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Expression of glucocorticoid receptor is associated with aggressive primary endometrial cancer and increases from primary to metastatic lesions

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HIGHLIGHTS

- Expression of GR is associated with aggressive endometrial cancer and poor survival.
- GR expression is associated with poor survival regardless of hormone receptor status.
- The majority of metastatic endometrial cancer lesions express GR.

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ABSTRACT

Background. Glucocorticoid receptor (GR) has emerged as an important steroid nuclear receptor in hormone dependent cancers, however few data are available regarding a potential role of GR in endometrial cancer. The aim of this study was to investigate expression of GR in primary and metastatic endometrial cancer lesions, and to assess the relationship between GR expression and clinical and histopathological variables and survival.

Methods. Expression of GR was investigated by IHC in 724 primary tumors and 289 metastatic lesions (from 135 patients), and correlations with clinical and histopathological data and survival were explored.

Results. Expression of GR was significantly increased in non-endometrioid tumors compared to endometrioid tumors, and was associated with markers of aggressive disease and poor survival both in univariate and multivariate analysis after correcting for age, FIGO stage and histologic grade. Within the subgroups of hormone receptor negative tumors (loss of androgen receptor, estrogen receptor or progesterone receptor) expression of GR was highly significantly associated with poor disease specific survival. There was an overall increase in GR expression from primary to metastatic lesions, and the majority of metastases expressed GR.

Conclusion. GR expression in primary endometrial cancer is associated with aggressive disease and poor survival. The majority of metastatic endometrial cancer lesions express GR; therefore GR may represent a therapeutic target in the adjuvant therapy of poor prognosis early-stage as well as metastatic endometrial cancer.

1. Introduction

Endometrial cancer is the sixth most common malignancy among women worldwide, and accounts for approximately 5% of cancers in females [1]. The incidence varies throughout the world, and in industrialized countries it is the fourth most common cancer after breast, colorectal and lung cancer [1]. The overall incidence of endometrial cancer is increasing, and this increase can partly be explained by increased life

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expectancy, but the increasing rate of obesity is also thought to be a contributing factor [2]. Although the prognosis is good for patients with localized disease, patients with recurrent or metastatic disease at diagnosis have few treatment options and a poor prognosis [3]. Little progress has been made in development of treatment options for these patients over the past decades; and to improve treatment for this patient group, both the identification of new treatment targets, and identification of good biomarkers to aid patient stratification are vital [3].

The endometrium is highly hormone sensitive. Estrogen and progesterone are involved in controlling the normal cyclic changes in the endometrium, and also in development of endometrial cancer [4]. The role and expression of estrogen and progesterone receptor (ER and PR) have therefore been extensively studied in endometrial cancer [4–7]. A member of the steroid nuclear receptor superfamily less studied in endometrial cancer is glucocorticoid receptor (GR). The glucocorticoid receptor is a ligand dependent transcription factor involved in regulation of key signaling pathways through activation and repression of gene expression [8]. GR is involved in many aspects of human physiology including cellular homeostasis and development and regulation of metabolic and immune function [9]. The effect of GR activation is found to vary depending on tissue type. While GR activation in hematological malignancies has been associated with increased apoptosis [10], GR activation in epithelial tumor cells inhibits apoptosis [11].

The role of GR in hormone-dependent breast and prostate cancer appears to depend on concomitant ER and AR status, respectively, suggesting that crosstalk between the different receptors is important for their function [12]. GR expression is associated with good survival in breast cancer patients in the presence of ER [13], while in the absence of ER, GR is associated with poor prognosis and induces expression of pro-survival genes that inhibit chemotherapy effectiveness [14,15]. In prostate cancer with intact AR signaling, GR activation can be anti-proliferative, while after inhibition of AR signaling GR contributes to therapy resistance through activation of cell survival mechanisms [16,17]. Interestingly, high expression of GR was recently also shown to predict short progression free survival in ovarian cancer patients [18]. This indicates a role for GR in promoting tumor aggressiveness and therapy resistance in these cancers, and several clinical trials are investigating its potential as a therapeutic target in breast, ovarian, and prostate cancer.

The aim of this study was to investigate GR expression in both primary and metastatic endometrial cancer lesions, and assess the relationship between GR expression and clinicopathological variables and survival in general, and especially within subgroups depending on hormone receptor status.

