeneous group of cells, in which subsets with potentially different functions are characterized by the expression of a variety of markers [4,5]. The fibroblast activation protein (FAP) is a cell surface serine protease that is overexpressed by fibroblasts present in the microenvironment in most tumors. Experimental tumor biology studies have linked FAP-positive fibroblasts to tumor growth and invasion [6–8]. Recent data from experimental cancer models have shown pro-inflammatory and immune suppressive phenotype of FAP-positive CAFs, involving various molecular mechanisms [2,7–10]. From a clinical point of view, high FAP expression has been shown to be a negative prognostic factor in several malignancies [6,11,12]. In HGSC high expression of FAP has been associated with shorter OS and PFS [13] and with shorter time to recurrence in ovarian epithelial cancer [14].

Tumor infiltration of immune cells has been shown to be associated with favorable prognosis in multiple solid tumors [15,16] and the presence of intra-tumoral T-cells has been shown to be associated with improved clinical outcome in epithelial ovarian cancer [17]. The CD8 marker is predominantly expressed on the surface on cytotoxic T-cells which are a crucial component of the cellular immune system involved in cell-mediated antitumor immune responses [18]. Tumor biology studies have implied FAP+ fibroblasts as negative regulators of T-cell-activity, which could control T-cell-dependent effects on natural course and response to treatment. Previous data suggest that FAP-positive CAFs exert suppressive immune-modulatory mechanisms on tumor cells, leading to an immune-suppressive microenvironment with impaired T-cell mediated immune response [9,19–22]. Furthermore, it has also been implied that CAFs have potential to diminish nuclear accumulation of platinum in ovarian cancer cells, resulting in stromal-mediated resistance to platinum [23]. However, the presence of CD8+ T cells has been reported to abrogate this resistance mechanism, and hence make the cells more sensitive to platinum [23]. In addition, CAFs have also been suggested to be involved in the regulation of response to immune therapy in various tumor types [12,24,25].

The effect of FAP+ CAFs on prognosis in a clinical setting of HGSC is unknown. This study explores the possible associations between FAP intensity and tumor infiltration of CD8+ T cells in a well annotated cohort of patients with HGSC. The results were then validated in an independent cohort of patients with HGSC as well as in publicly available databases (including the TCGA) of gene expression of ovarian cancer [26].

2. Material and methods

2.1. Study population, discovery cohort

The Swedish Cancer Registry was used to identify all patients diagnosed with ovarian, fallopian tube or primary peritoneal carcinoma, or carcinoma of undesignated primary site, in Stockholm County between 2002 and 2006. Inclusion criteria included age above 18 years, HGS histology, FIGO stage IIC-IV (according to the current 1988 FIGO system), no administration of chemotherapy prior to surgery or diagnostic biopsy and availability of tissue. Exclusion criteria included administration of chemotherapy prior to diagnostic biopsy or surgery, history of previous malignant disease (except for in situ cancer and basalioma), diagnosis at autopsy, and previous treatment with chemotherapy. All cases were re-examined by an expert gynecological pathologist, and re-classified from the older three-tier differentiation grade to the two-tier grade system [27].

Of the 401 patients screened, 135 met the inclusion criteria and were included in the study (Supplementary Fig. S1). Only patients with tissue available from adnexal site were included in the analysis ($n = 113$, Table 1). Medical records were reviewed to retrieve relevant clinical data. Response to treatment was defined according to the RECIST criteria together with CA-125 as established by the Gynecological Cancer Intergroup [28]. Follow-up (date of diagnosis of last participant included in the study to last date of follow-up) was 114 months. Ethical permission for the study was approved by the Regional Ethics Committee of the Karolinska Institutet (ethical permit number 2012/539–31/1).

2.2. The tissue microarray (TMA), discovery cohort

The building of the tissue microarray (TMA) was done as previously described [29]. In short, formalin-fixed, paraffin-embedded (FFPE), tumor tissue was collected, and a fresh 4 μm section was obtained, stained with hematoxylin & eosin and a representative tumor area was chosen. Core biopsies with 1 mm diameter were punched and brought into a recipient TMA. For each case (when possible), two punches were obtained, one from the primary adnexal site and one from the metastatic site (omentum or peritoneum). A fresh 5 μm section from the TMA block was mounted on glass slides and used for immunostaining.

2.3. Patients, clinical data and TMA, validation cohort

To validate the findings revealed in the discovery cohort, an independent cohort of 121 patients with HGSC (Lund, Sweden) was analyzed (Table 1). A detailed description of the inclusion criteria and TMA building of the validation cohort can be found in Martin de La Fuente et al. 2020 [30]. In short, FFPE biopsies were retrieved from tumor tissue obtained at staging or primary cytoreductive surgery from 141 chemo-naive patients affected by HGSC, at the Gynecology Department in the southern Swedish healthcare region between 2011 and 2015 (Lund, Sweden). From the tumor tissue, two to four blocks (primary site, lymph node metastases and peritoneal metastases, respectively whenever available) were available and one to two punches

Table 1
Clinical and pathological characteristics.

