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High glucocorticoid receptor expression predicts short progression-free survival in ovarian cancer

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Abstract

Objective—To investigate the association of tumor glucocorticoid receptor (GR) expression and patient outcome in ovarian cancer.

Methods—GR expression was evaluated by immunohistochemistry using tissue microarrays of specimens from 481 patients with ovarian cancer and 4 patients with benign conditions. Low GR expression was defined as an intensity of 0 or 1+ and high GR as 2+ or 3+ in >1% of tumor cells. Analyses were performed to evaluate the relationship of GR expression with clinical characteristics, progression-free survival (PFS) and overall survival (OS).

Results—GR protein was highly expressed in 133 of 341 (39.0%) tumors from patients who underwent upfront cytoreduction surgery followed by adjuvant chemotherapy. High GR expression

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Conflict of interest statement

SDC and The University of Chicago have been issued patents on methods of measuring GR expression in TNBC and using GR antagonists in TNBC, which while not directly related to this work on ovarian cancer, could be considered broadly relevant. The spouse of JTV is an employee of Abbott Laboratories.

was more common in serous tumors ($p < 0.001$), high grade tumors ($p < 0.001$), and advanced stage tumors ($p = 0.037$). Median PFS was significantly decreased in cases with high GR (20.4 months) compared to those with low GR (36.0 months, HR = 1.66, 95% CI 1.29–2.14, $p < 0.001$). GR remained an independent prognostic factor for PFS in multivariate analysis. OS was not associated with GR status.

Conclusions—These data suggest that high GR expression correlates with poor prognosis and support the hypothesis that modulating GR activity in combination with chemotherapy may improve outcomes.

GRAPHICAL ABSTRACT

**Progression-Free Survival in Ovarian Cancer
Stratified by Glucocorticoid Receptor Expression**

Keywords

Epithelial ovarian cancer; Hormone receptor; Glucocorticoid receptor; Tumor markers; Survival

1. Introduction

Invasive epithelial ovarian cancer (EOC) is the leading cause of mortality among gynecologic malignancies in the developed world, and is estimated to cause >14,000 deaths in the United States in 2017 [1]. The majority of cases are diagnosed at an advanced stage, when disease has spread outside of the ovary or fallopian tube. Despite cytoreductive surgery and platinum-based chemotherapy, most tumors ultimately recur and develop chemotherapy resistance [2]. Patients with platinum resistant or refractory disease have poor response rates to standard chemotherapy regimens [2]. Novel therapeutic approaches are needed to improve patient outcomes.

The glucocorticoid receptor (GR) is a nuclear hormone receptor activated by endogenous cortisol and synthetic glucocorticoids. Several lines of evidence suggest that signaling through GR may play a role in tumorigenesis and tumor progression. Endogenous cortisol levels are disrupted in states of physical and psychosocial stress. Epidemiologic studies have suggested that stressful events associated with disruption of the neuroendocrine axis (including chronic stress, depression, and other psychological events) may be associated with increased risk of cancer onset, progression, and mortality (reviewed in [3,4]).

GR-mediated signaling has also been shown to have direct effects in tumor cells. Administration of glucocorticoids reduces the effectiveness of chemotherapy in cell line and xenograft models of several solid tumor types including breast [5–7], pancreatic [8] and ovarian cancer [9–11]. Treatment with clinically relevant concentrations of dexamethasone in triple-negative breast cancer (TNBC) and ovarian cancer decreases chemotherapy efficacy [11] and inhibits apoptosis [6,12]. In contrast, treatment with a glucocorticoid receptor (GR) antagonist, such as mifepristone, potentiates the antitumor efficacy of chemotherapy in mouse xenograft models of TNBC [7], and EOC [9].

