Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium

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In women, a single dose of the antiprogestin mifepristone (RU486) in the secretory phase rapidly renders the endometrium unreceptive and is followed by endometrial breakdown and menstruation within 72 h. This model provides a system to identify progesterone-regulated genes, which may be involved in endometrial receptivity and the induction of menstruation. We used cDNA microarrays to monitor the response of the endometrium over 24 h following administration of mifepristone in the mid-secretory phase. We identified 571 transcripts whose expression was significantly altered, representing 131 biochemical pathways. These include new progesterone regulated members of the Wnt, matrix metalloproteinase (MMP), prostaglandin (PG) and chemokine regulatory pathways. Transcripts involved in thyroid hormone metabolism and signalling such as type II iodothyronine deiodinase and thyroid receptors were also found to be highly regulated by progesterone antagonism in the endometrium. Transcripts required for thyroid hormone synthesis such as thyroid peroxidase (TPO) and thyroglobulin (TG) were also expressed, indicating that the endometrium may be a site of thyroxin production. These results add to the existing knowledge of the role of the Wnt, chemokine, MMP and PG pathways in receptivity and early menstrual events. They provide in vivo evidence supporting direct or indirect regulation of many new transcripts by progesterone. We have also identified for the first time the very early transcriptional changes in vivo in response to progesterone withdrawal. This greatly increases our understanding of the pathways leading to menstruation and may provide new approaches to diagnose and treat menstrual disorders.

Keywords: endometrium; microarray; progesterone; mifepristone

Introduction

The endometrium undergoes dramatic and characteristic changes during the menstrual cycle, in preparation for implantation of an embryo. During a natural cycle these changes are driven by estrogens and progesterone produced by the ovary. Following ovulation, during the secretory phase of the cycle, the glands and stroma undergo maturation in response to progesterone from the corpus luteum resulting in an endometrium which is receptive to embryonic implantation. This period of receptivity lasts ~6 days during the mid-secretory phase, coinciding with peaking of serum progesterone levels. In the absence of embryo implantation, the corpus luteum involutes and progesterone levels decline, triggering menstruation. It is recognized that progesterone acts on an estrogen primed endometrium to initiate a pattern of gene expression important for implantation and the early stages of pregnancy. Conversely, withdrawal of progesterone results in changes in gene expression that rapidly render the endometrium unreceptive followed by endometrial breakdown and menstruation (Critchley et al., 2001; Jabbour et al., 2006). However in women, only a limited number of progesterone-regulated genes have been identified, and our understanding of the molecular mechanisms by which these functional changes in endometrium occur is primitive. If these specific, progesterone-dependent molecules are identified, there is the potential for their use as markers of uterine receptivity, as targets for contraception or to better understand and treat menstrual disorders.

Progesterone exerts its effects mainly by binding the nuclear progesterone receptor (PR), which exists in two isoforms PRA and PRB. Both isoforms are expressed in the stroma and epithelium of the endometrium during the proliferative phase, however, expression of both decreases sharply in the epithelium in early to mid-secretory phase (Wang et al., 1998). Endometrial receptivity appears to be tightly associated with the down-regulation of epithelial PR, and the stroma maintains expression only of PRA in the secretory phase (Lessey et al., 1988; Wang et al., 1998). The antiprogestin mifepristone blocks the biological effects of progesterone by binding to the PR with high affinity. In women, a single dose of mifepristone (200 mg) in the secretory phase of the cycle rapidly renders the endometrium unreceptive, and has been shown to alter gene expression in the uterus within 6 h of oral administration (Hapangama et al., 2002; Danielsson et al., 2003). These rapid actions are almost certainly due to direct effects on the endometrium since there is no detectable effect on circulating progesterone levels by this time (Gastro et al., 1988). A direct effect of progesterone withdrawal on epithelial receptivity is supported by the finding that attachment of human embryos to endometrial epithelium in vitro is inhibited by prior treatment with antiprogestin (Petersen et al., 2005). Following these effects on receptivity,
the artificial progesterone withdrawal results in endometrial breakdown and menstruation within 72 h (Garzo et al., 1988). It has been suggested that this menstrual bleeding is due to effects on endometrial blood vessels (Johannisson et al., 1989). Mifepristone administered in the mid-secretory phase induced changes in capillary lumen area and in endothelial cells lining these vessels. Some of these effects may be due to the progestosterone antagonist acting directly on endothelial cells, since human microvascular endometrial endothelial cells have been shown to be responsive to both estrogen and progestins (Krikun et al., 2005). However, the majority of PR expression is in stromal cells and mifepristone has been shown to alter the expression of regulators of blood vessel function expressed in stroma and glands (Ghosh et al., 1998; Narvekar et al., 2006). Many of the effects of mifepristone on blood vessels are therefore likely to be indirect due to blocking of the actions of progesterone in stromal cells.

This model therefore provides a system to identify progesterone-regulated genes, which are involved in endometrial receptivity and the induction of menstruation in women. This approach has recently been successfully used in mice to identify many new progesterone-regulated genes and pathways during the receptive period. If mifepristone is administered to mice on day 3 of pregnancy, ~24 h prior to embryo attachment, the endometrium does not become receptive and implantation is prevented. Microarray analysis identified 140 transcripts altered by this treatment. Regulation of many of these genes by progesterone was confirmed using PR knockout mice (Cheon et al., 2002). Significantly, several of the novel genes identified in this way have been shown to be functionally important in implantation in the mouse (Li et al., 2004). However, extrapolation of the results from such animal experiments to humans is not easy because the effects of mifepristone depend upon the location of the isoforms of PR, which differ between species. Interpretation of the effects of mifepristone on the endometrium must also consider that mifepristone has both antagonist and agonist activities on the PR but also exhibits anti-glucocorticoid and anti-androgenic activities (reviewed in Chwalisz et al., 2000). These receptors have subtly different distributions between species, and even within primates. For these reasons, we have sought to investigate the responses of human endometrium to progesterone withdrawal, using mifepristone in vivo.

We used cDNA microarrays to identify genes whose expression was altered by the administration of a single dose of mifepristone (200 mg) to women 8 days after the luteinising hormone surge (LH + 8). Endometrial responses were measured using biopsies taken 6 or 24 h later. This strategy identified more than 500 genes altered by mifepristone in the endometrium. Many of these represent mediators of the rapid effects of mifepristone action on receptivity and vascular integrity. Using pathway analysis, we have identified biochemical pathways whose members show coordinate regulation. We describe five pathways in greater detail, four of which have been described previously in the endometrium and which may play a role in receptivity or the initiation of menstruation. These are the Wnt, chemokine, MMP and PG pathways. In this study, we identified new members of each pathway, whose expression in endometrium appears to be regulated by progesterone withdrawal. In addition, we provide novel evidence for the expression and steroid regulation in endometrium of thyroid hormone signalling and metabolism. We also show expression of all of the transcripts required for thyroxin production and discuss the possible role of thyroid hormone synthesis in the endometrium. For the first time, we are able to delineate in detail the early molecular responses to progesterone withdrawal in vivo. This has provided new insights into the mechanisms by which progesterone regulates endometrial receptivity and the early events leading to menstruation.