Characteristic	Discovery Cohort $n = 113$	Validation Cohort n = 121
Median age at diagnosis, years (range)	64 (36.5–84.2)	67 (43–86)
Diagnosis		
Ovarian Cancer	82 (72.6 %)	62 (51.2 %)
Fallopian Tube cancer	12 (10.6 %)	51 (42.1 %)
Peritoneal cancer	17 (14.0 %)	6 (5.0 %)
Undesignated site	2 (1.8 %)	2 (1.7 %)
Missing	0	0
FIGO stage		
IIC[a]-IIB[b]	2 (1.8 %)[a]	4 (3.3 %)[b]
IIIA	1 (0.9 %)	6 (5.0 %)
IIIB	5 (4.4 %)	7 (5.8 %)
IIIC	83 (73.5 %)	79 (65.3 %)
IV	22 (19.3 %)	25 (20.6 %)
Missing	0	0
Type of surgery		
Primary debulking surgery	93 (82.3 %)	119 (98.4)
Delayed primary/interval	14 (12.4 %)	2 (1.6)
No surgery	6 (5.3 %)	0
Missing	0	0
Macroscopic residual disease after surgery		
Absent	32 (28.3 %)	77 (63.6 %)
Present	75 (66.4 %)	44 (36.4 %)
Missing	0	0
Chemotherapy first line		
Platinum based	105 (92.9 %)	115 (95.0 %)
No platinum	1 (0.9 %)	3 (2.5 %)
No chemo	6 (5.3 %)	3 (2.5 %)
Missing	1 (0.9 %)	0
Response at EOT[3]		
CR	64 (56.6 %)	83 (68.6 %)
PR	24 (21.2 %)	26 (21.5 %)
SD	2 (1.8 %)	1 (0.8)
PD	14 (12.4 %)	5 (4.1 %)
Missing	2 (1.8 %)	3 (2.5 %)
Survival		
Alive with no evidence of disease	4 (3.5 %)	23 (19.0 %)
Alive with evidence of disease	5 (4.4 %)	25 (20.7 %)
Dead from ovarian cancer	97 (85.8 %)	71 (58.7 %)
Dead from other causes	4 (3.5 %)	2 (1.7 %)
Lost at follow up	3 (2.7 %)	0
Median follow up	36.4 months (0.4–171.9)	39.1 months (0.3–79.9)
Missing	0	0
Time from EOT to recurrence/progression		
≥6 months (platinum sensitive)	66 (58.4 %)	89 (73.6 %)
< 6 (platinum resistant)	39 (34.5 %)	26 (21.5 %)
Missing	0	0

FIGO = International Federation of Gynecology and Obstetrics, NACT = Neoadjuvant chemotherapy, EOT = End of treatment, CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease.
[a] FIGO staging system 1988.
[b] FIGO staging system 2013.

per block were obtained and included in the TMA. Only patients with stage IIB-IVB according to the FIGO classification of 2013 participated in the study and only tissues from adnexal tumoral site where considered [31]. Of the 141 cases of the validation cohort, 121 patients were included in the validation analysis. Follow-up was 32 months or the patients included in the analysis ($n = 121$). The study was approved by the ethics committee at Lund University (ethical permit number 2014/717).

2.4. Immunohistochemical analyses

From the TMA blocks 4 μm thick slides were cut and stained as following. FAP staining was performed with a rat antibody against human FAP (1:200, MABS1001, Vitatex, Stony Brook, NY) using the Ventana machine by a protocol provided by Roche (see protocol in Supplementary Material and Methods). For the CD8 staining, a mouse antibody against CD8 was used (1:100, M7103, DAKO Agilent Technologies, Santa Clara CA) and immunohistochemistry was performed with the Ventana machine (see protocol in Supplementary files, Material and Methods).

2.5. Image analyses and scoring

FAP scoring was evaluated separately by two of the authors, a pathologist and an oncologist (trained in pathology), who were blinded to clinical information at the time of assessment. FAP fraction positive stroma on total stroma and FAP intensity of positive stained stroma, were scored independently on a semi quantitative scale and a consensus was found between the two observers. FAP fraction was scored on a 5 points scale (0: 0 % of stroma area stained, 1: 1–10 %, 2: 11–50 %, 3: 51–95 %, 4: 96–100 %) and FAP intensity was scored on an optical 4 points scale (0 to 3); descriptive images are provided in Fig. 1. Two metrics were then produced: FAP positive fraction in the primary tissue and FAP intensity in the primary tissue. For the survival and association analyses, FAP positive stroma intensity was dichotomized in low (score 0 and 1) and high (score 2 and 3). To evaluate if one core from a whole tumor section can be considerate representative of the tumor expression of FAP, we measured FAP positive stroma intensity from four different cores of each case of an independent cohort ($n = 40$) of serous ovarian cancers (See Supplementary files, Materials and Methods). In all forty cases we noticed a homogeneous expression of stromal FAP; only ten cases showed one core as an outlier.

