Heterogeneity in endometrial expression of aromatase in polyp-bearing uteri

Lubna Pal1,4, Andrea L. Niklaus2, Mimi Kim3, Staci Pollack1 and Nanette Santoro1

1Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology and Women’s Health, Albert Einstein College of Medicine, Bronx, NY, USA; 2Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA; 3Department of Epidemiology and Population Health, Bronx, NY, USA

4Correspondence address. Tel: +1-718-4303152; Fax: +1-718-430-8586; E-mail: lubnapal@hotmail.com

BACKGROUND: Comparative assessment of expression of aromatase (CYP19A1) messenger RNA (mRNA) in pathological and non-pathological sites within the uterine cavity of mid reproductive age women diagnosed with endometrial polyp (EP). METHODS: We report a case series of seven premenopausal infertile women undergoing hysteroscopic removal of EP. Directed endometrial biopsies were collected from the EP (P), from the endometrium immediately adjoining the EP (A) and from a normal appearing site remote from the EP (R). Expression of CYP19A1 mRNA within the respective samples in each patient was evaluated by quantitative real-time PCR. Fold changes in the mRNA expression of CYP19A1 within the P versus the R and A endometrial sites were calculated to assess the hypothesis of a ‘field effect’ in the expression of aromatase within EP bearing endometria. RESULTS: Overall, similar mRNA expression of CYP19A1 gene was demonstrated between P and A endometrial samples. In only one of the seven patients, aromatase expression within P was enhanced by almost 4-fold compared with R (P = 0.14 for comparison with the difference in CYP19A1 expression in P versus R in the remainder of the patients). In contrast, in three of the seven patients, P demonstrated a marked (>1000-fold) under-expression in CYP19A1 mRNA levels compared with the R endometrium (P = 0.22). CONCLUSIONS: We herein provide evidence of heterogeneity in the expression of endometrial aromatase in premenopausal uteri bearing EPs. Our data suggest that an overexpression of endometrial aromatase may underlie pathogenesis of EP at least in a subset of cases.

Keywords: endometrium; polyp; aromatase; CYP19A1; PCR

Introduction

Endometrial polyps (EPs) belong to a spectrum of proliferative endometrial pathologies that includes endometrial hyperplasia (EH) and endometrial adenocarcinoma (EAC) (Nagele et al., 1996). Epidemiological data suggest that EPs may be regarded as a risk factor for EAC (Pettersson et al., 1985; Anastasiadis et al., 2000; Baskin et al., 2002). In a post-menopausal woman ever diagnosed with an EP, the risk of subsequent EAC may be nine times greater than that of a woman without EP (Armenia, 1967). A point prevalence of EP of up to 16% is reported, both in premenopausal (DeWaay et al., 2002) as well as asymptomatic post-menopausal women (Fay et al., 1999).

Prolonged endometrial exposure to mitogenic effects of estrogen (E), unopposed by progestin, is recognized as a contributor to the various proliferative endometrial disorders (Maia et al., 1998, 2001; Thijs et al., 2000). An increased likelihood of endometrial disorders with advancing age is an apparent paradox, since trophic stimulation from circulating E is minimal in the post-menopausal period, and may be partly explained by local sequestration or synthesis of E within the endometrium.

Aromatase (CYP19A1), a ubiquitous P450 enzyme responsible for the conversion of C19 steroids to E, is a major contributor to circulating E in the post-menopausal women (Bulun et al., 2001). A pathogenic role for an exaggerated local aromatase activity within a spectrum of E-dependent proliferative disorders including breast cancer, mammary ductal hyperplasia, endometrial cancer, endometriosis, adenomyosis and uterine leiomyomas is suggested (Edman and MacDonald, 1978; Kitawaki et al., 1997; Tekmal et al., 1999; Tarkowski et al., 2000; Bulun et al., 2001). Aromatase immunoreactivity has been demonstrated within endometrial stromal cells both in the secretory and proliferative phases of the menstrual cycle (Tseng, 1984; Bulun et al., 1994).

As the current literature supports a role for dysregulated aromatase expression in proliferative endometrial pathologies, we hypothesized that there would be a differential messenger RNA (mRNA) CYP19A1 expression between the EP and the adjoining (A) and remote (R) sites within the same endometrial
The presented data describe endometrial expression of aromatase gene from samples collected from the P, A and R...
endometrial sites in each of the seven participants (1, 3, 4, 9, 11, 12 and 13). The mean values (± SD) for age and BMI of the participants were 35.43 ± 3.7 years and 25.2 ± 7.84 kg/m², respectively.