Despite preclinical data supporting a role for GR signaling in tumor cell survival and tumor progression, little is known about the characteristics of GR expression in primary solid tumors. In one analysis, high expression of GR (*NR3C1*) messenger RNA correlated with decreased progression-free survival in early stage breast cancer patients with estrogen receptor (ER)-negative [5] but not ER-positive breast cancer, suggesting a subtype-specific mechanism for GR activity. A much earlier study in ovarian cancer used a dextran-coated charcoal technique to determine tumor expression of ER, progesterone receptor (PR), androgen receptor (AR), and GR in 36 ovarian specimens [13]. These authors found GR expression in 88% of ovarian cancers, but clinical outcome data were not reported [13]. To our knowledge, no study has previously examined the relationship between GR expression and clinical outcome in ovarian cancer. An in depth immunohistochemistry (IHC) study was therefore undertaken to expand on earlier work and investigate the association of GR expression in primary ovarian cancers with subsequent relapse and survival times in patients with both early and advanced stage EOC.

2. Materials and methods

2.1. Patients

Tissue microarrays (TMAs) were constructed from 481 patients with ovarian cancer collected at the time of definitive debulking surgery at Oregon Health & Science University Hospital and the University of Southern California during a 15-year period from 1995 to 2010. An additional 4 patients had benign conditions. Specimen collection and retrospective review of patient medical records were performed under a protocol approved by the local Institutional Review Boards (IRB). The review included outpatient and inpatient treatment, including surgery and chemotherapy. Study outcomes included overall survival (OS) and time to recurrence or progression, each measured from the time of definitive surgery. The duration of OS was the interval between definitive surgery and death. The duration of progression-free survival (PFS) was the interval between definitive surgery and first recurrence or progression. Observation time was the interval between definitive surgery and last contact (death or last follow-up). Data were censored at 120 months for patients who did not reach a study endpoint of recurrence, progression, or death. Among the 341 patients who received primary cytoreductive surgery followed by adjuvant chemotherapy (Cohort 1) there were 243 progression events and 201 deaths with a median follow up time of 43.4 months (Interquartile range 26.3–71.2). An additional 144 cases included in the tissue microarrays (Cohort 2, OHSU & USC) consisted of an expanded complement of cases described in further detail below.

A separate group of 20 patients who received neoadjuvant chemotherapy was selected from the University of Chicago tissue bank (Cohort 3). Overall, a total of 130 cases received neoadjuvant chemotherapy. Of these, 35 cases had a biopsy specimen prior to receiving chemotherapy in addition to post-treatment surgical specimen at the time of interval cytoreductive surgery. Twenty cases were identified with adequate tumor cellularity in both specimens (pre- and post- chemotherapy) for IHC analysis.

2.2. Tissue microarray construction

TMAAs were constructed as described previously by Kononen et al. [14]. Briefly, after carefully choosing the morphologically representative region from the hematoxylin and eosin (H&E) section, a one-millimeter core was punched from the individual paraffin-embedded block (donor block), and transferred to the receiver paraffin-embedded block (receiver block). To overcome tumor heterogeneity, core biopsies were performed from three different areas of each tumor. One section was stained with H&E to confirm the presence of the tumor by light microscopy. The tumor subtypes and grade were re-reviewed for confirmation by a single experienced pathologist (PMF). Histologic subtypes were based on the World Health Organization (WHO) guidelines. Histologic grading was based on the Silverberg grading system [15]. Staging was performed according to the 1988 FIGO classification [16].