CD8 density was also scored on a semi-quantitative five points scale (0: 0 % of stroma area covered by CD8 positive cells, 1: 1–10 %, 2: 11–50 %, 3: 51–95 %, 4: 96–100 %). CD8 density was evaluated in the epithelial and stromal areas of the tumors respectively, and only patients with available adnexal tumor were included in the analysis (Fig. 1). Grade 4 was never reached in stromal CD8 density scoring, so for survival analyses CD8 stromal density was used as dichotomized in low (score 0 and 1) and high (score 2 and 3).

For FAP and CD8 scoring in the validation cohort, we used the same grade categories previously used for the discovery cohort. A mean of the scoring from each core provided the case-based values.

2.6. Statistical analyses

Overall survival (OS) was defined as survival from date of diagnosis to date of death of any cause. Progression free survival (PFS) was defined as the time from the date of diagnosis to progression, recurrence or death from any cause (whichever came first).

Significant differences in OS and in PFS were estimated using Log Rank tests and Cox Regression proportional hazard models. All variables showing a significant p value (< 0.05) at the univariable analysis were entered into the multivariable model. FIGO stage, age at diagnosis and residual tumor after primary surgery were the clinical variables included in the multivariable Cox regression model. Survival correlation analyses were performed through Pearson Chi-square test. Association between FAP expression and clinico-pathological parameters (age at diagnosis, FIGO stage and residual tumor after primary surgery) was analyzed using Fisher's exact test. Statistics were performed in SPSS, version 23.0 and in R.

The design of the study respected the guidelines for biomarker studies (REMARK) [32] (Supplementary Table S1). Data that support the findings of this study are available from the corresponding author upon reasonable request.

2.7. FAP and CD8 gene expression related to survival in publicly available databases

To confirm the findings obtained in the discovery and validation cohorts regarding CD8 and FAP protein expression, we performed survival