The QR–PCR data, presented as CT values for individual samples, and the ΔCT (CT values for CYP19A1–18S rRNA) values for the individual patient samples are presented in Table I. The mean ‘fold change in gene expression’ of CYP19A1 in P versus R and A samples was calculated based on 2^−ΔCT method ((2−ΔCT+CYP19A1−18S_rRNA)−ΔCT_CYP19A1−18S_rRNA) and 2^−ΔCT(CYP19A1−18S_rRNA)−(CTA_CYP19A1−18S_rRNA)), respectively (Livak and Schmittgen, 2001).

As shown in the Table I, a patient and site-dependent heterogeneity in the expression of CYP19A1 is noted. A progressive up-regulation in aromatase expression, i.e. P > A > R, was hypothesized, as noted in only one patient (#9), in whom an almost 4-fold up-regulation in CYP19A1 expression within EPs (Dal Cin et al., 2000), monoclonal EH (Jovanovic et al., 1996) and genetic causes, which have demonstrated a narrow spectrum of mutations including 6p21–22, 7q22 and 12q13–15 within EPs (Dal Cin et al., 1995). A recent report focusing on aromatase expression within EPs, utilizing histological specimens, demonstrated aromatase protein overexpression within EPs (by immunohistochemistry), compared with normal endometria, albeit obtained from different uterine specimens (Maia et al., 2006). Our findings of EPs yielding positive expression of the aromatase gene are thus consistent with protein data reported by Maia et al. (2006).

We demonstrate herein a pathogenic heterogeneity within the EPs in premenopausal women as well as relates to expression of aromatase gene. Our hypothesis of a ‘localized overexpression’ of endometrial aromatase within the EP was validated only in a single patient (i.e. aromatase expression in P > A > R, patient #9). Notable is the morbidity high BMI of patient #9 (as well as the relatively older age) who is clearly an outlier in this group of otherwise normal weight individuals. The association

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age (years)</th>
<th>BMI kg/m²</th>
<th>Gene</th>
<th>CYP19A1</th>
<th>CtP</th>
<th>CTR</th>
<th>ΔCT</th>
<th>PversusR</th>
<th>PversusA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>38</td>
<td>21.3</td>
<td>CYP19A1</td>
<td>33.51</td>
<td>13.41</td>
<td>20.40</td>
<td>3.73-fold</td>
<td>33.55</td>
<td>22.63</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>22.3</td>
<td>CYP19A1</td>
<td>33.48</td>
<td>11.02</td>
<td>19.47</td>
<td>2.51-fold</td>
<td>27.74</td>
<td>17.60</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>42.4</td>
<td>CYP19A1</td>
<td>33.65</td>
<td>13.05</td>
<td>26.62</td>
<td>3.73-fold</td>
<td>35.58</td>
<td>21.21</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>22.1</td>
<td>CYP19A1</td>
<td>33.67</td>
<td>11.65</td>
<td>20.73</td>
<td>1.85-fold</td>
<td>32.75</td>
<td>20.85</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>22.4</td>
<td>CYP19A1</td>
<td>33.67</td>
<td>11.65</td>
<td>20.73</td>
<td>1.85-fold</td>
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<td>11.65</td>
<td>20.73</td>
<td>1.85-fold</td>
<td>32.75</td>
<td>20.85</td>
</tr>
<tr>
<td>14</td>
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<td>20.4</td>
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<td>33.67</td>
<td>11.65</td>
<td>20.73</td>
<td>1.85-fold</td>
<td>32.75</td>
<td>20.85</td>
</tr>
</tbody>
</table>

ΔCT represents the relative expression of aromatase gene CYP19A1 compared with 18S rRNA for each sample, i.e. CT of CYP19A1−CT of 18S rRNA. A differential expression of CYP19A1 gene in P versus R and A samples was noted. No relationship was noted between CYP19A1 ΔCT values for the respective sites and patient’s age or BMI (P > 0.05).

Discussion

Pathophysiology of EPs remains elusive. Proposed mechanisms that may contribute to development of EPs include a constitutive lack of progesterone receptor (Maia et al., 1998; Thijs et al., 2000), monoclonal EH (Jovanovic et al., 1996) and genetic causes, which have demonstrated a narrow spectrum of mutations including 6p21–22, 7q22 and 12q13–15 within EPs (Dal Cin et al., 1995). A recent report focusing on aromatase expression within EPs, utilizing histological specimens, demonstrated aromatase protein overexpression within EPs (by immunohistochemistry), compared with normal endometria, albeit obtained from different uterine specimens (Maia et al., 2006). Our findings of EPs yielding positive expression of the aromatase gene are thus consistent with protein data reported by Maia et al. (2006).

