Steroid Hormone Receptor Profile of Premenopausal Endometrial Polyps

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The etiology and pathogenesis of endometrial polyps (EPs) are only partially understood. To better understand how sex steroids regulate polyp growth, we investigated the messenger RNA (mRNA) expression of the genes of reproductive steroid hormone receptors (estrogen receptors alpha [ERα] and beta [ERβ], G protein-coupled receptor 30 [GPR30], and progesterone receptor [PR]) in EP tissue and autologous normal appearing endometrium (R) Within each patient, the normal appearing endometrial tissue remote from the site of the endometrial polyp (R) was taken as an internal control. Relative expressions of genes of interest within the endometrial polyp were compared to expressions of respective genes within the internal control tissue (i.e. R). R is the abbreviation for normal appearing endometrium in the later calculation formula. Ten patients diagnosed with EP in a tertiary care center were included in this study. Directed biopsies were obtained under hysteroscopy from the EP and from a normal appearing site remote from EP along the opposite uterine wall in each patient. Quantitative real-time reverse transcription–polymerase chain reaction (qRT-PCR) was used for gene expression profiling in the paired tissue samples. The relative gene expression between EP and normal appearing endometrium in each patient was analyzed with $2^{-\Delta\Delta Ct}$ method. We found that ERα, ERβ, GPR30, and PR were expressed in both normal appearing endometrium and EP in each patient. ERα, ERβ, GPR30, and PR showed no difference in relative expression in EP samples compared with paired normal endometrial samples from the same uterine cavity. However, the relative expression of PR correlated with that of GPR30 (r = .70, P = .023), suggesting that the co-expression of PR and GPR30 may be a contributory mechanism in the pathogenesis of EPs at least in a subset of women.

Key words: Endometrial polyp, progesterone receptor, estrogen receptor, G protein–coupled receptor 30.

INTRODUCTION

Endometrial polyp (EP) is a commonly encountered gynecological condition, with a reported prevalence of up to 16% in premenopausal asymptomatic women.[1,2] Although a majority of EPs were found in asymptomatic women older than 35,[1] their prevalence in symptomatic women is much higher.[1,2] Clinical symptomatology attributable to EP ranges from abnormal uterine bleeding to infertility.[1,3]

Despite a similar clinicopathological presentation, EPs present several cytogenetically different subgroups.[4] The common denominator of these aberrations at the molecular level, however, is still not clear. The role of steroid hormones, especially the expressions of estrogen and progesterone receptors (ER and PR, respectively) in the pathogenesis of EP has previously been evaluated with equivocal results (Table 1).[5–11] In postmenopausal EPs, ER has been consistently shown to be overexpressed compared to normal endometrial glands,[5–8] and this is

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Table 1. Relative Expression of Steroid Receptors in EP Than Normal Endometrium

Author	EP Patients' Age	Control Samples	Sample Collection	Control Samples	Relative Expression in EP Than NE		Conclusions
					EG	ES	
Mittal et al[9]	PreMP	Matched	patients		Curettage	Matched patients	ER: same; PR: same
ER: ↓; PR: ↓	EPs may result from a decrease in ER and PR expression in stromal cells. EPs may be relatively insensitive to cyclic hormonal changes.						
Taylor et al[10]	PreMP	Matched	patients		Unknown	Matched patients	ER: same in PP; ER: ↑ in SP; PR: ↓ in PP; PR: same in SP
ER: same in PP; ER: same in SP; PR: ↓ in PP; PR: same in SP	EP is not shed along with the rest of the endometrium during menstruation.						
Ye et al[11]	PreMP	Same	patients	Hysteroscopy; NE: unknown	location	Same patients	ERβ: same in PP
ERβ: ↑ in PP; ERα: ↓ in PP	Overexpression of ERβ in stroma might play an important role in the pathogenesis of EP.						
Lopes et al[8]	PreMP; PosMP	Same patient	Hysteroscopy; NE: opposite wall to EP	Same patient	ER: ↑; PR: ↑	ER: same; PR: same	
Sant'Ana et al[7]	PosMP	Same patient	Hysteroscopy; NE: adjacent to EP	Same patient	ER: ↑; PR: ↑	ER: ↑; PR: same	Steroids receptors present a crucial role in the pathophysiology of the EPs in postmenopausal women, especially the ER.
Inceboz et al[5]	PosMP	Matched	patients		hysteroscopy	Matched patients	ER: ↑; PR: ↑
ER: ↑; PR: ↑	Estrogen may have a role in the development of PosMP EPs.						
Belisario et al[6]	PosMP	Same	patients	Hysteroscopy; NE: unknown	location	Same patients	ER: ↑; PR: same
ER: ↑; PR: same	The higher proportion of positive gland cells for ER in EP supports an implication of ER in the pathogenesis of EP.						