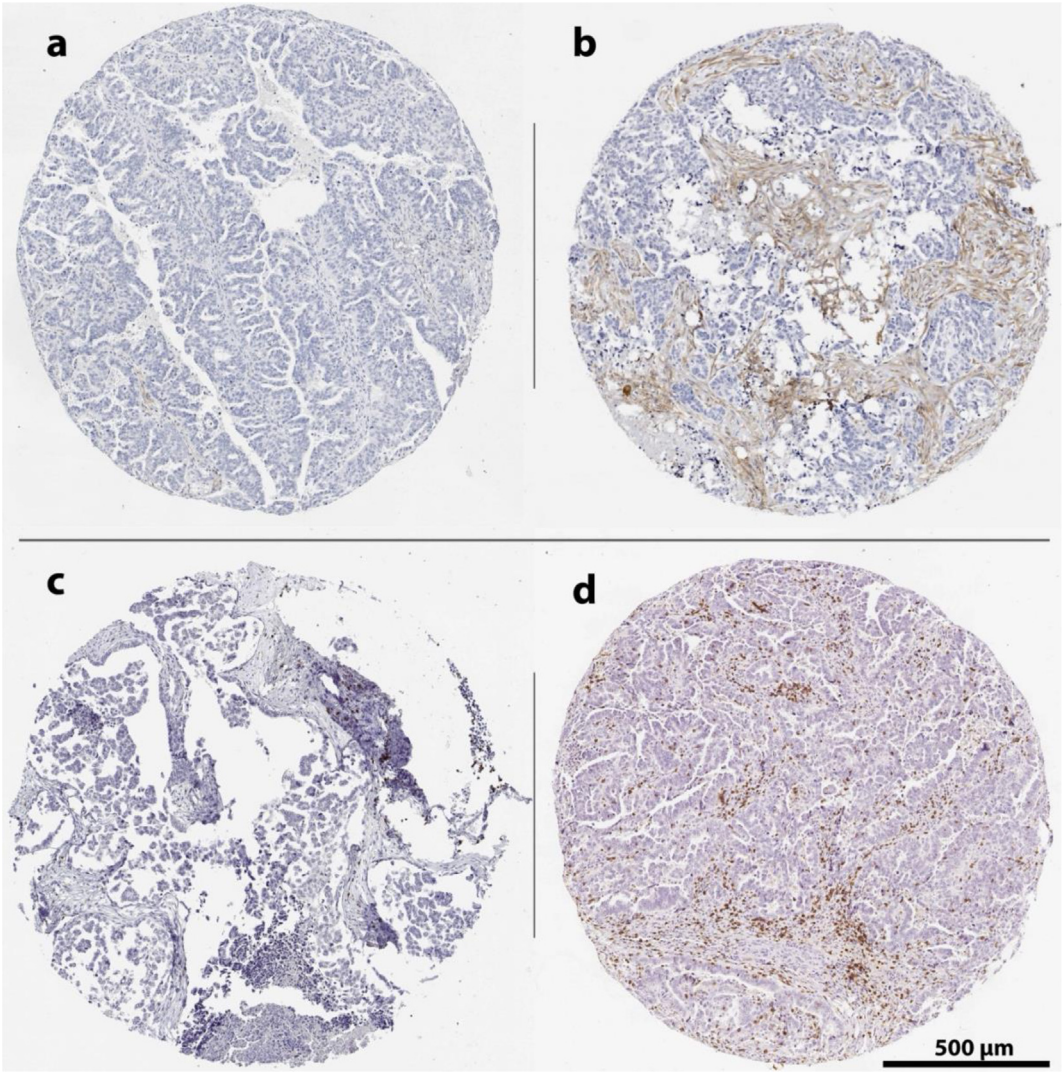

Fig. 1. Microphotographs of examples of low (a) and high (b) FAP stroma intensity, and low (c) and high (d) CD8 stromal density.

analyses associated to FAP (209955_s_at) and CD8A gene (205758_at) expression in fifteen publicly available databases of ovarian cancer patients (TCGA, GSE9891, GSE65986, GSE63885, GSE51373, GSE3149, GSE30161, GSE27651, GSE26712, GSE26193, GSE23554, GSE19829, GSE18520, GSE15622 and GSE14764). Patients with HGSC, stage III and IV, who were treated with platinum-based therapy were selected for evaluation ($n = 681$ for PFS, $n = 705$ for OS). Patients were split according to the median value of CD8A expression. Survival analyses were performed considering patients with high FAP and low FAP gene expression cut-off defined by the software (https://kmplot.com/analysis/).

3. Results

3.1. Patient characteristics

Among the 135 patients in the discovery cohort, primary adnexal tumor was available in 113 cases. Nine patients were excluded due to absence of tumor cells in the TMA, and for 13 patients only tumor from the metastatic site (omentum) was available (Supplementary Fig. S1). The clinical characteristics of the 113 patients included in the analysis are described in Table 1. Median age was 64 years (range 37 to 84), patients had mostly stage IIIC disease (73 %) and the majority of the patients underwent primary debulking surgery (82 %). Macroscopic radical surgery was obtained in 28 % of the cases (Table 1).

Clinical data from the 121 patients included from the validation cohort is summarized in Table 1. Median age was 67 years (range 43–86), stage IIIC disease was most common (65 %) and primary debulking surgery was performed in 98 % of the cases. Macroscopic radical surgery was obtained in a greater portion of the patients (64 %) than in the discovery cohort.

3.2. Immunohistochemical staining of FAP and CD8

Immunohistochemical staining of FAP and CD8 staining was performed. Six cores stained with FAP, and six cores stained with CD8 were excluded due to poor staining quality. The FAP marker was mostly represented in the stroma and showed a considerable inter-individual variation (Fig. 1).

The procentage of tumors with high FAP intensity was 35.5 % in the discovery cohort and 38.3 % in the validation cohort. No significant associations between FAP intensity and clinico-pathological parameters were found in the discovery cohort (Supplementary Table S2).

CD8 scoring also showed significant inter-individual variability (Fig. 1) Scoring was performed separately in the epithelial rich areas and in the stroma rich areas.

Tumors which showed high CD8 stromal density were 32.1 % and 32.4 % in the discovery and validation cohort respectively.

3.3. Overall survival and progression-free survival in discovery cohort

OS and PFS analyses according to FAP intensity and fraction and CD8 epithelial and stromal density were performed. The analyses were performed both on the whole patient cohort as well as for the group of patients with evaluable disease (patients with residual disease after surgery or patients who did not undergo surgery) at start of primary chemotherapy.

High CD8 stromal density in the whole patient cohort was significantly correlated to a longer OS than low CD8 stromal density (median OS 54.1 versus 34.4 months, $p = 0.01$) (Fig. 2). The improved patient survival rate for HGSC with high CD8 density in stromal tissue was confirmed in univariable Cox regression (HR 0.55; 95 % CI 0.35–0.86; p = 0.01) and multivariable analysis (HR 0.55; 95 % CI 0.33–0.85; p = 0.01) (Supplementary Table S3). FAP intensity and fraction and CD8 epithelial density displayed no effect on OS. None of the four markers showed any effect on PFS for the whole cohort.