Materials and Methods

Patient treatment and tissue collection

To study the effects of mifepristone in vivo, women were recruited into a randomised study in Edinburgh as previously described (Hapangama et al., 2002). Additional subjects were recruited in Helsinki. All subjects were healthy fertile women with a mean age of 34 years (range 26–45) who had regular menstrual cycles of 27–30 days, and had not used hormonal contraception, intrauterine device (IUD) or received hormone therapy for at least 2 months prior to the endometrial sampling. Timing of the LH-surge was identified by using a daily urinary dipstick. A single dose of 200 mg mifepristone was administered on cycle day LH + 8 and endometrial biopsies taken either 6 (n = 5) or 24 h (n = 4) later. Endometrial biopsies (n = 15) taken at cycle day LH + 8 from untreated women were used as controls. Each specimen was assessed as normal by histological examination. For evaluation of gene expression changes throughout the cycle, we used endometrial samples taken from 36 normal fertile women attending Addenbrookes’ Hospital, Cambridge for sterilization and who were not using hormonal contraception or IUD. In this case, endometrial biopsies were taken throughout the cycle and dated by a pathologist. All biopsies were divided into two pieces, one part being flash frozen in liquid nitrogen for RNA isolation, and the second was fixed in formalin for routine histology. Written informed consent was obtained from all patients, and this study was approved by the local research ethics committee of the hospitals involved.

Microarray gene profiling

Microarray analysis was performed using two custom made arrays representing a total of 16 000 different human cDNA clones, spotted in duplicate. The microarrays were produced in the microarray core facility, Department of Pathology, University of Cambridge. Details of the cDNA clones and the methods used in the array production have previously been reported (Rossi et al., 2005). Total RNA was extracted from each biopsy as previously described (Catalano et al., 2003). A reference cDNA was made from pooled endometrial total RNA samples from 15 different control individuals sampled during the mid-secretory phase. This was labelled with Cy3-deoxyxuridine triphosphate. Endometrial samples taken from control (untreated) subjects and those administered mifepristone were labelled with Cy5-deoxyuridine triphosphate (Amersham-Pharmacia, Little Chalfont, UK), using the BioPrime DNA labelling kit (Invitrogen, Paisley, UK). Each Cy5-labelled mifepristone-treated or control sample was combined with an equal amount of the pooled Cy3-labelled reference. Labelled cDNA was purified using Atooseq G50 columns (Amersham), and hybridized in hybridization buffer containing 40 µg/ml human cot-1 DNA, 160 µg/ml poly dA and 80 µg/ml yeast tRNA for 16 h at 50°C. Arrays were washed twice in 2× SSC, 0.5% SDS and twice in 0.1× SSC, 0.1% SDS for 5 min and twice in 0.1× SSC, each at room temperature. The fluorescence signal was acquired with a Genechip 4100 microarray scanner (Axon Instruments, Foster City, CA, USA) allowing the Cy5 hybridization signal for each spot to be compared to the Cy3 signal from the common reference.

Array analysis

Hybridization signals were quantified using BlueFuse 2.1 software (BlueGnome Ltd, Cambridge, UK). The raw spot intensity data were normalized per spot and per chip using GeneSpring 7.2 software with intensity dependent (Lowess) normalization (percentage of the data used for smoothing 10% and cut-off value of 0.01). Spots with poor morphology and low hybridization signals were removed using default confidence values with BlueFuse software. Genes that showed statistically significant changes were identified using the parametric Welch t-test and using Benjamini and Hochberg false discovery rate correction for multiple testing. GeneSpring 7.2 software was utilized for pathway identification.

Real-time RT–PCR

Array verification was preformed by real-time RT–PCR analysis of selected genes using an ABI PRISM 7700 sequence detection system according to the manufacturer’s instructions (Applied Biosystems, Warrington, UK). The sequences of primers and Taqman probe for IGFBP3 were as published previously (Horcajadas et al., 2006). For ribosomal 18S RNA, MMP7 and
CXCL1, prevalidated primers and probes were purchased (Assays-on-Demand, Applied Biosystems). Primers for real-time RT–PCR using SYBR Green detection for Wnt5A, thyroid hormone receptors alpha 1 (THRA1), THRA2, thyroid hormone receptors beta 1 (THRB1), DIO2 and TG were also designed using Primer Express v5.0 (Table 1). Standard curves were generated by serial dilution of cDNA prepared from mid-secretory endometrium. Data are expressed in arbitrary units relative to the level of the same gene in this standard RNA. cDNA was produced from each endometrial sample by reverse transcription using 0.5 μg of total RNA with 200 units of SuperScript III (Invitrogen) primed with random hexamers according to the manufacturer’s instructions. The values obtained were normalized against those from ribosomal 18S RNA to account for differing amounts of starting material. Expression levels in endometrial biopsies from different subject groups were compared using the non-parametric Mann–Whitney test; statistical significance was accepted when P < 0.05. Presence or absence in endometrium of transcripts encoding TPO, the thyroid stimulating hormone receptor (TSHR), thyroid stimulating hormone beta subunit (TSHB) and the common alpha subunit (CGA) was investigated by RT–PCR and gel-based analysis of the amplified products. These additional primers are also shown in Table 1.

Results
Identifying genes altered by mifepristone administration in vivo
Endometrial biopsies were collected at either 6 or 24 h after a single dose of 200 mg of mifepristone administered on cycle day LH + 8. Endometrial biopsies collected from women at LH + 8 who did not receive mifepristone were used as controls. RNA from each sample was labelled with Cy5 and hybridized together with a common Cy3 labelled reference (pooled mid-secretory endometrium) to two microarrays containing ~16 000 human cDNAs. Gene profiling identified 103 transcripts that were significantly down-regulated and 186 transcripts that were up-regulated by ≥2-fold at 6 h following mifepristone administration, compared with the control group (P < 0.05). At 24 h, 126 transcripts were down-regulated and 242 transcripts were up-regulated compared with the control group. In total, 571 genes (~3.6%) displayed significant changes of ≥2-fold, following administration of mifepristone in the mid-secretory phase (Supplementary material, Table S4). The results of this analysis are shown in representative scatter plots (Fig. 1). Mean intensity values for each of the 289 significantly changing transcripts at 6 h (Fig. 1A) and the 368 transcripts altered at 24 h (Fig. 1B) are shown compared with values from the control subjects. Of the 289 transcripts, whose expression changed ≥2-fold at 6 h following mifepristone administration, only 86 of these have sustained a ≥2-fold change in expression at 24 h; the remaining 203 transcripts returned to similar levels as in the control (untreated) subjects. Table 2 shows genes that significantly change ≥2.5-fold at either time point after administration of mifepristone (P < 0.05). The table includes 181 genes that are grouped according to their expression profiles based on whether they show a significant change in expression at each time point.