We demonstrate herein a pathogenic heterogeneity within the EPs in premenopausal women as well as relates to expression of aromatase gene. Our hypothesis of a ‘localized overexpression’ of endometrial aromatase within the EP was validated only in a single patient (i.e. aromatase expression in P > A > R, patient #9). Notable is the morbidity high BMI of patient #9 (as well as the relatively older age) who is clearly an outlier in this group of otherwise normal weight individuals. The association
of obesity with peripheral circulating estradiol is well characterized and attributed to enhanced peripheral conversion of androgens by aromatase within the adipose tissue (Edman and MacDonald, 1978) and extreme obesity has been identified as an independent risk factor for endometrial proliferative pathologies including EPs (Hale et al., 2002). A progressive increase in aromatase CP450 transcriptive activity is described, within the adipose tissue at the various body sites with advancing age (Bulun et al., 2001), thus defining a tendency for an overall unopposed estrogenic environment with aging. An association between obesity, aging and endometrial expression of aromatase however is yet to be described.

Much more interestingly, and contrary to our expectations, a converse pattern in the endometrial expression of aromatase gene was noted in three of the seven patients (#3, 1 and 11). In these subjects, a markedly robust expression in the aromatase gene was noted within the grossly normal endometrium remote endometrium compared with the polyp. Our data thus suggest that pathogenesis of at least some EPs may be independent of aromatase-dependent focal excesses of E within the endometrium. These data thus imply that mechanisms other than chronic exposure to focal E excess maybe at play, at least in some patients with EPs. Our findings therefore are supportive of a mechanistic diversity in contributors to the development of EP.

The study design, specifically procurement of hysteroscopically directed tissue biopsies from multiple sites within the same woman’s uterus, allowed each patient to serve as her own control and is a strength of our design. Our data are the first to suggest not only the occurrence of a ‘focal overexpression’ of endometrial aromatase within EP in at least some women with EPs, but also to demonstrate a heterogeneity in the underlying pathogenic mechanisms. We are intrigued by the marked differential in the expression of aromatase gene within the endometrium obtained from P compared with the grossly normal appearing tissue from the R site, as seen in three of the seven patients. Previous reports have described a differential expression of aromatase within the endometrial tissue compartments; the stroma has been shown to preferentially express aromatase, both the gene as well as the enzyme, compared with the glandular tissue (Huang et al., 1991). Different histological characteristics of the respective tissue sites such as an ‘under’ or ‘over’ representation of the stroma from respective biopsy sites may account for the noted heterogeneity in the expression of aromatase gene within the patients and samples. We are however unable to substantiate this conjecture for the presented cases, due to limitations intrinsic to the methodology employed for sample collection for the purpose of gene expression analysis.

Despite the systematic nature of our study, we acknowledge several limitations that hamper our ability to draw firmer conclusions, including the small sample size, and our inability to substantiate our findings by confirmatory assessment of enzymatic expression at the tissue level. We are unable to unambiguously conclude that the alterations in aromatase gene expression within the polyp-bearing uterus is in fact succeeded by concomitant changes in the aromatase protein and enzymatic activity (as reported by others). Nonetheless, we believe that our findings suggest that all endometria-bearing EP may not be considered a uniform entity. Our data suggest that mechanisms other than ‘focal excess of E’ need to be considered as contributors to the genesis of proliferative endometrial pathologies including EP. It remains to be seen whether the prognosis for EP-bearing endometria demonstrating a ‘gradation’ in the expression of endometrial aromatase is more reassuring than those exhibiting a ‘global overexpression’ of the enzyme. Continued overexpression of aromatase has been demonstrated in vitro to be sufficient for maintaining mammary hyperplasia without the need for large amounts of E (Tekmal et al., 1999). Based on existing data linking chronic exposures to E excess with proliferative endometrial disorders, we speculate that polyp-bearing uteri which demonstrate an overexpression of aromatase within the normal appearing endometrium (R) may be at risk for subsequent development of additional proliferative pathologies including recurrent EPs, EH or even EAC (Bakour et al., 2000); this inference merits substantiation by longitudinal follow up studies. If indeed the latter is true, then given the favorable responses to aromatase inhibitor therapy, as seen in women with EAC (Bonte, 2001), these treatments may be of interest as preventive and adjunctive therapies for lesser proliferative lesions of the endometrium.

In conclusion, our data suggest a pathogenic heterogeneity, in terms of endometrial expression of aromatase gene, in premenopausal uteri with EPs. Our findings, if substantiated by future studies, may help identify a disturbed endometrial milieu destined for progression to more sinister pathologies. Future studies need to appreciate this pathogenic diversity within the entity ‘endometrial polyp’.

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