In patients with evaluable disease at start of chemotherapy ($n = 81$), HGSC with high intensity of FAP was significantly correlated with shorter PFS than for HGSC with low FAP (median PFS 11.3 versus 14.5 months, $p = 0.02$) (Fig. 3A). Similar results were seen when FAP fraction was analyzed, with a significant shorter PFS in cases with high FAP fraction than in low (median PFS 11.4 versus 14.7 months, $p = 0.03$) (data not shown). The shorter PFS for HGSC with high FAP intensity and fraction was confirmed in univariable (for FAP intensity HR 1.73; 95 % CI 1.07–2.79; $p = 0.03$, and for FAP fraction HR 1.67; 95 % CI 1.05–2.65; p = 0.03 respectively) and multivariable Cox regression analyses (for FAP intensity HR 2.09; 95 % CI 1.24–3.53; $p = 0.01$ for FAP fraction HR 2.13; 95 % CI 1.26–3.60; p = 0.01, Table 2).

Further analyses in patients with evaluable disease based on CD8 (high vs low stromal density) demonstrated that high FAP intensity was associated with shorter PFS in HGSC with high CD8 density (median PFS 11.4 versus 18.6 months, $p = 0.01$, Fig. 3B) but not for HGSC with low CD8 density (Fig. 3C). Similar results were found when FAP fraction was analyzed (data not shown). The FAP-intensity effect on PFS in HGSC with high CD8 stromal density,

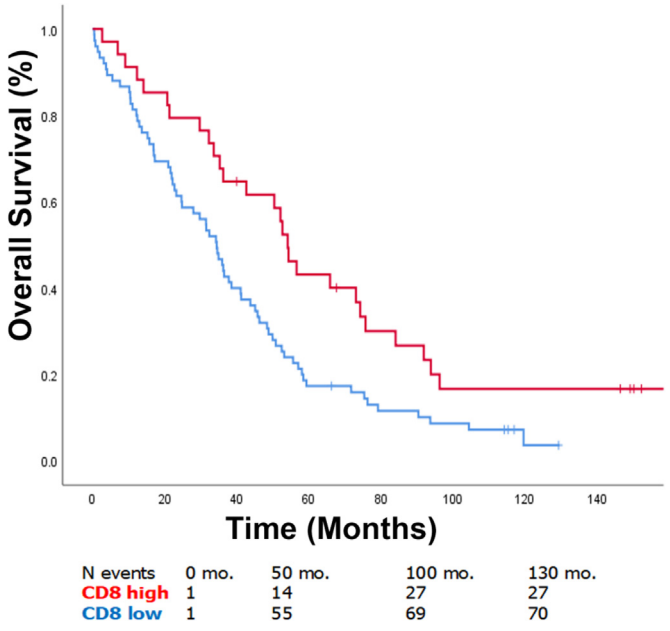

Fig. 2. Overall survival (OS) according to CD8+ stromal density among patients operated for HGSC in the discovery cohort. Log-rank test ($p = 0.01$) showed that patients with high CD8+ stromal density (red label) had a longer OS compared to patients with low CD8+ stromal density (blue label) with a median OS of 54.1 compared to 34.4 months (HR 0.55, 95 % CI, 0.35–0.86, p 0.01).

