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Original Research

Loss of progesterone receptor links to high proliferation and increases from primary to metastatic endometrial cancer lesions

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Abstract ***Objective:*** In endometrial cancer loss of progesterone receptor (PR, gene name *PGR*) is associated with aggressive disease and altered response to hormonal treatment. The aim of this study was to investigate changes in PR expression level with disease progression, and explore whether differences in gene expression according to PR status can be linked to processes involved in cancer development elucidating new therapeutic opportunities.
Methods: 686 primary endometrial cancers and 171 metastatic lesions were investigated for PR expression in relation to clinical and histopathological data. Protein levels were investigated by immunohistochemistry and reverse phase protein array, and mRNA levels by DNA oligonucleotide microarray.
Results: PR protein level was significantly associated with *PGR* mRNA expression ($P < 0.001$) and patient survival ($P < 0.001$). Loss of PR increased with disease progression, with 23% of the primary tumours and 76% of metastases demonstrating PR loss. Using a cell cycle progression signature score, PR loss was associated with increased proliferation for both oestrogen receptor (ER) positive and negative tumours. Through a Connectivity Map search, CDK inhibitors and other drugs with anti-proliferative effects were suggested in particular for treatment of patients with loss of PR.

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Conclusion: Loss of PR in endometrial cancer is associated with increased proliferation, poor survival, and increases from primary to metastatic lesions. Based on expression profiles, CDK inhibitors may have activity in PR negative tumours, supporting further testing in clinical trials for patients with systemic endometrial cancer dependent on PR status.

1. Introduction

When endometrial cancer is identified and treated at an early stage there is subsequent good prognosis. However, for patients with systemic disease either recurrent or metastatic at presentation, the prognosis is poor, unchanged over the last several decades, and with limited treatment options [1]. Although endometrial cancer is the most common gynaecological malignancy in developed countries and the incidence is increasing [2], the progress in development of treatment for advanced or recurrent disease has been slow. Effective new targeted therapies, combined with robust biomarkers to identify patient subgroups that will benefit most from emerging as well as available treatments will improve patient care.

Progesterone is important for regulation of normal reproductive function, and is involved in controlling changes in the uterus and ovaries during the menstrual cycle. The effect of progesterone is mediated through progesterone receptor (PR), and PR is expressed in a variety of human tissues, including the uterus, mammary gland and ovary [3].

In breast cancer progesterone plays a role in controlling tumour promotion [4], whilst in the endometrium and the ovaries it has a suppressive effect on tumour development [5,6]. Although the effect of progesterone differs depending on the target tissue, the PR expression profile has demonstrated a prognostic value in uterine, breast and ovarian malignancies, and loss of PR is associated with worse outcome [7–10].

In the endometrium oestrogen induces proliferation whilst progesterone suppresses the oestrogen mediated signals and has a differentiating effect [6]. Oestrogen dependent endometrial cancers are thought to arise from unopposed oestrogen exposure, not balanced by the differentiating effect of progesterone [11]. Currently, both drugs antagonising oestrogen effects, and progesterone analogues are used in endometrial cancer treatment. In advanced or recurrent disease, treatment with progesterone has shown modest response rates [12]. However, in premenopausal woman with well differentiated endometrial cancer the response rates are reported to be higher, allowing fertility preserving treatment [13,14]. Although response to progesterone therapy is reported to be dependent on progesterone receptor (PR) status [12,15], and PR is reported to be a prognostic marker in endometrial cancer [9,10,16], evaluation of PR expression is not routinely performed in endometrial cancer to guide treatment decisions.

The aim of this study was to investigate changes in progesterone receptor expression during disease progression, and to explore if genes differentially expressed according to PR status, can be linked to biological processes involved in cancer development. The identified genetic alterations were explored to search for new potential drug candidates.

2. Materials and methods

2.1. Patient series

A population based patient series was prospectively collected from 2001 to 2013, and includes 686 primary tumours from patients diagnosed with endometrial cancer in Hordaland County (Norway). Patients were surgically staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2009 criteria. Clinical data were collected as described earlier [17]. Biopsies from metastatic tissue were available from 76 patients (in total 171 lesions). When available, fresh frozen tissue was collected in parallel with formalin fixed paraffin embedded (FFPE) tissue and used for mRNA and protein extraction. Tissue microarrays (TMA) were generated from FFPE tissue as previously described [18]. The independent endometrial cancer patient series with data for Ki-67 and PR has previously been described and published [19,20]. All parts of the study have been approved according to Norwegian legislation, including the Norwegian Data Inspectorate, Norwegian Social Sciences Data Services and the Western Regional Committee for Medical and Health Research Ethics, (NSD15501; REK 052.01). Participants gave written informed consent.

2.2. Protein detection

TMAs were dewaxed in xylene, and rehydrated in ethanol before microwave antigen retrieval and stained for PR expression using M3569 (Dako). The staining was evaluated as previously described [19]. Staining index 0 was considered PR negative, and 1–9 PR positive. Kappa value was calculated to be 0.82 for PR in two groups. Oestrogen receptor (ER) was stained and scored as previously described [21,22]. When evaluating multiple metastatic lesions from the same patient, PR was defined as lost if any of the metastatic lesions demonstrated loss. Reverse phase protein array (RPPA) was performed on 358 primary tumours as previously described [23,24].