2.3. Immunohistochemistry

GR IHC was performed as follows. Four-micron sections were cut from formalin-fixed, paraffin-embedded whole tissue sections or TMAAs. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 5 min. Antigen retrieval was carried out in high pH (pH 8) citrate buffer for 3 min in a steam-cooker. Then, sections were incubated for 1 h with GR antibody (rabbit monoclonal antibody, clone# D8H2 XP, Cell Signaling, Danvers, MA) at 1:500 dilution at room temperature. A subsequent reaction was performed with the biotin-free HRP enzyme labeled polymer of the Envision plus detection system (Dakocytomation, Carpinteria, CA). Diaminobenzidine complex was used as chromogen. Breast and ovarian cancer tissues were used as positive controls. In negative controls, normal goat serum was substituted for primary antibody resulting in a lack of detectable staining. Ten whole sections of ovarian cases that were included in the array were also stained for comparison and showed homogenous nuclear staining throughout the tumor indicating that the TMA cores were representative of the tissue. One pathologist (PMF) reviewed the IHC slides twice at an interval of two months. Staining intensity and percentage of positive tumor cells were recorded. The staining intensity was interpreted as none, weak, moderate, or strong (*i.e.* 0, 1+, 2+, 3+). Percentage of tumor staining was categorized as follows: 0 = no positive staining; 1 = 1–5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–100%. Final analyses focused on categorization of tumor expression of GR as low when tumor cells exhibited 0 or 1+ intensity staining or as high when tumor cells exhibited 2+ or 3+ in >1% of tumor cells.

2.4. Statistical analysis

Of the 485 specimens in the TMA, the primary analysis focused on 341 specimens from patients with invasive EOC who received primary debulking surgery followed by adjuvant chemotherapy and had complete clinical follow up data available, including residual disease

status (Cohort 1, OHSU & USC). The remaining 144 cases included in the tissue microarrays (Cohort 2, OHSU & USC) consisted of 12 borderline tumors, 42 early stage (I and II) EOC (23 treated with surgery alone, 19 treated with surgery and adjuvant chemotherapy for whom follow up data was incomplete), 26 advanced stage (III and IV) EOC cancers treated with primary surgery and adjuvant chemotherapy for whom follow up data was incomplete, 7 carcinosarcoma cases, 53 advanced stage cases treated with neoadjuvant chemotherapy followed by interval debulking surgery and adjuvant chemotherapy, and 4 cases with benign disease. A schema of study cohorts is shown in Supplemental Fig. S1. All 485 specimens in Cohorts 1 and 2 were collected at the time of primary or interval debulking surgery at OHSU & USC, including the specimens from the 53 patients who received neoadjuvant chemotherapy, whose specimens were collected after exposure to chemotherapy. Finally, Cohort 3 consisted of samples from 20 patients from the University of Chicago tissue bank who received neoadjuvant chemotherapy and for whom pre- and post-chemotherapy specimens were available.

To evaluate the relationship between GR expression and clinical characteristics, the Pearson's Chi-square method or Fisher's Exact test was used to test for association with age group, histologic subtype, FIGO stage (early *versus* advanced) and presence of residual disease after debulking surgery. For our analysis, early stage was defined as FIGO stage I/II and advanced as FIGO stage III/IV. In our dataset, gross residual disease was defined as ≥ 1 cm remaining and no gross residual disease was defined as <1 cm disease remaining. A Mantel-Haenszel test of trend was used to test for correlation of GR expression with grade. PFS and OS were estimated using the Kaplan-Meier method, with the difference between groups compared by log-rank test. Cox proportional hazards model was also performed to assess the association of GR expression with PFS or OS, with multivariate analysis adjusted for age (in increments of 10 years), histological cell type, grade, stage (early *versus* advanced), and the presence of gross residual disease after surgical debulking. Pre- and post-chemotherapy cases (Cohort 3) were compared by Wilcoxon rank sum test using STATA. All other statistical analyses were performed by SAS version 9.4 (SAS Institute, Cary, NC). All p-values are two-sided and a p-value of 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 shows clinical and pathologic tumor characteristics for Cohort 1. This cohort consisted of 341 patients who received upfront debulking surgery followed by adjuvant chemotherapy and for whom complete clinical and follow up data were available. The median age was 59 years (range 24–89 years). The majority of the tumors ($n = 240$ (70.9%)) were of serous histology; nine cases were low grade serous (Grade 1) and 231 were high grade serous (Grade 2 or Grade 3). The remaining cases included clear cell ($n = 42$ (12.3%)), endometrioid ($n = 32$ (9.4%)), mucinous ($n = 17$ (5.0%)), and other ($n = 10$ (2.9%)). Most tumors were high grade (Grade 2 or Grade 3, $n = 311$ (91.2%)) and advanced stage (FIGO III or IV, $n = 245$ (71.8%)).