Verification of microarray changes by real-time RT–PCR
Real-time RT–PCR was used to verify the changes in RNA expression levels for selected transcripts identified by the cDNA array analysis. Four genes: MMP7, CXCL1, Wnt5A and IGFBP3, representing different expression profiles after mifepristone administration, were chosen for verification. cDNA was synthesized from the same total RNA samples used for the array analysis. MMP7 expression increased by a mean of 3.9-fold, IGFBP3 by a mean of 3.5-fold, Wnt5A by a mean of 1.7-fold and CXCL1 decreased by a mean of 3.1-fold compared with control samples at 24 h (Fig. 2). The difference in expression level 24 h after mifepristone administration was statistically significant compared to controls for all four transcripts (P < 0.03). These results are in good agreement with the microarray analysis. We therefore believe the gene list generated by the cDNA array analysis reflects reliable changes in gene expression in vivo.

Pathway analysis
Pathway analysis was used to identify if particular biochemical pathways or protein families were being co-regulated. This allows large gene lists to be ordered into a smaller list of pathways, and genes can be visualized as part of a network of interactions. The KEGG database was searched with the 571 transcripts shown to be altered by mifepristone. The analysis revealed 131 different regulatory and biochemical pathways to be affected with mifepristone. Many are involved in metabolic processes, such as fatty acid and carbohydrate metabolism (Supplementary material, Table S5). Pathways that showed the greatest numbers of genes affected included cytokine/receptor interactions, Wnt signalling, cell communication and extracellular matrix (ECM)-receptor interaction. We have focused on five pathways, in particular, in which several members showed altered gene expression following mifepristone administration: the MMPs, chemokines, PG metabolism, thyroid hormone metabolism and Wnt pathways (Table 3). Several MMPs were dramatically up-regulated, including MMP10 and MMP3 by 99- and 75-fold, respectively. ECM degradation and MMP up-regulation are well-documented
### Table 2: Genes significantly changing 2.5-fold or more after RU486 administration in vivo

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene description</th>
<th>Fold change</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>6 hr</td>
<td>24 hr</td>
<td></td>
</tr>
</tbody>
</table>

**Group 1: Down at 6 h, down at 24 h**

| ACTB | Actin, beta | -2.2 | -3.9 |
| ARF4L | ADP-ribosylation factor 4-like | -3.0 | -4.1 |
| B3GAT3 | Beta-1,3-glucuronitransferase 3 (glucuronosyltransferase 1) | -2.3 | -2.7 |
| BCAT2 | Branched chain aminotransferase 2, mitochondrial | -2.1 | -3.1 |
| CKB | Creatine kinase, brain | -2.0 | -5.5 |
| COLA4A6 | Collagen, type IV, alpha 6 | -2.0 | -5.0 |
| CXCL13 | Chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant) | -2.3 | -13.3 |
| CYP4B1 | Cytochrome P450, family 4, subfamily B, polypeptide 1 | -2.3 | -7.6 |
| LMC1 | LIM and cysteine-rich domains 1 | -2.4 | -2.8 |
| MTA1 | Metastasis associated 1 | -2.4 | -2.7 |
| MYC | V-myc myelocytomatosis viral oncogene homolog (avian) | -2.2 | -2.5 |
| PC | Pyruvate carboxylase | -2.2 | -2.5 |
| PEMT | Phosphatidylethanolamine N-methyltransferase | -3.4 | -5.1 |
| POLD2 | Polymerase (DNA directed), delta 2, regulatory subunit 50kDa | -2.0 | -2.9 |
| ROG | Ras homolog gene family, member G (rho G) | -4.7 | -6.0 |
| RPRM | Reprimo, TP53 dependant G2 arrest mediator candidate | -4.9 | -5.6 |
| TH | Tyrosine hydroxylase | -2.3 | -3.5 |

**Group 2: Down at 6 h, unchanged at 24 h**

| ANPEP | Alanyl (membrane) aminopeptidase (CD13, p150) | -2.6 | n/s |
| APOC1 | Apolipoprotein C-I | -3.5 | n/s |
| C6orf19 | Chromosome 6 open reading frame 19 | -2.5 | n/s |
| CAPN5 | Calpain 5 | -2.6 | n/s |
| CCL13 | Chemokine (C-C motif) ligand 13 | -2.7 | n/s |
| CCL3 | Chemokine (C-C motif) ligand 3 | -2.7 | n/s |
| CCL8 | Chemokine (C-C motif) ligand 8 | -2.8 | n/s |
| CRISPLD2 | Cysteine-rich secretory protein LCCL domain containing 2 | -3.6 | n/s |
| CST7 | Cystatin F (leukocystatin) | -2.8 | n/s |
| DDT14 | DNA-damage-inducible transcript 4 | -3.0 | n/s |
| FAH | Fumarylacetoacetate hydrolase (fumarylacetoacetase) | -2.6 | n/s |
| FBXO46 | F-box protein 46 | -2.9 | n/s |
| FLRT2 | Fibronectin leucine rich transmembrane protein 2 | -2.8 | n/s |
| GMPPA | GDP-mannose pyrophosphorylase A | -2.5 | n/s |
| ML12 | Myeloid/lymphoid or mixed-lineage leukemia 2 | -2.5 | n/s |
| MPRA | Progestin and adipoQ receptor family member VII | -2.5 | n/s |
| RODH | Hydroxysteroid (17-beta) dehydrogenase 6 | -2.7 | n/s |
| TXLNA | Taxilin | -3.2 | n/s |