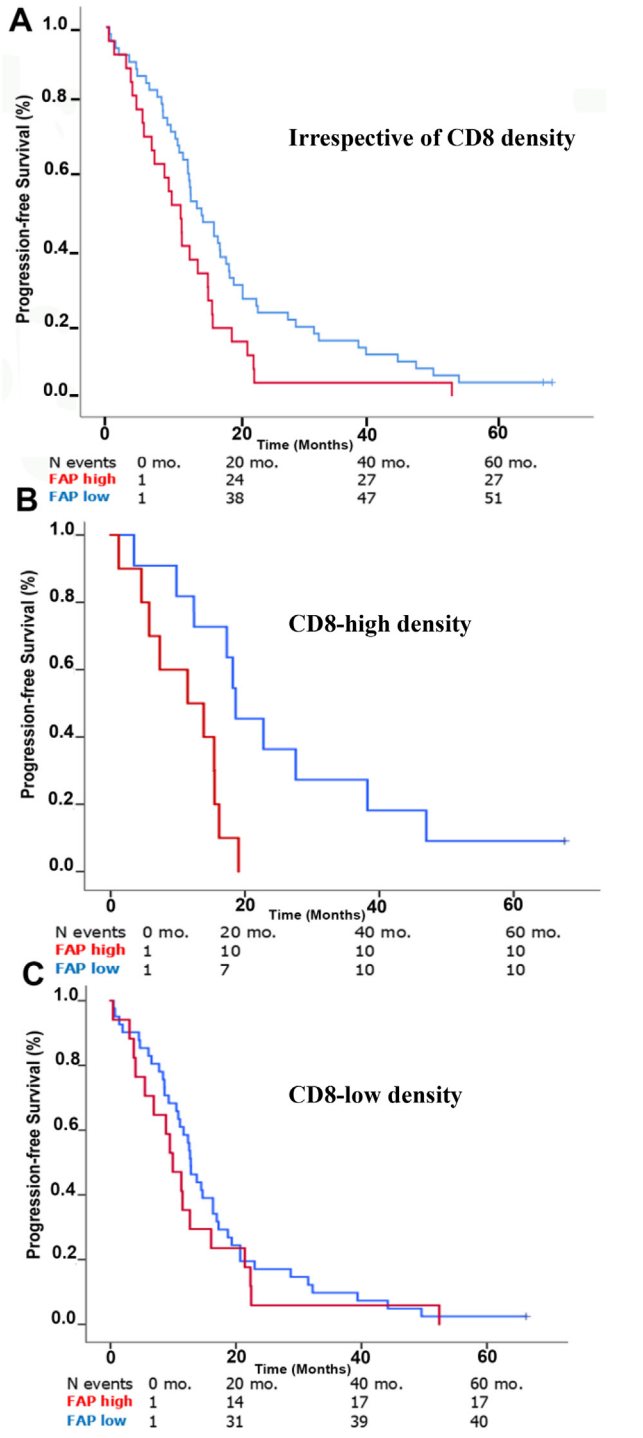

Fig. 3. A-C: Progression-free survival (PFS) among patients in the discovery cohort with HGSC with evaluable disease at start of platinum-based chemotherapy ($N = 81$), according to (A) FAP high (red label) and low intensity (blue label) (11.3 vs 14.5 months, p 0.02), (B) FAP high (red label) and low intensity (blue label) in the group with high density of CD8 (11.4 vs 18.6 months, p 0.01), and (C) FAP high (red label) and low intensity (blue label) in the group with low density of CD8 (9.9 vs 12.8 p 0.35).

Table 2
Uni- and multivariable analyses of progression-free survival in patients with measurable disease at start of platinum-based chemotherapy (n = 81) in the discovery cohort.

Variables (n)	Univariate		Multivariate	
	HR (95 % CI)	*p*-value	HR (95 % CI)	*p*-value
Age at diagnosis				
<64 (38)	1 (reference)	0.17	1 (reference)	0.03
>64 (43)	1.37 (0.88–2.13)		1.77 (1.07–2.94)	
FIGO stage				
IIC + IIIA+IIIB (1)	1 (reference)	0.47	1 (reference)	0.84
IIIC+IV (80)	2.09 (0.29–15.13)		1.23 (1.64–9.30)	
FAP stromal intensity				
Low (53)	1 (reference)	0.03	1 (reference)	0.01
High (27)	1.73 (1.07–2.79)		2.09 (1.24–3.53)	
FAP stromal fraction				
Low (49)	1 (reference)	0.03	1 (reference)	0.01
High (31)	1.67 (1.05–2.65)		2.13 (1.26–3.60)	

was confirmed in univariable (HR 4.03, CI 95 % 1.38–11.72, p = 0.01) and in multivariable Cox regression analyses (HR 3.74, 95 % CI 1.12–12.52, p = 0.03, Supplementary Table S4). FAP intensity did not show a correlation to OS in either the high or low stromal CD8 density groups (data not shown).

3.4. Validation of the survival association in an independent cohort

To validate the survival-related associations of CD8 and FAP-related metrics revealed in the discovery cohort, a TMA derived from a cohort of 121 patients with HGSC was analyzed (Table 1).

Patients affected by HGSC with high density of CD8 in epithelial areas had longer OS than patients affected by HGSC with low CD8 epithelial density (median OS 55.7 versus 43.3 months; p = 0.01, Log Rank test (data not shown). The results were confirmed at the univariable (HR 0.5, 95 % CI 0.26–0.98, $p = 0.04$), but not at the multivariable (HR 0.56; 95 % CI 0.28–1.12; $p = 0.10$) (data not shown), Cox Regression analysis.

FAP intensity and fraction had no impact on OS or PFS (data not shown). However, high FAP intensity showed a significant negative impact on OS in cases with high CD8 stromal density, with a median OS of 31.5 months compared to 76.9 months in cases with low FAP intensity ($p = 0.02$ at Log Rank test) (Fig. 4A). This result was confirmed in univariable (HR 2.83; 95 % CI 1.17–6.86; p = 0.02) and in multivariable Cox regression analyses (HR 2.60; 95 % CI 1.05–6.47; p = 0.04). In the group with low CD8 stromal intensity, high FAP intensity had no impact on OS (Fig. 4B). Similar results were found when FAP fraction was analyzed (data not shown). High FAP intensity did not affect PFS in either the high or in low stromal CD8 density groups respect (data not shown).