3.2. GR expression varies with histologic subtype, grade and stage

High GR expression, defined as >1% of tumor cells with 2+ or 3+ intensity staining, was present in 133 of 341 patients (39%) of tumors in Cohort 1 (Table 1). Representative stained sections of the TMAs are illustrated in Fig. 1. There was no association between GR expression and patient age at diagnosis ($p = 0.772$). High GR expression was significantly associated with histologic subtype ($p < 0.001$), with the highest prevalence observed in the serous tumors ($n = 115/240$ (47.9%)) and the lowest prevalence observed in the endometrioid tumors ($n = 2/32$ (6.3%)). Clear cell and mucinous tumors had high GR expression in 11 of 42 (26.2%) and 2 of 17 (11.8%) cases, respectively. High GR expression also correlated positively with increasing grade ($p = 0.001$) and with advanced stage at diagnosis (early *versus* advanced, $p = 0.037$).

GR expression was analyzed in the remaining cases included in the TMAs (Cohort 2) in order to evaluate GR expression in premalignant cases and other histologies (carcinosarcomas), increase the number of early stage tumors, and evaluate tumors collected at the time of interval debulking surgery. These cases were excluded from the primary statistical analyses, which were performed exclusively in Cohort 1. Clinical characteristics for all 481 EOC patients in Cohorts 1 and 2 are shown in Supplemental Table S1. Carcinosarcomas and serous cases had the largest proportion of cases with high GR expression ($n = 4/7$ (57.1%), and $n = 128/265$ (48.3%), respectively, Fig. 2A). As was observed when just Cohort 1 cases were considered, high GR expression was rare in endometrioid cases ($n = 5/47$ (10.6%)). Few borderline tumors exhibited high GR expression ($n = 2/12$ (16.7%), Fig. 2B). Early stage tumors treated with surgery alone (earlyS) or surgery and adjuvant chemotherapy (earlySCT) had a similar proportion of cases with high GR expression ($n = 7/23$ (30.4%) and $n = 37/116$ (31.9%), respectively). Advanced stage tumors treated with neoadjuvant chemotherapy before interval debulking surgery had an increased rate of high GR expression compared with unmatched advanced stage cases treated with primary debulking surgery (62.3% *versus* 41.9%, $p = 0.006$, respectively). In the 4 benign cases, no GR staining was observed in the ovarian epithelial cells.

The prevalence of GR expression in neoadjuvant cases may be due to high GR expression pre-treatment; alternatively, chemotherapy may cause selection for tumor cells with high GR expression. To examine whether high GR expression in neoadjuvant cases is present pretreatment or whether chemotherapy selects for chemotherapy-resistant cells with high GR, GR expression was examined in an independent cohort of 20 patients for whom paired pre- and post-neoadjuvant chemotherapy specimens were available (Cohort 3, Supplemental Table S2). Of the 20 pairs, 18 had evaluable staining. GR expression was not statistically different between paired pre-treatment and post-treatment specimens ($p = 0.583$, Fig. 2C). High GR expression (2+ or 3+ staining) was present in 15 out of 18 (83%) specimen in both groups.

3.3. High GR expression is associated with decreased PFS

High GR expression predicted an increased risk of disease progression in Cohort 1 (hazard ratio (HR) = 1.66, 95% confidence interval (CI) = 1.29–2.14, Fig. 3A). Median PFS was 15-months shorter in patients whose tumors had high GR expression compared to those whose

tumors had low GR expression (20.4 *versus* 36.0 months respectively, $p < 0.001$, Fig. 3A). There was no significant difference in OS by GR status (Fig. 3B). When adjusted for other clinical variables in multivariate analysis, high GR expression remained an independent predictor of PFS (HR = 1.41, 95% CI 1.08–1.84, $p = 0.012$, Table 2) but not of OS. Other clinical variables with independent prognostic value for PFS and OS, respectively, included age at diagnosis ($p = 0.024$ and $p = 0.007$), stage ($p < 0.001$ and $p < 0.001$), and the presence of residual disease after primary surgery ($p < 0.001$ and $p < 0.001$), consistent with prior studies [17].