**Group 3: Unchanged at 6 h, down at 24 h**

<p>| ABCCS | ATP-binding cassette, sub-family C (CFTR/MRP), member 8 | n/s | -3.1 |
| ALDH3B2 | Aldehyde dehydrogenase 3 family, member B2 | n/s | -5.5 |
| ALDOC | Aldolase C, fructose-bisphosphate | n/s | -3.1 |
| B3GNT6 | UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6 | n/s | -2.7 |
| BSCL2 | Bernardinelli-Seip congenital lipodystrophy 2 (seipin) | n/s | -3.1 |
| C10orf116 | Chromosome 10 open reading frame 116 | n/s | -3.1 |
| C19orf32 | Chromosome 19 open reading frame 32 | n/s | -2.9 |
| CD36 | CD36 antigen (collagen type I receptor, thrombospondin receptor) | n/s | -4.8 |
| CNTNAP1 | Contactin associated protein 1 | n/s | -2.6 |
| COLA4A5 | Collagen, type IV, alpha 5 | n/s | -4.3 |
| EXOC5 | Exosome component 5 | n/s | -2.6 |
| FBLN1 | Fibrillin 1 | n/s | -3.0 |
| FSTL3 | Follistatin-like 3 (secreted glycoprotein) | n/s | -3.5 |
| GAGEC1 | P antigen family, member 4 (prostate associated) | n/s | -3.6 |
| GPR56 | G protein-coupled receptor 56 | n/s | -3.4 |
| GRIA1 | Glutamate receptor, ionotropic | n/s | -3.9 |
| GSTT2 | Glutathione S-transferase theta 2 | n/s | -3.0 |
| HLA-DOB | Major histocompatibility complex, class II, DO beta | n/s | -12.3 |
| HPGD | Hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD) | n/s | -2.8 |
| HS17B8 | Hydroxysteroid (17-beta) dehydrogenase 8 | n/s | -3.2 |
| IGFBP5 | Insulin-like growth factor binding protein 5 | n/s | -4.7 |
| LDOC1 | Leucine zipper, down-regulated in cancer 1 | n/s | -2.6 |
| MPZL1 | Myelin protein zero-like 1 | n/s | -2.7 |
| MPZL1 | Myelin protein zero-like 1 | n/s | -2.7 |
| NQO1 | NAD(P)H dehydrogenase, quinone 1 | n/s | -4.0 |
| PRODH | Proline dehydrogenase (oxidase) I | n/s | -3.1 |
| PTGDS | PG-H-2 D-isomerase precursor | n/s | -4.6 |
| RARG | Retinoic acid receptor, gamma | n/s | -3.6 |
| S100A6 | S100 calcium binding protein A6 (calcyclin) | n/s | -2.7 |
| SFT4 | Septin 4 | n/s | -2.7 |</p>
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<th>Gene symbol</th>
<th>Gene description</th>
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<tr>
<td>SERPINF1</td>
<td>Serine (or cysteine) proteinase inhibitor, clade F member 1</td>
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<tr>
<td>SLC22A3</td>
<td>Solute carrier family 22 (extraneuronal monoamine transporter), member 3</td>
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<td>SLC27A5</td>
<td>Solute carrier family 27 (fatty acid transporter), member 5</td>
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<td>SLC33A1</td>
<td>Solute carrier family 43, member 1</td>
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</tr>
<tr>
<td>SLC7A4</td>
<td>Solute carrier family 7 (cationic amino acid transporter, y + system), member 4</td>
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</tr>
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<td>SLIT3</td>
<td>slit homolog 3</td>
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<td>SNX12</td>
<td>Sorting nexin 12</td>
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</tr>
<tr>
<td>SRPX</td>
<td>Sushi-repeat-containing protein, X-linked</td>
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</tr>
<tr>
<td>STAR</td>
<td>Steroidogenic acute regulator</td>
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<td>SYNGR1</td>
<td>Synaptogyrin 1</td>
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</tr>
<tr>
<td>TIPARP</td>
<td>TCDD-inducible poly(ADP-ribose) polymerase</td>
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**Group 4**  
*Up at 6 h, up at 24 h*

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<td>ABLIM1</td>
<td>Actin binding LIM protein 1</td>
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<td>ARHE</td>
<td>Rho family GTPase 3</td>
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<tr>
<td>ARHGAP28</td>
<td>Rho GTPase activating protein 28</td>
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<tr>
<td>CD69</td>
<td>CD69 antigen (p60, early T-cell activation antigen)</td>
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<tr>
<td>CDH1</td>
<td>Cadherin 1, type 1, E-cadherin (epithelial)</td>
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</tr>
<tr>
<td>CHES1</td>
<td>Checkpoint suppressor 1</td>
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<tr>
<td>CLECSF2</td>
<td>C-type lectin domain family 2, member B</td>
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<td>DJO2</td>
<td>Deoxynucleotide, deoxynucleosine, type II</td>
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<tr>
<td>ENC1</td>
<td>Ectodermal-neural cortex (with BTB-like domain)</td>
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<tr>
<td>F13A1</td>
<td>Coagulation factor XIII, A1 polypeptide</td>
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<td>FZD4</td>
<td>Frizzled homolog 4 (Drosophila)</td>
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<td>GI1A</td>
<td>Gap junction protein, alpha 1, 43kDa (connexin 43)</td>
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<td>HDAC2</td>
<td>Histone deacetylase 2</td>
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<td>INHBA</td>
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<td>Laminin, gamma 2</td>
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<tr>
<td>LUM</td>
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<td>LNX</td>
<td>Latexin</td>
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<td>MAPK6</td>
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<td>MAPK14</td>
<td>Monocyte to macrophage differentiation-associated</td>
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<td>P2RY14</td>
<td>Purinergic receptor P2Y, G-protein coupled, 14</td>
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<td>PSMC6</td>
<td>Proteasome (prosome, macropain) 26S subunit, ATPase, 6</td>
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<td>RB1CC1</td>
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<td>SH3-domain GRB2-like (endophilin) interacting protein 1</td>
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<tr>
<td>ZNF22</td>
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**Group 5**  
*Up at 6 h, unchanged at 24 h*

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<td>Annexin A1</td>
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<td>C20orf19</td>
<td>Chromosome 20 open reading frame 19</td>
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<td>CABIN1</td>
<td>Calcineurin binding protein 1</td>
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<tr>
<td>CTNNAL1</td>
<td>Catenin (cadherin-associated protein), alpha-like 1</td>
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<tr>
<td>GSTA1</td>
<td>Glutathione S-transferase A1</td>
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<td>ITGA6</td>
<td>Integrin, alpha 6</td>
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<td>MARCKS-like 1</td>
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<tr>
<td>NEURL</td>
<td>Neuralized-like (Drosophila)</td>
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<tr>
<td>NOV1A</td>
<td>neuro-oncological ventral antigen 1</td>
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<td>NTN4</td>
<td>Nettin 4</td>
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<td>NUBP2</td>
<td>Nucleotide binding protein 2 (MinD homolog, E. coli)</td>
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<tr>
<td>PP1R1C</td>
<td>Protein phosphatase 1, regulatory (inhibitor) subunit 1C</td>
<td>3.1</td>
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<td>PPT2</td>
<td>Palmitoyl-protein thioesterase 2</td>
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</tr>
<tr>
<td>PTMRK</td>
<td>Protein tyrosine phosphatase, receptor type, K</td>
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<tr>
<td>RPL39</td>
<td>Ribosomal protein L39</td>
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<td>RPS10</td>
<td>Ribosomal protein S10</td>
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<td>SCYE1</td>
<td>Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)</td>
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<td>Secreted frizzled-related protein 1</td>
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<td>SOX9</td>
<td>SRY (sex determining region Y)-box 9 ( autosomal sex-reversal)</td>
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<td>Sperm associated antigen 1</td>
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<td>TLOC1</td>
<td>Translocation protein 1</td>
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<td>TRIB2</td>
<td>Tribbles homolog 2 (Drosophila)</td>
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<td>UQCRB</td>
<td>Ubiquinol-cytochrome c reductase binding protein</td>
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**Group 6**  
*Unchanged at 6 h, up at 24 h*

