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Downregulation of FOXO1 mRNA levels predicts treatment failure in patients with endometrial pathology conservatively managed with progestin-containing intrauterine devices

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HIGHLIGHTS

- We analyzed hormone receptor levels/activity following progestin-containing IUD.
- PR target gene FOXO1 was significantly downregulated in patients who progressed.
- FOXO1 mRNA levels may reflect PR function and response to progestin-based therapy.

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ABSTRACT

Objective. To examine hormone receptor expression levels and downstream gene activation in pre-treatment and post-treatment biopsies in a cohort of patients with endometrial pathology who were being conservatively managed with a progestin-containing intrauterine device (IUD). A molecular signature of treatment failure is proposed.

Methods. A retrospective analysis of pre- and post-treatment biopsy specimens from 10 women treated with progestin-containing IUD for complex atypical hyperplasia (CAH) or grade 1 endometrioid adenocarcinoma was performed. Expression of estrogen receptor (ER), progesterone receptor (PR) and PR target genes was examined by immunohistochemistry (IHC) and quantitative RT-PCR.

Results. The mean treatment duration was 14.3 months. Four CAH patients had stable disease or regressed after treatment, and four progressed to endometrioid adenocarcinoma. Both patients with an initial diagnosis of endometrioid adenocarcinoma regressed to CAH or no disease. In general, hormone receptor levels diminished post-treatment compared to pre-treatment biopsies; however, we noted unexpected higher expression of the B isoform of PR (PRB) as well as ER in those patients who progressed to frank cancer. There was a trend towards a non-nuclear cytoplasmic location of PRB in these patients. Importantly, the differentiating impact of PR signaling, as determined by the expression of the progestin-controlled tumor suppressor FOXO1, was lost in individuals who progressed on therapy.

Conclusions. FOXO1 mRNA levels may serve as a biomarker for response to therapy and an indicator of PR function in patients being conservatively managed with a progestin-containing IUD.

1. Introduction

Endometrial cancer is the most common gynecologic malignancy in women, with 54,870 estimated new cases in the US in 2015 [1]. The majority of patients with endometrial cancer have Type 1 disease, which arises primarily through excess estrogen unopposed by progesterone [2]. Patients with endometrial hyperplasia, or increased glandular proliferation, are at increased risk of developing endometrial cancer [3]. Hyperplasia is most often caused by unopposed estrogen stimulation and occurs in the following four histologic categories: 1) simple hyperplasia; 2) simple hyperplasia with atypia; 3) complex hyperplasia (CH); and 4) complex hyperplasia with atypia (also complex atypical hyperplasia, CAH) [4]. Approximately 25% of patients with endometrial hyperplasia with atypia will develop endometrial cancer if left

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untreated [3]. Indeed, one study reported that 43% of cases had a concomitant diagnosis of carcinoma when hysterectomy was performed within 12 weeks of initial biopsy [5]. However, the molecular mechanisms underlying the progression from atypical hyperplasia to endometrioid adenocarcinoma remain incompletely understood.

Current guidelines suggest that patients diagnosed with endometrial cancer or the precursor CAH should be managed by hysterectomy. However, for patients who are poor surgical candidates due to medical comorbidities or those that still desire child bearing, hormonal therapy with progesterone has been suggested as an alternative treatment for CAH or low-grade endometrioid adenocarcinoma [6–14]. For example, one study demonstrated a 65% complete response, 23% with complete response followed by recurrence, and only 28% with persistent or progressive disease following progestin treatment [15]. Another study suggested that management with a progestin-containing intrauterine device (IUD) may be superior to oral progestins (94.8% vs. 84.0% regression rate) [16].

Despite the relatively high response rates of CAH and endometrial endometrioid adenocarcinoma patients to progestin-based therapy, some patients eventually relapse or develop progressive disease. Therefore, it is important to identify which patients are at a higher risk of treatment failure. Patient characteristics such as body mass index and histopathologic features such as glandular cellularity have been correlated with response to progestin therapy [15,17]. For endometrial cancer, expression of the hormone receptors estrogen receptor (ER) and progesterone receptor (PR) has been positively correlated with response [18–21]. However, identification of molecular biomarkers for progression from CAH to endometrial cancer has proven more challenging, with some studies providing conflicting results [15,22]. The objective for this study was to examine hormone receptor expression levels and downstream gene activity in pre-treatment and post-treatment biopsies in a cohort of patients with CAH or grade 1 endometrioid adenocarcinoma who were being conservatively managed with a progestin-containing IUD. These data shed light on the molecular signature of therapeutic response vs. failure to conservative management in such patients.