3.5. FAP and CD8 gene expression related to survival in publicly available databases

To confirm the prognostic findings obtained in the discovery and validation cohorts regarding CD8 and FAP protein expression, we performed PFS ($n = 681$) and OS ($n = 705$) analyses associated to FAP and CD8A gene expression in fifteen combined publicly available databases, including the TCGA data set. In the subgroup of HGSC with high CD8A gene expression, high FAP gene expression had a significantly worse OS compared to HGSC with low FAP gene expression with a median OS of 35 compared to 45 months (HR 1.6; 95 % CI 1.19–2.15, $p = 0.002$, Supplementary Fig. S2A). Worse outcome was also demonstrated when PFS was analyzed; PFS was found shorter in HGSC with a high vs low FAP gene expression in the CD8A high group (median PSG 11.1 vs 12 months, HR = 1.38; 95 % CI 1.06–1.78, $p = 0.02$, Supplementary Fig. S2B). FAP gene expression had no statistically significant impact either on OS or PFS the CD8A low group (data not shown).

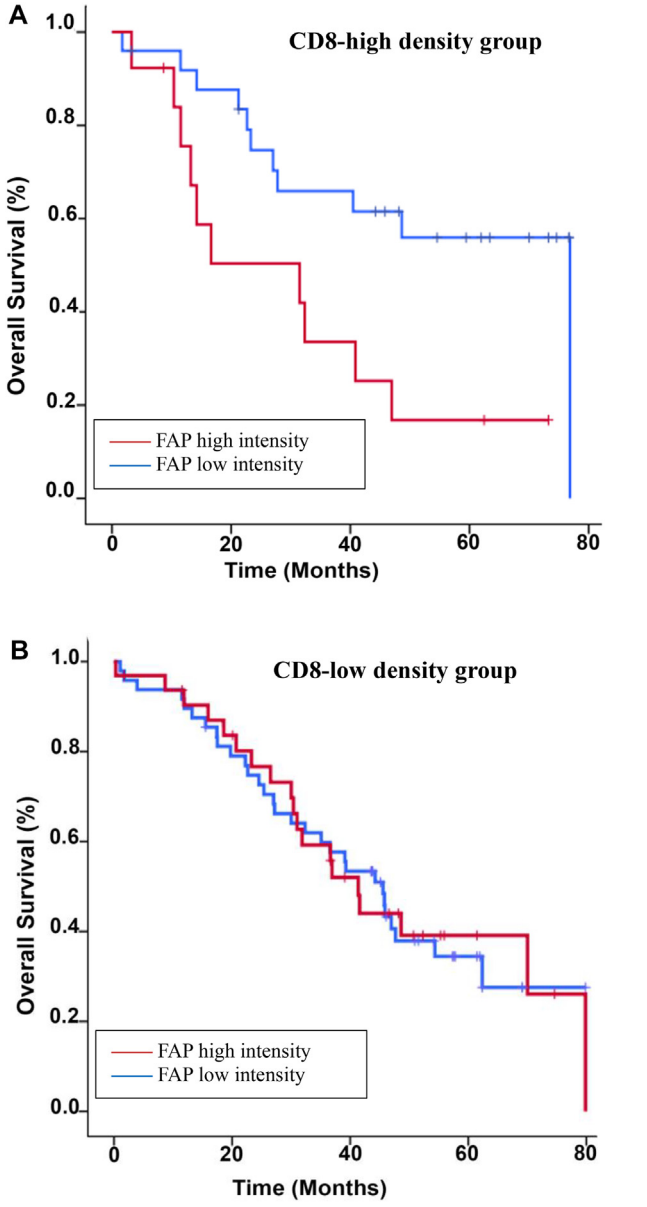

Fig. 4. A-B: Overall survival among patients in the validation cohort according to (A) FAP-high (red label) and low (blue label) intensity in the group with high density of CD8 (31.5 vs 76.9 months, p 0.02) and (B) FAP high (red label) and low (blue label) intensity in the group with low density of CD8 (41.4 vs 45.6, p 0.95). This was confirmed in univariate (HR 2.83; 95 % CI 1.17–6.86; $p = 0.02$) and multivariate (HR 2.60; 95 % CI 1.05–6.47; $p = 0.04$) Cox regression analyses.

4. Discussion

Our study presents novel data on HGSC, demonstrating prognostic significance of FAP+ fibroblasts, restricted to patients with high density of CD8+ cells, compatible with mechanisms proposed from preclinical models. We confirm previous findings that high tumor infiltration of CD8+ cells is related to favorable prognosis in patients with HGSC, but our results show that this positive effect is hampered in FAP high tumors. This study provides the most extensive clinical sample-derived data suggesting a clinically relevant interaction between CD8+ lymphocytes and FAP+ fibroblasts.

Preclinical studies linking FAP-positive fibroblasts with T-cells has been performed in models of lung and pancreas cancer. Using the Lewis lung carcinoma model, Kraman et al. reported that depletion of

FAP positive cells causes growth arrest in tumors with an immunogenic response [8]. Furthermore, in another mouse model of pancreas cancer, Feig et al. reported that immune control of tumor growth by T-cell checkpoint antagonists was achieved only under depletion of FAP positive cancer-associated fibroblasts [2]. More recent data has described the role of different marker-defined CAF subsets, including FAP positive fibroblasts, in various immune-modulatory mechanisms, generating a microenvironment characterized by immune suppression and leading to impaired prognosis of the patient [19–22]. This is also supported by findings in the current study, suggesting a regulatory effect of CAFs on tumor immunosuppression.