Abbreviations: EG, endometrial gland; ES, endometrial stroma; NE, normal endometrium; PreMP, premenopause; PosMP, postmenopause; EB, endometrial biopsy; ER, estrogen receptor; PR, progesterone receptor; PP, proliferative phase; SP, secretory phase; EP, endometrial polyp; ↑, increase; ↓, decrease.

suggested as a possible pathogenic mechanism for postmenopausal EP. Inconsistencies in the expression of PR within endometrial glands and in the expression of both ER and PR within the endometrial stroma are identified in postmenopausal EP.[5-8] Inconsistencies are also described in the endometrial expression of ER and PR within premenopausal EPs.[8-11]

The role of G protein-coupled receptor 30 (GPR30), a novel membrane bound ER, in the context of EP has not been previously explored.[12,13] Estrogens traditionally control transcriptional activation through the classic nuclear ERs (ERα and ERβ).[14] Recent studies demonstrate that estrogens may bind GPR30 to initiate many rapid nongenomic signaling events.[12,13] Unlike the rest of GPRs expressed in the cell membrane, GPR30 is uniquely localized to the endoplamic reticulum.[13] Activating GRP30 by estrogen resulted in intracellular calcium mobilization and release of heparin-binding epidermal growth factor (EGF)−like growth factor.[12,13]

We had previously proposed the concept of an endometrial field effect as a pathophysiologic mechanism in the genesis of EP.[15] Field effect phenomenon in the context of gene expression may be defined as a progressively altering (declining or increasing) gradient in the relative expression of one or more genes of interest when sampling endometrial tissue from sites within the same uterine cavity that are progressively removed from the EP; if indeed one or more of the studied genes may be of pathogenic significance for EP, the field effect hypothesis dictates the gene to be markedly over- or underexpressed within the polyp with gradually attenuating or escalating expression in the normal appearing tissue that is progressively removed from the lesion within the same uterus. The field effect hypothesis thus pursues the concept that local environmental change caused by abnormal endometrial expression of one or more genes may be contributory to the development of EP. Hypothesizing a ''field effect'' phenomenon within the premenopausal uterine endometrium, we herein propose that an imbalance in the relative gene expression between EP and the grossly normal appearing endometrium in the same uterus, rather than the absolute expression levels of target steroid hormone receptor genes may underlie the development of EP. To address this hypothesis, we investigated the relative expressions of genes for steroid hormone receptors (ERα, ERβ, GPR30, and PR) between the EP and the autologous, normal appearing endometrium quantitative real-time reverse transcription−polymerase chain reaction (qRT-PCR). A correlation in the relative expressions of GPR30 and PR in the EPs compared to the remote normal endometrium is identified and suggested as possibly contributory to the pathogenesis of premenopausal EP.

MATERIALS AND METHODS

Cases

Premenopausal patients diagnosed with EP during routine evaluation for either infertility or abnormal uterine bleeding and anticipating hystersocopic polypectomy per standard clinical care were offered participation. The protocol was approved by the Center for Clinical Investigation at the Albert Einstein College of Medicine and the Institutional Review Board at the Montefiore Medical Center. Written informed consent was obtained from each participant prior to surgery. Patients with co-existing submucous uterine myoma, breast cancer, or antiestrogen therapy (such as tamoxifen, raloxifene or aromatase inhibitors) were excluded as were those with evidence of multiple EPs (more than 2).

Under hysteroscopic guidance, endometrial specimens (>2 mm) were collected directly from the EP and from a normal appearing endometrial site along the opposite endometrial wall remote from the EP. All the samples were collected in the follicular phase of the menstrual cycle. The specimens were placed in RNA-later (Ambion, Inc., Austin, Texas) in marked vials identifying the participant and site of collection and were stored at −80°C until subsequent RNA analyses.