The level of GR expression needed to have an impact on outcomes is not known. The relationship of PFS with staining intensity and percent staining as nominal variables was also examined (Supplemental Table S3). A strong correlation was observed between the magnitude of GR intensity and the percentage of tumor cell nuclei with positive GR staining ($p < 0.001$). Supplemental Fig. S2A illustrates the ranked relationship between the intensity of GR staining and PFS with the longest PFS observed in patients with 0 or 1+ intensity staining and the shortest PFS observed with 2+ or 3+ intensity staining ($p < 0.001$, Supplemental Fig. S2A). A statistical trend was also observed when PFS was categorized by the percentage groups from 0 to 5 ($p = 0.022$, Supplemental Fig. S2B). Patients with zero GR positive tumor cells had the best outcomes and patients with any percentage of GR positive tumor cells had worse outcomes. Because of the strong correlation of intensity with percentage staining, we chose to characterize our cases by intensity using a dichotomous variable for ease of interpretation.

3.4. High GR expression shows a trend toward decreased PFS across histologic subtypes, grades and stage

As expected, the majority of cases in the study were of high grade serous histology. However, when only advanced stage high grade serous cases were considered, there was only a marginal difference in PFS (HR = 1.34, 95% CI 1.00–1.78, $p = 0.048$, Fig. 3C) and no difference in OS (HR = 0.95, 95% CI 0.68–1.30, $p = 0.740$, Fig. 3D) between cancers with high and low GR expression.

Further subgroup analyses were then performed, starting initially with patients diagnosed with early stage disease. Fig. 3E shows that among the 96 cases of early stage disease in Cohort 1 those with high GR expression had decreased PFS and increased risk of disease progression compared to those with low GR expression (HR = 2.68, 95%CI 1.16–6.19, $p = 0.016$). To examine this further, the 42 early stage cases in Cohort 2 were evaluated separately. Fig. 3F shows that a strong correlation of high GR expression with poor PFS was again observed in this cohort (HR 8.35, 95% CI 0.93–74.79, $p = 0.023$), independently validating this finding. Clinical characteristics for early stage patients are shown in Supplemental Table S4.

High GR expression also correlated with decreased PFS in the subsets with advanced stage disease, grade 2, and no gross residual disease (Fig. 4A). A trend toward decreased PFS was observed for high GR expression across all histologic subtypes (Fig. 4A). The trend was also present for all grades, however confidence intervals for the subgroups are wide for many variables examined. While there is no association of high GR expression with OS for Cohort

1 as a whole, or in the early stage cases, Fig. 4B demonstrates that high GR expression is associated with a shorter OS for the subset of patients with no gross residual disease.

Supplemental Table S5 displays the results of univariate and multivariate modeling for PFS for high *versus* low GR expression for all patients in Cohort 1, and in the subsets with early stage, advanced stage, advanced stage high grade serous ovarian cancer, or no gross residual disease. These analyses were also performed for the relationship of PFS with GR expression categorized as 0, 1+, 2+ or 3+ intensity (Supplemental Table S5).

4. Discussion

We found high GR expression in primary EOC to be associated with known features of aggressive behavior, including higher grade and more advanced stage. There was also an association between GR and histologic subtype with the highest frequency of GR expression in high grade serous tumors. High GR expression was an independent prognostic factor for PFS when adjusting for the impact of age at diagnosis, grade, stage, and histologic subtype on PFS. We did not find any overall correlation between GR expression and OS, although there was an association between GR expression and OS in the group with no gross residual disease.