Previous studies have used different approaches for targeting of FAP positive fibroblasts in various experimental tumor models [33,34]. Studies have mostly been done as mono-treatments and have not specifically explored roles of this fibroblast subset in interactions with immune cells or involvement in immunogenic cell death. The findings of the present study encourage to such analyses. Furthermore, it will be of interest to see if the associations detected in the present study also can be seen in other tumor types.

CAFs have been reported to influence T cell function through expression of checkpoint ligands, such as PD-L1 and PD-L2. Both PD-L1 and PD-L2 bind to the PD-1 receptor expressed by T cells and drive their dysfunction resulting in suppression of immune response and enhanced tumor growth. High PD-L2 expression in FAP+ CAFs could be a new mechanism of primary resistance to immunotherapies [19,35]. Furthermore, studies on breast cancer at single-cell level, identified fibroblast subsets associated with immunosuppression and immunotherapy resistance [9,36,37].

CAFs can also regulate tumor-infiltrating lymphocytes (TILs) and their role in tumor immunosuppression via the inflammatory cytokines [38]. Cytokines are major regulators of immunity that enables cells of the immune system to communicate. IL6 and other cytokines are known to play an important role in tumorigenesis, proliferation, invasion and immunosuppression via signaling pathways in the tumor microenvironment [39,40]. In preclinical experiments, approaches of targeting cytokines in cancer treatment have shown promising results. The role of cytokines should be further explored in future studies [22,41].

One strength of this study is the use of the Swedish cancer registry to identify eligible patients for the study. The Swedish Cancer Register has a high coverage (94 %) and histologic verification of diagnosis of 99 % [42]. In addition, clinical and tumor characteristics were reviewed in medical charts in a standardized way using Case Report Forms. In addition, a reference pathologist reviewed all tumor tissue. The findings of this report were validated and confirmed in an independent cohort of HGSC patients. The baseline characteristics of the two cohorts were similar, however some important differences were noted. The lower percentage of complete cytoreductive surgery with no residual disease in the discovery cohort compared with the validation cohort is because the cohorts are from different time periods. The validation cohort was collected after a practice change towards more aggressive cytoreductive surgical approach in ovarian cancer was implemented, resulting in the higher rate of no residual disease in the validation cohort. This is reflected, as expected, in the worse clinical outcome prognosis in the discovery cohort (Table 1). Furhermore, this may explain why the prognostic impact of FAP in CD8-high group is reflected in PFS in the discovery cohort while in OS in the validation cohort. The study has also some other limitations. The use of TMA can raise concerns if the analyses of one core can give representative information for the whole tissue. To support the representativeness of our TMA material, we validated FAP scoring on a TMA composed of tissues from HGSC previously used in our group [29]. This analysis allowed us to conclude that the availability of only one core per case should not affect the results in a significant way. Notably, all immunohistochemical analyses were performed at the same time and under the same conditions, minimizing sources of errors related to different experimental conditions. Moreover, our data was supported by similar results of FAP and CD8A gene expression analysis in fifteen combined publicly available databases, including the TCGA data set.

In conclusion, our study reveals a previously unknown, potentially clinically relevant prognostic interaction between CAFs and a high tumor infiltration of CD8+ T cells in patients with HGSC. The results were confirmed in an independent cohort as well as in two publicly available databases of gene expression in ovarian cancer. Although the results need to be further validated, our data suggest that FAP should be considered as a possible therapeutic target to improve tumor immunity and enhance the efficacy of conventional cytotoxic therapies, including immunotherapies. This strategy may also be useful to explore in T cell directed immunotherapy in HGSC.

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CRediT authorship contribution statement

Sara Corvigno: Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. **Josefin Fernebro:** Writing – review & editing, Writing – original draft. **Josefin Severin Karlsson:** Writing – review & editing. **Artur Mezheieusky:** Writing – review & editing, Validation, Methodology, Investigation. **Alfonso Martín-Bernabé:** Data curation. **Laura Martin De La Fuente:** Writing – review & editing. **Sofia Westbom-Fremer:** Writing – review & editing. **Joseph W. Carlson:** Writing – review & editing. **Christian Klein:** Writing – review & editing, Methodology. **Paivi Kannisto:** Writing – review & editing. **Ingrid Hedenfalk:** Writing – review & editing. **Susanne Malander:** Writing – review & editing. **Arne Östman:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Hanna Dahlstrand:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

CK declare employment, patents/royalties and stock ownership with Roche. AÖ has during last three years received research support from IPSEN, and is founder of TECKNET AB. The other authors declare that they have no known competing financial interests or personal relationships that creates potential conflicts of interest to the work reported in this article.

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