Quantitative Real-Time RT-PCR

Quantitative real-time reverse transcription−polymerase chain reaction was performed as previously described.[16] In brief, total RNA was extracted by RNeasy Mini kit (Qiagen, Valencia, California) according to the manufacture's instructions. RNA samples were treated with DNase I (Invitrogen, Carlsbad, California) to ensure removal of any contamination with genomic DNA. The RNA integrity was confirmed by gel electrophoresis. The RNA yield ranged from 200 ng to 3 μg. First strand complementary DNA (cDNA) was reverse transcribed with a SuperScript II RNase (Invitrogen). SYBR GreenER qPCR SuperMix Universal Kit (Invitrogen) was used for PCR according to the manufacturer's instruction. Real-time PCR reactions were run in duplicates with Prism Gene Amp 5700 (Applied Biosystems, Foster City, California).

The specimens of normal endometrial tissue and EP collected from 10 patients were included in the analysis.

Table 2. Primers Sequences, Product Size, and Anneal Temperature for RT-PCR Amplication of ERα, ERβ, GPR30, PR, RPL 19 mRNA

	Forward Primer 5′-3′	Reverse Primer 5′-3′	Anneal Temperature (°C)	Amplicon Length (bp)
ERα	agcacccagtgaagctact	tgaggcacacaaactcct	57	155
Erβ	aagaatatctctgtgtcaaggccatg	ggcaatcacccaaaccaaag	65	143
GPR30	agtcggatgtgaggttcag	tctgtgtgaggagtgcaag	58	240
PR	gaaccagatgtgatctatgcagga	cgaaaacctggcaatgatttagac	60	122
RPL 19	gtaagcggaagggtacagcca	ttgtctgccttcagcttgtg	58	211

Abbreviations: bp, base pair; ER, estrogen receptor; GPR30, G protein-coupled receptor 30; PR, progesterone receptor; mRNA, messenger RNA; RPL 19, ribosomal protein L19; RT-PCR, reverse transcription−polymerase chain reaction.

Human placental messenger RNA ([mRNA] 15 week gestation) and RNA alone (no primers) constituted positive and negative controls, respectively. The primers sequence for amplification, annealing temperature, and product sizes are listed in Table 2. The housekeeping gene ribosomal protein L19 (RPL 19) was used as a normalizer gene.[17] The thermal cycling conditions included an initial denaturation step at 95°C for 10 minutes, then 40 cycles at 95°C for 15 seconds annealing for 30 seconds. Polymerase chain reaction products were then migrated on 2% agarose gel to confirm the presence of a single band of expected size for each of the specified genes of interest.

Statistics

The threshold cycle (C_T), defined as the cycle number at which the amount of amplified target reaches a fixed threshold, was obtained for each gene in each sample in duplicate, and the mean C_T values were used for analysis. First, the target gene expression was normalized relative to the internal control, RPL 19. The relative expression of all the target genes (TG) in each pair of samples (EP versus R) was determined by $2^{-\Delta\Delta Ct}$ method,[18] where

$$\Delta\Delta C_T = \Delta C_T [TG_{EP}] - \Delta C_T [TG_R]$$

$$\Delta C_T [TG] = C_T [TG] - C_T [RPL19]$$

The mean fold change in expression of each target gene was calculated and pairwise correlation between the relative expressions of the specified steroid hormone receptors within EP compared to the normal appearing eutopic endometrium were calculated using STATA (version 10.0; College Station, Texas). Data for relative gene expression were log transformed for PR and GPR30 to meet assumptions for proceeding with parametric analyses; pairwise Pearson correlation analysis used to assess the relationship between the relative expressions of the specified steroid hormone receptors within EP compared to the normal appearing eutopic endometrium. Greater than 2-fold or less than −2-fold difference in the relative gene expression was considered biologically relevant.[19] All statistical tests were 2-sided and $P < .05$ was considered statistically significant.

RESULTS

The average age (±SD) of participants was 35.8 ± 4.9 years. Genes for all the 4 studied steroid receptors, ERα, ERβ, GPR30, and PR, were expressed in both the EP and the grossly normal appearing remote endometrium. The relative expression of mRNA for the individual genes in EP is listed in Table 3 (as derived by $2^{-\Delta\Delta CT}$ method). There were no significant differences in the relative expression of the genes of interest within EP compared to the normal endometrium from the same uterine cavity ($P > .05$).