2. Materials and methods

See Supplementary methods for detailed descriptions of methods.

2.1. Human subjects

The study was reviewed and approved by the University of Iowa Institutional Review Board (IRB, ID#201211782). Retrospective chart review identified 10 patients with abnormal endometrial biopsy findings that were managed with a progestin-containing IUD (Mirena IUD) at the University of Iowa Hospitals and Clinics (Fig. 1). The sample set included 8 cases with complex atypical hyperplasia (CAH) and 2 cases of grade 1 endometrial endometrioid adenocarcinoma pre-IUD insertion. All subjects had been evaluated with an endometrial biopsy via dilatation and curettage before IUD placement. Biopsies were also available for repeat sampling. This study utilized the last retrieved biopsy sample. The duration between the initial biopsy and the latest biopsy ranged from 6.0–29.6 months, with a mean interval of 14.3 months. "No progression" was defined as regression to normal (N = 3), complex hyperplasia (N = 1) or stable disease (N = 1). Three patients progressed from CAH to endometrioid adenocarcinoma. One CAH patient had benign endometrium at the final biopsy, but subsequently developed a mixed grade 3 endometrioid/undifferentiated adenocarcinoma of the uterus. She was diagnosed with a metastatic axillary lymph node from a core biopsy that subsequently prompted a hysterectomy. This patient was thus included in the "Progression" group in this study for a total of four "Progression" patients. Patient characteristics for all 10 patients are provided in Table 1.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) endometrial biopsy and hysterectomy tissue sections were assessed for expression of hormone receptors and FOXO1 via immunohistochemistry (IHC) and quantitated as described [23,24] by three investigators blinded to sample identity.

2.3. Quantitative RT-PCR

Quantitative RT-PCR was performed on available pre- and post-IUD insertion biopsy samples (N = 9) as previously described [25].

2.4. Statistical analysis

IHC data were analyzed using paired t-test. For quantitative RT-PCR studies, fold change and statistical significance were determined using the ΔΔCt method [26,27] and standard t-test, respectively, with unequal variances [28]. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Treatment with a progestin-containing IUD is associated with a decrease in ER, PR and PR isoform B levels

Study design and patient characteristics are provided in Fig. 1 and Table 1, respectively. We first examined hormone receptor expression by IHC in paired endometrial biopsies obtained at the time of initial diagnosis ("pre-IUD insertion") and the last patient visit ("post-IUD insertion"). The mean interval between IUD placement and last clinic visit was 14.3 months. Of the 10 patients that were included in the study, most demonstrated a significant reduction in PR protein expression post-IUD insertion as compared to pre-insertion (Fig. 2A–C). Levels of the progesterone receptor isoform B (PRB) were also markedly decreased in post-treatment biopsies (Fig. 2A–C), consistent with a previous report demonstrating downregulation in response to progestin-based therapy [20]. Pearson correlation analysis demonstrated that post-IUD insertion expression of ER significantly correlated with PRB (Fig. 2D), in line with the reported relationship between hormone receptors in endometrial tumors [29].

3.2. Analysis of hormone levels in patients who progressed vs. those that did not progress on therapy

We next asked how hormone receptor levels change in patients who progressed vs. those who did not. "No progression" was defined as stable disease (N = 1) or regression to normal or complex hyperplasia (N = 3; N = 1, respectively). In the "No progression" group, we detected a significant decrease in levels of ER, PR and PRB in the post-IUD insertion biopsies as compared to pre-IUD insertion (Fig. 3A), indicating that progestin-containing IUD mediates canonical ligand-mediated receptor downregulation. However, ER and PRB levels in the post-IUD insertion biopsies were significantly higher in the "Progression" group as compared to the "No progression" group (Fig. 3B–D). These data suggest that the expected ligand-mediated downregulation of PR [25] is lost in patients with CAH that progress to endometrioid adenocarcinoma. It was next important to determine whether the increase in PRB expression correlated with enhanced or loss of expression of the expected progestin-controlled genes associated with endometrial differentiation.

3.3. Gene expression changes associated with progestin-containing IUD

Progesterone promotes differentiation of the uterine endothelium through PR-mediated transcription of several pro-differentiation genes, including amphiregulin (*AREG*), progesterone-associated endometrial protein (*PAEP*, also termed "glycodelin"), and FOXO1 [21]. Of