GR had only a marginal statistically significant impact on PFS in advanced high grade serous cases, the most prevalent subgroup. This suggests that the effect of GR on low grade or non-serous histologies may be driving the effect on PFS of high GR expression seen in the main analysis. If GR has a different magnitude of effect on different histologic subtypes, this could also confound determining an effect on OS in the overall analysis. The small number of cases in the non-serous subgroups limits the ability to explore effect of GR on OS in these subgroups further.

High levels of tumor GR expression were seen among patients who received neoadjuvant chemotherapy. No significant difference in GR expression was observed between the paired pre- and post-neoadjuvant chemotherapy samples in Cohort 3. This supports the argument that high GR expression in this group reflects an underlying phenotype rather than selection for increased GR expression following chemotherapy. Moreover, all of the patients with high GR expression (2+ or 3+ intensity staining) in Cohort 3 exhibited high expression in both the pre- and post-treatment tumor sample, further indicating that chemotherapy did not induce a dramatic increase in IHC expression of GR.

A strength of this study is that the main cohort appropriately represents the population seen in clinical practice. The distribution of histologic subtypes and stage at presentation are similar to the prevalence in the general population [18]. Moreover, variables with known prognostic significance in other studies of epithelial ovarian cancer including age at diagnosis, stage, and the presence of residual disease were also prognostic in this study. A potential weakness of our study is that we did not look at other nuclear hormone receptors, such as estrogen receptor (ER) and progesterone receptor (PR). Data in breast cancer has suggested that high GR expression is associated with poor prognosis in ER/PR-negative disease, but conversely with favorable prognosis in ER-positive disease [5]. Several studies

have investigated the prognostic significance of other nuclear hormone receptors including ER and PR in various histologic subtypes of ovarian cancer with mixed results [19–23]. It is possible that the absence or expression of other receptors may affect GR function in ovarian cancer, particularly in potentially estrogen-sensitive subtypes such as endometrioid cancer. Exploration of the influence of other nuclear hormone receptors on the prognostic significance of GR is planned in future studies.

The current study lends support to previous work suggesting that glucocorticoid signaling is unfavorable in solid tumors. The observation that high GR expression was associated with worse PFS is consistent with the hypothesis that the level of GR correlates with the effectiveness of frontline chemotherapy, as observed in preclinical work by our group and others. The negative effects of GR signaling on response to chemotherapy *in vitro* have led to clinical trials of GR antagonists in patients with solid tumors. Preclinical studies suggest that increased GR expression correlates with increased GR activity. We hypothesize that GR antagonists could decrease GR activity and promote increased sensitivity to chemotherapy, thus increasing response rates to chemotherapy and prolonging PFS. A small phase I trial of nab-paclitaxel with the GR/PR antagonist mifepristone in breast cancer patients was recently reported [24], and a randomized phase II of this combination in GR-expressing TNBC is being initiated. This concept is also being studied in ovarian cancer. A phase I study of carboplatin, gemcitabine, and mifepristone in TNBC and recurrent or persistent epithelial ovarian cancer recently completed accrual. Interest is also growing in novel non-steroidal selective GR antagonists; a phase I trial combining nab-paclitaxel with the selective GR antagonist CORT 125134 (Corcept Therapeutics) in advanced solid tumors including ovarian cancer is currently underway (NCT02762981).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- Glucocorticoid receptor (GR) is expressed in 39.0% of invasive epithelial ovarian cancers.
- GR expression is associated with histologic subtype, higher grade, and advanced stage.
- GR expression correlates with decreased PFS, but not OS, in patients receiving cytoreductive surgery and adjuvant chemotherapy.

Fig. 1.