No correlation was noted between the relative expressions of steroid receptors and patient age, nor with body mass index (BMI; data not shown). A significant correlation was observed between the relative expressions of GPR30 and PR in EPs compared to the normal eutopic endometrium ($r = 0.70$, $P = 0.023$; Figure 1); there was no observed correlation among the remainder of the studied genes ($P > .05$).

DISCUSSION

While estrogen is traditionally thought to control transcriptional activation through the classical nuclear ERs,[12,13] rapid nongenomic signaling effects of estrogen via GPR30, a novel membrane bound ER in the endoplasmic reticulum have recently been described.[12,13] We herein demonstrate expression of GPR30 within

Table 3. The Patient's Demographic Characteristics and Relative Expressions of mRNA for ERα, ERβ, GPR30, and PR in EPs Compared to Normal Appearing Endometrium[a]

			Relative expression (Fold Change) of Individual Genes in EP's Compared to Control Eutopic Endometrium			
Patient ID	**Age (years old)**	**BMI (kg/m^2)**	**ERα**	**ERβ**	**GPR30**	**PR**
11	36	25.10	0.57	0.79	0.53	3.71
12	29	20.13	1.48	1.44	0.45	0.90
13	31	20.65	0.29	0.83	1.24	0.74
16	35	19.27	0.51	2.40	0.35	0.42
17	44	32.82	19.09	0.69	0.47	0.66
18	38	31.18	0.69	3.38	0.75	0.34
19	37	31.20	0.09	1.04	2.73	12.86
20	30	21.01	0.27	2.85	6.54	7.59
21	42	21.82	0.36	0.55	0.32	1.00
22	36	20.43	0.77	0.73	1.32	1.21
Mean[b]	35.8 ± 4.89	24.36 ± 5.33	2.41 ± 5.87[c]	1.47 ± 1.03[d]	1.47 ± 1.93[e]	2.94 ± 4.15[f]

Abbreviations: BMI, body mass index; GPR30, G protein-coupled receptor 30; EP, endometrial polyp; ER, estrogen receptor; mRNA, messenger RNA; PR, progesterone receptor.

[a] The relative expression (as a fold change) of steroid receptors in EPs in each patient was calculated by $2^{-\Delta\Delta Ct}$ method.

[b] Mean fold change in relative expression of genes of interest.

[c] $P = .32$.

[d] $P = .62$.

[e] $P = .69$.

[f] $P = .72$.

premenopausal EPs as well as in the grossly normal appearing eutopic endometrium. While the relevance of GPR30 in the context of EP has not been previously explored, a negative correlation between GPR30 and PR expression has previously been described within endometrial cancer.[20] In breast cancer cell lines, progestin induces an increase in GPR30 expression that is mediated via PR and is suggested to contribute to growth inhibition.[21,22] The significant correlation observed between the relative expressions of PR and GPR30 within EP relative to the normal appearing eutopic premenopausal endometrium has not previously been described. The observed relationships noted in our population are of specific interest, given the absence of any identifiable progestin response elements within the promoter and regulatory region of the GPR30 gene.[22] We conjecture that the correlation between relative expressions of PR and GPR30 in the EP tissue compared to the normal appearing endometrium may be relevant to the pathogenesis of premenopausal EP.

A role for steroid hormones has long been proposed in the pathogenesis of EP.[5-11] However, our findings suggest that the mechanisms through which steroid hormones might influence the growth of EP are indirect and complex. Our study design involved sampling the EP and the normal appearing eutopic endometrium that served as an "internal control" from the same uterine cavity. Existing studies on molecular targets in EP have mostly used tissue procured from surgical specimens of EP and heterologous controls; in this regard, our methodology allows an opportunity to study focal gene alterations within the EP compared to the normal appearing eutopic endometrium, thus eliminating any

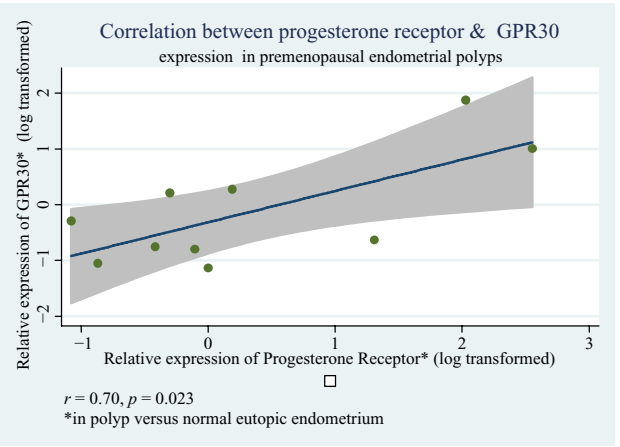

Figure 1. Relationship between relative expressions of PR and GPR30 within premenopausal endometrial polyps versus eutopic normal endometrium. GPR30 indicates G protein-coupled receptor 30; PR, progesterone receptor.