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<tr>
<td>ANKRD10</td>
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*Continued*
effects of progesterone withdrawal/antagonism (Lockwood et al., 1998; Critchley et al., 2003), but the arrays show that not only are MMPs increased but expression of their collagen substrates, collagen type II and type IV, was decreased. These novel data show that the expression profiles of MMP10 and MMP3 (expression group 6 of Table 1) are inversely correlated to their collagen and laminin substrates that they degrade (groups 1, 2 and 3, of Table 1), suggesting co-regulation. Interestingly, the collagen receptor CD36 is also down-regulated. We also showed up-regulation of tissue plasminogen activator (PLAT), activin A (INHBA) and follistatin related gene (FSTL3), which would enhance tissue remodelling. The Wnt family ligand Wnt5a and four Wnt receptors were up-regulated, together with β-catenin the downstream regulator of Wnt pathway activation and axin2, a regulator of β-catenin stability. Several key enzymes involved in PG synthesis and metabolism were down-regulated including PG D2 synthase (PTGDS), hydroxyprostaglandin dehydrogenase (HPGD) and PG-endoperoxide synthase 1 (PTGS1). Expression of members of the chemokine family was affected with the majority of C-C motif members being down-regulated and several C-X-C motif members being up-regulated after 6 h. Although

<table>
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<td>ARL6IP</td>
<td>ADP-ribosylation factor-like 6 interacting protein</td>
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<td>BID</td>
<td>BH3 interacting domain death agonist</td>
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<td>BNP2</td>
<td>BCL2/adenovirus E1B 19kDa interacting protein 2</td>
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<tr>
<td>BTK</td>
<td>Bruton agammaglobulinemia tyrosine kinase</td>
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<td>C1QTNF6</td>
<td>Clq and tumor necrosis factor related protein 6</td>
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<td>Cell division cycle 2, G1 to S and G2 to M</td>
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<td>Cadherin 11, type 2, OB-cadherin (osteoblast)</td>
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<td>Carboxypeptidase Z</td>
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<td>Matrix metalloproteinase 3 (stromelysin 1)</td>
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<td>Matrix metalloproteinase 7 (matrixin, uterine)</td>
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<td>Phospholipase D1, phosphatidylcholine-specific</td>
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<td>Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2</td>
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<td>Protein phosphatase 2 (formerly 2A), regulatory subunit B”, alpha</td>
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<td>Prolactin</td>
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<td>Polypyrimidine tract binding protein 2</td>
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<td>PG I2 (prostacyclin) receptor (IP)</td>
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<td>RAP1B, member of RAS oncogene family</td>
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<td>Ring finger protein 146</td>
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<td>Small EDRK-rich factor 2</td>
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<td>SMAD specific E3 ubiquitin protein ligase 2</td>
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<td>Stress 70 protein chaperone, microsome-associated, 60kDa</td>
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<td>Stathmin-like 2</td>
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<tr>
<td>SVIL</td>
<td>Supervillin</td>
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</tr>
<tr>
<td>TKE</td>
<td>TEK tyrosine kinase, endothelial</td>
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<td>TGFBIH4</td>
<td>TSC22 domain family, member 1</td>
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<td>THBS1</td>
<td>Thrombospondin 1</td>
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<td>TIMM17A</td>
<td>Translocase of inner mitochondrial membrane 17 homolog A (yeast)</td>
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<td>Tumor necrosis factor receptor superfamily, member 1A</td>
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<tr>
<td>Wnt5A</td>
<td>Wingless-type MMTV integration site family, member 5A</td>
<td>n/s</td>
</tr>
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</table>

Transcripts were classified into each group based on whether they showed a statistically significant change at that time point compared with the control patients. The fold change is only given when the change seen was statistically significant.
some members of these pathways have previously been identified as steroid regulated in endometrium, we have now identified new members of each family that appear to be altered by acute progesterone withdrawal during the mid- to late-secretory phase.

More significantly, the analysis revealed several uncharacterized pathways not previously known to be expressed in the endometrium or regulated by progesterone; in particular that of thyroid hormone metabolism and signalling (Table 3). Deiodinase, iodothyronine type II (DIO2), an enzyme that converts thyroxin (T4) to the more active metabolite T3, was up-regulated in both 6 and 24 h samples by up to 13-fold. THRA and THRB were shown to be differentially regulated by mifepristone. The analysis indicates that both thyroid hormone activation and thyroid receptor signalling are regulated in endometrial cells by progesterone.

Components of thyroid hormone signalling pathway are expressed in the endometrium

The thyroid signalling pathway consists of a complex group of proteins which regulate thyroid hormone synthesis, activation and trans-activation of gene transcription by the nuclear receptors. Transcripts representing the majority of the components of the thyroid signalling pathway were found to be expressed in the endometrium, although for most their expression was not altered by mifepristone (Fig. 3A).

Expression of the following genes was detected: several members of the thyroid interacting protein families 3, 4, 6, 10, 11, 12 and 15; thyroid transcription factor 1, thyroid hormone associated proteins 1 and 4 and the thyroid hormone receptors THRA and THRB. Interestingly, we also detected the expression of transcripts involved in thyroxin synthesis and activation including the sodium iodide symporter (SLC5A5) that actively transports iodide across the plasma membrane. P4HB a molecular chaperone involved in endocytosis of immature TG molecules and DIO2. The major proteins involved in thyroid hormone synthesis and signalling are shown in Fig. 4. Several key members of these pathways were absent from the array. To confirm their expression and to detect other key genes in the pathway not printed on the array, we performed RT–PCR using gene specific primers on the pooled reference mid-secretory endometrial cDNA (as used for the microarrays). Additional transcripts tested included TG, TPO, TSHB, CGA and TSHR, which stimulates iodide uptake. We also utilized primers able to distinguish the different splice variants of the THRA and THRB. The results verified endometrial expression of all transcripts tested except THRB2 and TSHB (Fig. 3B). The amplified product resulting from amplification with the TG specific primers was purified and sequenced, which confirmed the product as the transcript encoding TG.