Representative GR staining of tissue microarrays. (A) A representative case with negative GR staining (0 intensity in 0% of tumor cell nuclei) by IHC. (B) A high power magnification of the case shown in panel A. A region of lymphocytes with positive nuclear GR staining adjacent to negative tumor is present (brown, dashed outline). (C) A representative case with 3+ nuclear GR staining in >75% tumor cell nuclei. (D) A high power magnification of the case shown in panel C. Photomicrographs were taken with 10× magnification (A, C) or 40× magnification (B, D).

Fig. 2.

GR expression varies with histologic subtype and stage. (A) High GR expression varies significantly with histologic subtypes in invasive ovarian cancer. Serous ovarian cancer (Serous), endometrioid cancer (EC), clear cell cancer (CC), mucinous cancer (Mucinous), Carcinosarcoma (CS). Other includes mixed epithelial histology. (B) A trend of increasing GR is observed with increasing invasiveness in 481 ovarian cancer cases. Borderline tumors (Bd), early stage (stage I/II) treated with surgery alone (EarlyS), early stage treated with surgery and adjuvant chemotherapy (EarlySCT), advanced stage (stage III/IV) treated with surgery and adjuvant chemotherapy (AdvSCT), advanced stage treated with neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy (NeoSCT). Clinical characteristics for cases in panels A and B are shown in Supplemental Table S1. (C) Line plot of intensity of nuclear GR staining in tumor cells from specimen collected pre- and post-neoadjuvant chemotherapy in paired samples. 15 of 18 (83%) cases had high GR expression (intensity 2+ or 3+) pre-chemotherapy and post-chemotherapy ($p = 0.583$, not significant). Three cases exhibited low GR expression (0 or 1+ staining) both pre- and post-neoadjuvant chemotherapy. The 15 cases with high GR expression exhibited 2+ or 3+ staining both pre- and post-neoadjuvant chemotherapy.

Fig. 3.

High GR expression correlates with decreased PFS but not OS in Cohort 1. (A) Kaplan-Meier plot of PFS and (B) OS of cases with high GR (2+ or 3+ staining, black solid line) *versus* low GR expression (0 or 1+ staining, dashed line) in Cohort 1. (C) Kaplan-Meier plot of PFS and (D) OS in the 206 advanced stage (FIGO stage III/IV) high grade (Grade 2/3) serous cases (advanced HG-SOC) of Cohort 1. (E) Kaplan-Meier plot of PFS in early stage (FIGO stage I/II) cases from Cohort 1. (F) Kaplan-Meier plot of PFS in a separate group of 42 early stage (FIGO stage I/II) cases from Cohort 2 of the TMA. In the online version, high GR expression appears in red and low GR expression appears in green.

Fig. 4.

Forest plots demonstrate a trend toward increased hazard for progression for high GR expression in all subgroups. (A) Forest Plot of unadjusted hazard ratios (HR) for PFS (A) and OS (B) for the following subgroups: Early stage (Early), advanced stage (Advanced), Grade 1, Grade 2, Grade 3, serous ovarian cancer (Serous), endometrioid cancer (EC), clear cell cancer (CC), mucinous cancer (Mucinous), other histologies (Other), no gross residual disease (No Gross), any gross residual disease (Gross), or all patients (All) in Cohort 1 (n = 341). Forest plots present unadjusted hazard ratio (black box) with 95% confidence intervals (black lines). HR greater than one indicates a decreased survival for cases with high GR expression compared to low GR expression. Numbers of cases in each subgroup are as shown in Table 1. Selected adjusted and unadjusted HR are shown in Supplemental Table S5.

Table 1

Patient characteristics and glucocorticoid receptor (GR) staining, Cohort 1.

GR expression is categorized as low (0 or 1+) or high (2+ or 3+) intensity staining in >1% of tumor cell nuclei.

