Gynecologic Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

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

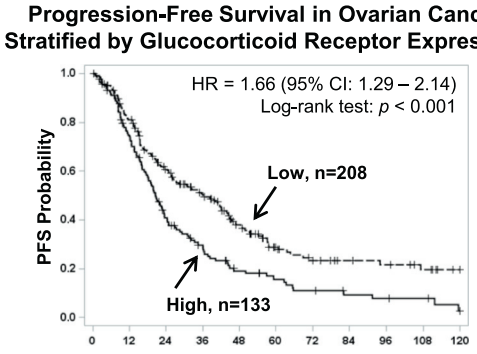
Jennifer Taylor Veneris [a,*], Kathleen M. Darcy [b], Paulette Mhawech-Fauceglia [c], Chunqiao Tian [b], Ernst Lengyel [d,h], Ricardo R. Lastra [e], Tanja Pejovic [f], Suzanne D. Conzen [a,g,h], Gini F. Fleming [a,h,*]

[a] Department of Medicine, Section of Hematology-Oncology, The University of Chicago, Chicago, IL, United States
[b] Women's Health Integrated Research Center, Inova Health System, Annandale, VA, United States
[c] Department of Pathology, University of Southern California, Los Angeles, CA, United States
[d] Department of Obstetrics and Gynecology, Section of Gynecologic Oncology, The University of Chicago, Chicago, IL, United States
[e] Department of Pathology, The University of Chicago, Chicago, IL, United States
[f] Department of Obstetrics and Gynecology, Section of Gynecologic Oncology, Oregon Health & Science University, Portland, OR, United States
[g] Ben May Department for Cancer Biology, The University of Chicago, Chicago, IL, United States
[h] The University of Chicago Comprehensive Cancer Center, Chicago, IL, United States

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.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 January 2017
Received in revised form 14 April 2017
Accepted 17 April 2017
Available online xxxx

Keywords:
Epithelial ovarian cancer
Hormone receptor
Glucocorticoid receptor
Tumor markers
Survival

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 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.

* Corresponding authors at: Department of Medicine, Section of Hematology-Oncology, The University of Chicago, 5841 S Maryland Avenue, Chicago, IL 60637, United States.
E-mail addresses: jveneris@medicine.bsd.uchicago.edu (J.T. Veneris), gfleming@medicine.bsd.uchicago.edu (G.F. Fleming).

http://dx.doi.org/10.1016/j.ygyno.2017.04.012

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.

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

TMAs 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 TMAs. 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

Please cite this article as: J.T. Veneris, et al., High glucocorticoid receptor expression predicts short progression-free survival in ovarian cancer, Gynecol Oncol (2017), http://dx.doi.org/10.1016/j.ygyno.2017.04.012