Effects of mifepristone on thyroid hormone signalling pathways in the endometrium

The array analysis indicated that expression levels of DIO2 and the two thyroid hormone receptors THRA and THRB were altered by mifepristone (Table 3). Real-time PCR was used to determine the effects of mifepristone on the different receptor isoforms, and on DIO2 and TG expression. Expression of THRA1 and THRA2 was significantly down-regulated at both 6 and 24 h after mifepristone administration, whereas THRB1 expression was increased (Fig. 5). The real-time PCR analysis verified that DIO2 expression was significantly increased, by 7.8-fold at 24 h compared with controls, in line with the array data. Expression of the transcript encoding TG was decreased at both 6 and 24 h by up to 3-fold (Fig. 5). The array data together with the RT–PCR indicate that mifepristone can modify expression of transcripts encoding key components of thyroid hormone synthesis and metabolism as well as thyroid hormone receptor expression.

Expression of thyroid hormone signalling components during the menstrual cycle

Quantitative analysis of expression of transcripts for thyroid hormone receptors and TG in the human endometrium through the menstrual cycle has not previously been reported. We examined their expression during the menstrual cycle by real-time PCR on endometrial samples from menstrual (n = 7), proliferative (n = 11) and secretory (n = 18) phases. THRA1 and THRB1 expression significantly increased.
1.6- and 5.5-fold, respectively, from proliferative to secretory phase. THRA2 expression did not significantly change between the two phases. TG expression significantly increased 4.4-fold from the proliferative to mid-secretory phase. Expression of all these transcripts fell dramatically after the secretory phase to low levels during menstruation (Fig. 6).

Discussion

We have exploited the properties of mifepristone to induce pharmacological withdrawal of progesterone in order to identify genes regulated during the loss of receptivity and early events leading to menstruation. These results are in marked contrast to those obtained when we used explant cultures of secretory phase endometrium to examine the effect of mifepristone on endometrial gene expression (Catalano et al., 2003). Although that study employed a much smaller array, only a small number of transcripts were identified, suggesting that explants do not reflect the dynamic responses to steroids that we have seen in vivo. An early response to progesterone withdrawal is a rapid influx of leukocytes such as neutrophils, macrophages and uterine NK cells prior to menstruation itself (Salamonsen and Lathbury, 2000). Since these endometrial leukocytes lack significant expression of genomic PR, this effect must be indirect (Henderson et al., 2003). Progesterone withdrawal is known to up-regulate a variety of inflammatory mediators such as the chemokines IL-8 (CXCL8) and MCP-1 (CCL2) (Critchley et al., 1999). Other chemokines increase in endometrium during the secretory phase, which suggests regulation by progesterone, but no direct evidence for this was produced in that study (Jones et al., 2004). Our array analysis has identified several chemokines, which appear to be regulated by progesterone withdrawal. The CXC chemokines CXCL5, CXCL12 and CX3CL1 were rapidly up-regulated by mifepristone. These CXC chemokines are probably directly regulated by progesterone and may be responsible for the perimenstrual influx of neutrophils. It has previously been suggested that CX3CL1 is involved in the initial recruitment of leukocytes premenstrually since CX3CL1 production increases in glands, stroma and in vascular endothelium at this time. The corresponding receptor CX3CR1 is also expressed on

<table>
<thead>
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<th>Gene symbol</th>
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<th>6 h</th>
<th>24 h</th>
<th>Group</th>
</tr>
</thead>
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<td>CCL13</td>
<td>chemokine (C-C motif) ligand 13</td>
<td>−2.7</td>
<td>n/s</td>
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<td>chemokine (C-X-C motif) ligand 12</td>
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<td>3.1</td>
<td>n/s</td>
<td>5</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>catenin beta 1</td>
<td>n/s</td>
<td>2.3</td>
<td>6</td>
</tr>
<tr>
<td>AXIN2</td>
<td>axin 2</td>
<td>n/s</td>
<td>2.1</td>
<td>6</td>
</tr>
<tr>
<td>PG pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTGIR</td>
<td>PG I2 (prostacyclin) receptor (IP)</td>
<td>n/s</td>
<td>4.8</td>
<td>6</td>
</tr>
<tr>
<td>HPGD</td>
<td>hydroxyprostaglandin dehydrogenase 15-(NAD)</td>
<td>n/s</td>
<td>−2.8</td>
<td>3</td>
</tr>
<tr>
<td>PTGDS</td>
<td>PG-H-2 D-isomerase precursor</td>
<td>n/s</td>
<td>−4.6</td>
<td>3</td>
</tr>
<tr>
<td>PTGS1</td>
<td>PG-endoperoxide synthase 1 (COX1)</td>
<td>n/s</td>
<td>−2.2</td>
<td>3</td>
</tr>
</tbody>
</table>

Groups correspond to those in Table 2. Fold change indicates mean change in expression relative to control patients.
endometrial neutrophils and macrophages, especially, those in or immediately surrounding vessels (Hannan et al., 2004). In contrast, chemokines MCP4 (CCL13), CCL3, MCP2 (CCL8), GRO1 (CXCL1) and GRO3 (CXCL13) were all down-regulated by mifepristone. The blastocyst and invasive trophoblast cells express chemokine receptors suggesting that some of these endometrial-derived chemokines may be involved in crosstalk between endometrium and embryo/trophoblast during the initial attachment (Sato et al., 2003). Down-regulation of these chemokines by mifepristone suggests that the role of these chemokines is rather in preparation for pregnancy than for menstruation. It is important to note that these effects, though rapid, may nonetheless be indirect. Progesterone withdrawal also alters levels of powerful inflammatory mediators such as IL-1β, and we have recently shown that IL-1β up-regulates multiple chemokines in endometrial stromal cells including CXCL5 (Rossi et al., 2005). It is likely that such indirect mechanisms contribute to the up-regulation of CXCL5 that we have shown by progesterone antagonism in vivo.