Characteristics	Total	GR low, n (%)	GR high, n (%)	p-value ^a
All cases	341	208 (61.0)	133 (39.0)	
Age (Median 59, range 24–89)				0.772
<50	73	41 (56.2)	32 (43.8)	
50–59	100	64 (64.0)	36 (36.0)	
60–69	89	55 (61.8)	34 (38.2)	
70	79	48 (60.8)	31 (39.2)	
Histology				<0.001
Serous	240	125 (52.1)	115 (47.9)	
Endometrioid	32	30 (93.8)	2 (6.3)	
Clear Cell	42	31 (73.8)	11 (26.2)	
Mucinous	17	15 (88.2)	2 (11.8)	
Other ^d	10	7 (70.0)	3 (30.0)	
Serous Subtype				0.503 ^b
Low grade serous (G1)	9	6 (66.0)	3 (33.3)	
High grade serous (G2/3)	231	119 (51.5)	112 (48.5)	
Grade				0.001 ^c
G1	30	26 (86.7)	4 (13.3)	
G2	62	41 (66.1)	21 (33.9)	
G3	249	141 (56.6)	108 (43.4)	
FIGO Stage				0.037
Early (Stage I/II)	96	67 (69.8)	29 (30.2)	
Advanced (Stage III/IV)	245	141 (57.6)	104 (42.5)	
Residual Disease				0.075
None or Microscopic (<1 cm)	159	105 (65.2)	54 (34.8)	
Gross	182	103 (56.6)	79 (43.4)	

^aDifference in proportions tested with Pearson Chi-square method unless otherwise noted.

^bDifference in proportions tested with Fisher's Exact method.

^cEffect of increasing grade tested with Mantel-Haenszel test of trend.

^dIncludes mixed epithelial cancers.

Table 2

Univariate and Multivariate Analysis of Association between GR and Progression-Free and Overall Survival, Cohort 1 (n = 341).

Univariate	PFS			OS		
	HR ^a (95% C.I.)	p-value	HR ^a (95% C.I.)	p-value	HR ^a (95% C.I.)	p-value
GR Expression	Low	Reference	Reference	Reference	Reference	Reference
	High	1.66 (1.29–2.14)	<0.001	1.18 (0.89–1.56)	0.248	0.248
Multivariate	PFS			OS		
	HR ^a (95% C.I.)	p-value	HR ^a (95% C.I.)	p-value	HR ^a (95% C.I.)	p-value
Age (years)						
	Inc. per 10 years	1.14 (1.02–1.27)	0.024	1.19 (1.05–1.35)	0.007	0.007
FIGO stage						
	Early (I/II)	Reference	Reference	Reference	Reference	Reference
	Advanced (III/IV)	4.00 (2.31–6.93)	<0.001	3.12 (1.67–5.83)	<0.001	<0.001
Grade						
	G1	Reference	Reference	Reference	Reference	Reference
	G2	2.27 (0.99–5.20)	0.054	1.59 (0.68–3.70)	0.285	0.285
	G3	2.02 (0.91–4.50)	0.085	1.30 (0.58–2.93)	0.524	0.524
Histology						
	Serous	Reference	Reference	Reference	Reference	Reference
	Endometrioid	0.89 (0.49–1.62)	0.709	0.88 (0.48–1.60)	0.672	0.672
	Clear cell	1.27 (0.76–2.12)	0.354	1.40 (0.79–2.48)	0.253	0.253
	Mucinous	2.40 (0.98–5.86)	0.055	2.58 (1.06–6.26)	0.036	0.036
	Others ^b	2.41 (0.83–6.99)	0.106	1.99 (0.46–8.64)	0.359	0.359
Residual disease						
	None or microscopic	Reference	Reference	Reference	Reference	Reference
	Gross	1.75 (1.28–2.40)	<0.001	2.11 (1.49–2.99)	<0.001	<0.001
GR expression						
	Low	Reference	Reference	Reference	Reference	Reference
	High	1.41 (1.08–1.84)	0.012	0.96 (0.71–1.30)	0.800	0.800

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Hazard ratio (HR) for disease progression estimated by Cox model adjusted for other clinical variables.
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Includes mixed epithelial cancers.