2. Methods

2.1. Patient series

A population-based patient series was prospectively collected from 2001 to 2015 and included 724 primary tumors from patients diagnosed with endometrial cancer in Hordaland County (Norway). Metastases were available from 135 patients, in total 289 metastatic lesions. Patients were surgically staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2009 criteria. The clinical and pathological variables, age at diagnosis, FIGO stage, histological subtype and grade and follow up data were collected by review of medical records as previously described [19].

The study has been approved according to Norwegian legislation by the Western Regional Committee for Medical and Health Research Ethics (REK 2009/2315). All included patients had given written informed consent.

2.2. Immunohistochemical staining

Formalin-fixed paraffin embedded (FFPE) tissue was used to construct tissue microarrays (TMAs) as previously described [20]. Briefly, three cylinders of 0.6 mm were retrieved from high tumor purity areas using a custom-made precision instrument (Beecher Instruments, Silver Spring, MD, USA) and mounted in a paraffin block. TMAs were made consecutively after inclusion of patients, and included samples of different grades and histologies. TMA sections were cut, dewaxed in xylene and rehydrated in graded ethanol series before microwave boiling in target retrieval buffer (pH 9) for 15 min. Slides were incubated with anti-GR primary antibody (GR D8H2 #3660, Cell Signaling), diluted 1:500, for 1 h at room temperature. Secondary antibody, anti-rabbit (Dako, Denmark), was applied for 30 min, followed by 8 min with Diaminobenzidine (DAB+, K4007, Dako, Denmark) before counterstaining with hematoxylin.

All slides were scored blinded for clinical and pathological data using a standard light microscope. To evaluate interobserver reproducibility a subset of random TMA slides were scored independently by two researchers, and the inter-evaluator κ-value was calculated to be 0.92 for GR in two groups. The staining was evaluated using a semi-quantitative system where both intensity and area of positive tumor cells are considered. Staining intensity was graded from 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining), and area from 0, 1 (<10%), 2 (10–50%) and 3 (>50%). A staining index was calculated as the product of staining intensity and area. In subsequent statistical analysis, indexes were grouped according to similarity in

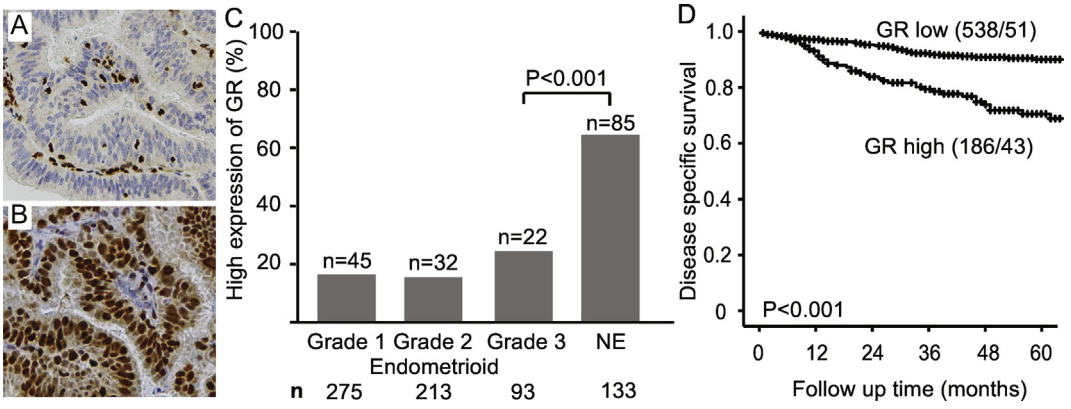

Fig. 1. GR expression in glandular cells was scored, with GR loss shown in A and strong immunohistochemical staining in B. A significant increase in GR protein expression was observed comparing endometrioid to non-endometrioid endometrial cancer primary tumors (C) (Number of patients included above the bars, and total number of patients indicated under the graph. Grade missing for 10 patients). High protein expression of GR significantly predicts poor survival compared to low expression of GR in endometrial cancer patients (in parantheses: number of patients/number of events) (D). Abbreviations: NE: non-endometrioid.

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