interindividual heterogeneity when interpreting relative expressions of genes of interest within EP compared to the normal endometrium.

The absence of any meaningful difference in the mRNA expression of ERα, ERβ, GPR30, and PR in EP compared to the normal appearing eutopic endometrium in our samples is of interest in itself and goes against any meaningful role for any single steroid hormone receptor in the pathogenesis of premenopausal EP. Alternatively, the observed lack of statistically significant differences in the expression of explored genes of interest may be a reflection of the small sample size, molecular level heterogeneity as well as heterogeneity within the study population. Although not of statistical significance, the mean relative expression of ERα (2.41) and PR (2.94) met the a priori defined threshold for biological relevance (ie, more than 2-fold difference, see METHODS), suggesting that the observed relationships merit further exploration. These questions and concerns may best be addressed by appropriately designed and suitably powered future studies.

Our findings are however inconsistent with some previous reports,[5-11] which showed differences in the expression of steroid receptors between EPs and the normal endometrium. Several reasons may underlie these observed differences. Sampling bias is a major concern in studies utilizing heterologous surgical samples of EPs and controls.[5,9,10] The use of control endometrium from a different woman may lead to unacceptable or systematic variation. The exact site of tissue sampling within a uterus may itself influence study results, given that within uterine tissue, ER and PR are topographically distributed with the highest expression at the level of the uterine fundus.[23] Thus, sampling EP and control tissue with disregard to the exact uterine site may have contributed some bias in prior studies. We attempted to sample the grossly normal eutopic endometrial tissue from the uterine wall opposite to the site of EP; thus, the tissues (EP and control) were collected from comparable topographic regions to standardize technique of collection and hopefully reduce sampling bias. Wide age ranges of women contributing EP samples in previously reported studies may be an additional contributor to the observed inconsistencies. Some of the previous reports studied premenopausal women,[8-11] whereas others focused on postmenopausal EP samples.[5-8] This heterogeneity in chronological aging as well as reproductive status may itself yield disparate results in the endometrial expression of genes of relevance. We herein focus our studies on premenopausal women diagnosed with EP.

Despite our attempts to define our study population and stringency in sample collection strategy, our study has several limitations. The small sample size and molecular level heterogeneity may have led to a failure to detect clinically significant differences between expression of the genes of interest within EP and the control eutopic endometrium. Unlike immunohistochemistry (IHC) that provides informative details relating to tissue compartment–specific expression, informative yield of qRT-PCR is limited to quantitative expressivity with disregard to tissue architecture. Hence, we are unable to comment on the tissue distribution of the steroid hormone receptors relating to the genes of interest. Given the study design and sample collection methodology, we are unable to expand our observations to include a study of expression of steroid hormone receptors for the specified genes by either IHC or Western Blot methodology, and hence cannot further explore whether the observed patterns of relative gene expression were paralleled by a variability in expression of the respective hormone receptors; these aspects deserve consideration in future studies. Finally, despite our attempts to focus on premenopausal EP, the age range of our study population is wide, an aspect that may itself have confounded our results. This latter concern, however, is unlikely to be of relevance, given that prior work from our group demonstrates an absence of significant age-related changes in the expression of steroid receptors within the human endometrium.[24]

Despite the limitations, our findings add to the existing literature on potential mechanisms that may underlie pathogenesis of EP, as well as support a molecular heterogeneity within premenopausal EPs. In contrast to previous studies that reported on the absolute expression of steroid receptors within EP, our results imply that the relative expression of steroid receptors in EP compared to the normal endogenous endometrial tissue may be a more meaningful approach to understand the pathogenic mechanisms contributing to EP. Positive correlation between PR and GPR30 expression within premenopausal EP compared to normal appearing autologous endometrium is a novel finding that merits further exploration in appropriately designed future studies.

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