MMPs play a critical role in tissue breakdown and repair and a marked increase in endometrial expression of MMPs occurs just before and during menstruation. As expected, the microarray analysis showed that MMPs 3, 7, 10, 14 and 16 were up-regulated following mifepristone administration. Progesterone is known to suppress MMP expression in endometrium by a number of mechanisms (Rodgers et al., 1994). Focal MMP7 expression in the epithelium is an early response to progesterone withdrawal and is due to stromal-to-epithelial signalling mediated by transforming growth factor-β (Bruner et al., 1995). MMP3 is up-regulated peri-menstrually in vascular structures such as pericytes and smooth muscle cells and it has been suggested that it initiates degradation of the vascular wall during menstrual breakdown (Hampton and Salamonson 1994; Freitas et al., 1999). MMPs perform a critical role in endothelial cell migration through the interstitial spaces (Sang, 1998). Fibrinolysis by MMP14 on endothelial cells is critical for neo-vascularization (Hiraoka et al., 1998) and MMP16 is thought to play a role in the regulation of tube formation by endometrial microvascular endothelial cells (Plaisier et al., 2004). MMP14 and MMP16 are membrane anchored MMPs both of which were up-regulated following mifepristone administration. MMP14 is expressed throughout the menstrual cycle with a moderate increase during menstruation and activates proMMP2 in endometrium following progesterone withdrawal (Zhang et al., 2000). MMP-10 has recently been demonstrated to be significantly up-regulated in response to physiological progesterone withdrawal (luteal regression) (Critchley et al., 2006; Talbi et al., 2006). The array data confirm not only that progesterone withdrawal regulates tissue breakdown but also up-regulates MMPs known to be involved in the development of new blood vessels.

Most MMPs are secreted as inactive zymogens (pro-MMPs). These are activated either by other MMPs or by the urokinase PA (uPA)-plasmin system (Carmeliet et al., 1997). PA activity in endometrial explants is regulated primarily by changes in the expression of PA inhibitor 1 (PAI1) (Lockwood et al., 1995). Progesterone inhibits the secretion of PAs (Casslen et al., 1986) and stimulates the expression of PAI1 (Casslen et al., 1992), indicating a control of this system by sex steroids during the menstrual cycle and its release before menstruation (Marbaix et al., 2000). The array data confirmed the down-regulation of PAI1 (official gene symbol SERPINE1) and up-regulation of PLAT, by mifepristone suggesting coordinated regulation of SERPINE1 and PLAT by the PR in vivo. Activin A (INHBA) is known to promote decidualization and stimulate production of proMMPs-2, -3, -7, -9 and active MMP2 and its activity is modulated by follistatin and FSTL3 (Mauger-Satta et al., 2001; Jones et al., 2002, 2006). We found that progesterone withdrawal up-regulated INHBA and down-regulated FSTL3. Indeed one of the striking findings from the array analysis is the coordinated up-regulation of MMPs and their activators such as PLAT (Table 3) and the down-regulation of MMP inhibitors such as SERPINE1 and various substrates of MMPs in the ECM such as collagens and laminins. Thus progesterone withdrawal induced by mifepristone results in co-regulation in vivo of several genes that contribute to increased ECM degradation.

Wnt proteins comprise a family of secreted ligands that bind to the frizzled (FZD) cell surface receptors, resulting in regulation of transcription by β-catenin. Wnt signalling plays important roles in the uterus both during development and at implantation, where they may be involved in embryo/endometrial signalling (Miller and Sassoon, 1998; Mohamed et al., 2005). We found several members of the Wnt signalling pathway were up-regulated by mifepristone administration including the Wnt receptors (FZD 4, 6, 9 and 10), ligands (Wnt5A and SFRP1) and the signal transduction molecules beta-catenin and axin2. This correlates with published data showing expression of several members of this family changes through the cycle, and demonstrates that they are regulated by progesterone in vivo (Talbi et al., 2006). The coordinated up-regulation of the Wnt receptors and Wnt5A in human endometrium by mifepristone suggests that this signalling system may mediate epithelial/mesenchyme interactions during menstruation and endometrial repair.

It is well established that the onset of menstruation is associated with increased local PG production in the endometrium (Baird et al., 1996). Mifepristone administration down-regulated several prostanooid metabolising enzymes and up-regulated the PG I2 receptor (PTGIR). This is consistent with previous reports that expression of

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**Figure 3:** Detection of transcripts in endometrium involved with thyroid hormone signalling and metabolism. Panel A shows normalized median intensity values for each spot on the microarray corresponding to the relevant transcript, for the control subjects. The intensity value of beta actin (ACTB) is shown for comparison; all intensities are well above background. Thyroid hormone receptor genes express variant splice forms which the array could not distinguish. Expression of these and other transcripts not printed on the microarray were assessed in endometrial tissue by RT–PCR and gel electrophoresis (Panel B). M, marker. For all transcripts tested by RT–PCR, only THRB2 and TSHB were not detected. All other primer pairs amplified DNA fragments of the expected size. The identity of the fragment amplified by primers for TG was verified by sequencing. Arrows indicate weak but clearly positive bands.
PTGIR increases during menstruation where it is localized to the glandular epithelium, stroma and endothelial cells (Battersby et al., 2004). At the same time, production of PG12 in the endometrium also rises and elevated levels of PG12 are associated with heavy menstrual bleeding (menorrhagia) (Cameron et al., 1987). Hydroxy-prostaglandin dehydrogenase 15 (HPGD) metabolises PGs to inactive metabolites and is known to be induced by progesterone. Mifepristone administration in vivo has previously been shown to decrease HPGD immunostaining in endometrium (Hapangama et al., 2002), which is consistent with the down-regulation of HPGD mRNA seen 24 h after mifepristone treatment in the luteal phase in agreement with our findings (Marions and Danielsson, 1999). PTGDS converts PGH2 to PGD2 and the down-regulation of PTGDS by mifepristone is similar to the decrease reported recently during physiological withdrawal of progesterone due to luteal regression (Critchley et al., 2006; Talbi et al., 2006). This would be expected to change the balance of production of these two PGs. The up-regulation of inflammatory mediators such as PGs by progesterone withdrawal at menstruation may have two roles: they may mediate the leukocyte influx and activation in the endometrium, and increased PG production is also almost certainly involved in the vasoconstriction of endometrial spiral arteries.

Figure 4: Diagrammatic representation of the expression of thyroid hormone synthesis and signalling components in the human endometrium. All boxed genes indicate expression was detected in the endometrium either by RT-PCR or microarray. Thyroid hormone synthesis involves the coupling of iodinated tyrosine residues by TPO and iodination of TG. In the thyroid gland, TG is stored extracellularly. Release of thyroid hormones requires uptake of TG by endocytosis aided by molecular chaperones such as P4HB. Hydrolysis of the TG releases predominantly tetra-iodothyronine (T4) and also tri-iodothyronine (T3). In target tissues, T4 is metabolised to T3 by DIO2 enabling T3 binding to thyroid hormone receptors. A host of co-regulatory receptor proteins assist in regulating gene transcription. Thyroid hormone synthesis and release is controlled by TSH, which is composed of two subunits TSHB and common CGA. Signalling through the TSHR controls the uptake of iodide through a sodium iodide symporter (SLC5A5) and the iodination of TG. These events are based on thyroid hormone synthesis in the follicular cells of the thyroid gland.
The array studies also showed that mifepristone altered the expression of transcripts involved in thyroid hormone metabolism and signalling. Thyroxin (T4) is the major secreted iodothyronine. In target tissues, DIO2 catalyses the deiodination of T4 to T3 as the first step in the activation of the prohormone. The T3 concentration in different tissues varies according to the balance of DIO2 and DIO3 (which inactivates T4 or T3). DIO2 expression in humans has been localized exclusively in the endometrial stroma throughout the menstrual cycle (Allan et al., 2003). The up-regulation of DIO2 by mifepristone, confirmed by real-time PCR, is the first report indicating progesterone regulation of DIO2 in the human endometrium. This would be expected to increase local levels of T3 available for receptor binding. Non-genomic actions of thyroid hormones have been described and thyroid hormone has been demonstrated to have a pro-angiogenic effect, initiated at the plasma membrane by interaction with integrin αvβ3 (Mousa et al., 2005). In rats, both T3 and GH are required for the full decidual response of the endometrium to an implantation stimulus (Kennedy and Doktorcik, 1988). DIO2 in the subepithelial stromal cells may be required for generating the appropriate levels of intracellular T3 required to coordinate the uterine decidual response to implantation.

The genomic actions of thyroid hormone are mediated by the receptors THRA and THRb. Although alternative splicing generates nine mRNA isoforms, only THRα1, α2, β1 and β2 are known to be expressed as proteins in vivo. Real-time RT–PCR showed that THRα1 and α2 were down-regulated and THRβ1 up-regulated by mifepristone, indicating regulation by PR. These transcripts also showed evidence of steroidal regulation during the menstrual cycle, with levels at their highest during the secretory phase. Using immunohistochemistry, THRα1 and THRβ1 expression has been reported localized in the stroma, luminal and glandular epithelium, increasing in the glandular and luminal epithelium in mid-secretory phase (Aghajanova et al., 2005). The protein expression data therefore correlate well with our real-time PCR. We also found many of the co-regulatory proteins that modulate the functions of DNA-bound THRαs were expressed in endometrium. Although mifepristone administration did not affect their expression, they may be involved in regulating thyroid receptor signalling in the endometrium. More surprisingly, we found expression of factors required for thyroid hormone synthesis in the endometrium including SLCT5A, a sodium iodide symporter; P4HB, a TG molecular chaperone and TG itself, which undergoes iodination and cleavage to produce the prohormone T4. Real-time PCR analysis of TG expression through the menstrual cycle revealed a significant increase in the secretory phase and down-regulation by mifepristone, suggesting that expression of TG is stimulated by progesterone. TG mRNA and immunoreactivity have been reported in tissues other than the thyroid gland, including the kidney and lymphocytes (Sellitti et al., 2000; Bugalho et al., 2001). However, we were unable to detect TG protein by immunohistochemistry or immunoblotting in either proliferative or secretory phase endometrium (data not shown). We were therefore not able to confirm the potential for local synthesis of T4 in non-pregnant endometrium in this study. Uptake of iodine and coupling to TG in the thyroid is controlled by thyroid secreting hormone (TSH) through activation of its receptor (Haraguchi et al., 2003). The existence of TSHR in extrathyroidal tissues has been reported, although their functional role has not been clarified (Haraguchi et al., 1996). The presence of TSHR transcripts that we found in the endometrium may underpin the abnormalities of menstrual bleeding and subfertility common in women with primary hypothyroidism, where systemic TSH is elevated and hypothesized to be acting directly on the uterus (Poppe and Velkensiers, 2004). Taken together, our expression data suggest that the molecular components necessary to synthesise and metabolise thyroid hormone are
expressed in the endometrium, together with THRs and the associated receptor modifiers.

Thyroid dysfunction is a condition known to reduce the likelihood of pregnancy, but is currently viewed in terms of effects on hypothalamic, pituitary or ovarian function by interfering with normal GnRH and LH secretion, FSH function and estrogen metabolism (reviewed in Poppe and Velkeniers, 2004). Hypothyroidism diminishes the growth response of all major uterine cell types to estrogen (Gardner et al., 1978) and hypothyroid women exhibit a number of reproductive disorders, including menstrual cycle aberration (often heavy and/ or irregular menses) and infertility. Thyroid hormone-deficient rats have abnormal uterus, with reduced endometrial thickness (Inuwa and Williams, 1996). Hyperthyroidism is associated with menstrual irregularities, although most hyperthyroid women remain ovulatory. The presence of antithyroid antibodies, specifically against TG and TPO has been significantly associated with pregnancy loss and poor IVF outcome (Bussen et al., 2000). Our results suggest the possibility that these effects could be due in part to the fact that T4 metabolism and signalling in the endometrium are important during the preparation for implantation and also in the endometrial response to progesterone withdrawal.

Several previous studies have employed microarrays to identify gene expression changes in endometrium in response to progesterone in vivo (Kao et al., 2002; Borthwick et al., 2003; Riesewijk et al. 2003; Ponnampalam et al 2004; Talbi et al., 2006). However, most have focused on transcripts which alter during the transition from the proliferative or early secretory phases (when oestrogen dominates) to the mid-secretory phase when the endometrium differentiates and becomes receptive in response to progesterone. Of these studies, only two appear comparable to ours. Both identified transcripts that alter between the mid- and late-secretory stages of natural cycles (Critchley et al., 2006; Talbi et al., 2006). Many of these changes are likely to be due to the natural decline in progesterone that occurs about this time, but there is inevitably heterogeneity in timing and levels of hormone between subjects. This makes it difficult to study the early events initiated by progesterone withdrawal. The use of timed biopsies after pharmacological progesterone withdrawal in our study has now allowed us to examine in detail the time-course of these early responses. Comparison of these three studies will allow identification of a subset of common genes for which our mifepristone administration data provide strong evidence of direct regulation by the PR in vivo. The detailed information we have now provided about the early events in response to progesterone withdrawal will be useful in generating a detailed understanding of the pathways by which this leads to menstruation and is likely to provide new avenues to diagnose and treat menstrual bleeding disorders.

**Supplementary Data**

Supplementary data are available at http://molehr.oxfordjournals.org

**Acknowledgements**

We would like to thank all the patients and staff at the participating hospitals, for their assistance, without which this study could not have taken place. This study was supported by the Rockefeller/World Health Organization initiative on implantation and the Medical Research Council, UK (programme grant No: G9623012 and MRC/DFID funded Special Project Grant No: G9523250). A.M.S. was supported by the Meres Research Studentship from St. John’s College, Cambridge. A clinical fellowship grant to O.H. was provided by the Finnish Medical Foundation.

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Submitted on January 9, 2007; resubmitted on March 6, 2007; accepted on March 8, 2007