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## Microarray profiling of progesterone-regulated endometrial genes during the rhesus monkey secretory phase

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Published: 07 July 2004

Received: 26 May 2004

*Reproductive Biology and Endocrinology* 2004, 2:54 doi:10.1186/1477-7827-2-54

Accepted: 07 July 2004

This article is available from: <http://www.rbej.com/content/2/1/54>

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### Abstract

**Background:** In the endometrium the steroid hormone progesterone (P), acting through its nuclear receptors, regulates the expression of specific target genes and gene networks required for endometrial maturation. Proper endometrial maturation is considered a requirement for embryo implantation. Endometrial receptivity is a complex process that is spatially and temporally restricted and the identity of genes that regulate receptivity has been pursued by a number of investigators.

**Methods:** In this study we have used high density oligonucleotide microarrays to screen for changes in mRNA transcript levels between normal proliferative and adequate secretory phases in Rhesus monkey artificial menstrual cycles. Biotinylated cRNA was prepared from day 13 and days 21–23 of the reproductive cycle and transcript levels were compared by hybridization to Affymetrix HG-U95A arrays.

**Results:** Of ~12,000 genes profiled, we identified 108 genes that were significantly regulated during the shift from a proliferative to an adequate secretory endometrium. Of these genes, 39 were up-regulated at days 21–23 versus day 13, and 69 were down-regulated. Genes up-regulated in P-dominant tissue included: secretoglobin (uteroglobin), histone 2A, polo-like kinase (PLK), spermidine/spermine acetyltransferase 2 (SAT2), secretory leukocyte protease inhibitor (SLPI) and metallothionein 1G (MT1G), all of which have been previously documented as elevated in the Rhesus monkey or human endometrium during the secretory phase. Genes down-regulated included: transforming growth factor beta-induced (TGFB1 or BIGH3), matrix metalloproteinase 11 (stromelysin 3), proenkephalin (PENK), cysteine/glycine-rich protein 2 (CSRP2), collagen type VII alpha 1 (COL7A1), secreted frizzled-related protein 4 (SFRP4), progesterone receptor membrane component 1 (PGRMC1), chemokine (C-X-C) ligand 12 (CXCL12) and biglycan (BGN). In addition, many novel/unknown genes were also identified. Validation of array data was performed by semi-quantitative RT-PCR of two selected up-regulated genes using temporal (cycle day specific) endometrial cDNA populations. This approach confirmed up-regulation of WAP four-disulfide core domain 2 (WFDC2) and SLPI during the expected window of receptivity.

**Conclusion:** The identification of P-regulated genes and gene pathways in the primate endometrium is expected to be an important first step in elucidating the cellular processes necessary for the development of a receptive environment for implantation.

## Background

The endometrium is a complex, specialized organ that plays critical roles in menstruation, embryo implantation and pregnancy in women. Normal human endometrium exhibits a 28 day cycle which is strictly controlled by the changing pattern of the ovarian sex steroids estrogen (E) and progesterone (P). Endometrial growth, differentiation, shedding and reconstruction are expected to be regulated by hormone-induced changes in gene expression during the menstrual cycle. It is the sequential secretion of E and P that leads to orderly regulation of signal transduction pathways invoking a wide variety of genes including ligands, receptors, cytokines, growth factors, extra-cellular matrix proteins and enzymes involved in cellular metabolism [1-5]. The action of these genes causes a shift from a proliferative to a secretory (differentiated) state that is accompanied by massive metabolic changes and structural remodelling necessary for correct maturation of a 'receptive' endometrium that can allow embryo implantation [1,2,6,7]. In humans the luminal endometrial epithelium becomes receptive due to the presence of P after E priming and the implantation window begins 4-5 days and ends 9-10 days after exposure to P, limiting this period to days 19-24 of the menstrual cycle [8]. The precise molecular mechanisms that act to achieve temporal and spatial control of gene expression in the primate endometrium to attain a state of receptivity (window of implantation) are largely unknown. P action has, however, been shown to be essential for proper endometrial maturation and the maintenance of pregnancy and these effects of P are expected to be mediated primarily through its cognate receptor (PR) [9,10].

One of the major objectives of our laboratory is the identification of P-regulated genes in Rhesus monkey endometrium and their potential function in endometrial maturation. Previous studies in humans [11-14] and other animal models [15-17] have identified several factors that may be implicated in endometrial maturation and receptivity. It is apparent that no single factor is responsible for preparation of the endometrium for receptivity, but more likely that controlled expression of many genes in a temporal fashion eventually leads to a proper uterine environment for implantation.

Recent technological advances in DNA microarrays has allowed the simultaneous monitoring of expression of thousands of genes in one experiment, facilitating a global view of molecular events during physiological functions and cellular processes. Prior to the publication of four recent papers [11,18-20] studies on human and primate endometrial gene expression during the menstrual cycle were primarily limited to individual genes. Correct maturation of the endometrium necessary for receptivity of the developing blastocyst and subsequent pregnancy is a pre-

cisely coordinated multi-step process involving many factors that requires regulated expression of large sets of genes. To date there is limited evidence for genetic markers that describe/predict a receptive endometrium capable of embryo implantation in the human and non-human primate.

We have previously described the construction of temporal (cycle day specific) endometrial cDNA populations from Rhesus monkey artificial menstrual cycles [21]. The current study used Affymetrix HG-U95A microarrays to simultaneously compare the expression of ~12000 genes between the E-dominant proliferative phase (day 13) and the P-dominant secretory phase (window of receptivity, days 21-23) in the Rhesus monkey. The use of human gene chips for non-human primate cross-species hybridization has been validated due to the high conservation of gene sequences between the species [22]. Our results show that the transition from an E-dominant to a P-dominant (receptive) endometrium involves both P-dependent up-regulation and down-regulation of genes.

## Methods

### **Animal Model – Rhesus Monkey**

The development and use of artificial menstrual cycles in the Rhesus monkey was first described by Hodgen [23]. These studies showed that simulation of the menstrual cycle by the timed insertion and removal of silastic implants of E or P was sufficient to allow the endometrium to support implantation and eventual delivery (IVF and surrogate transfer). Our previously published studies [24-27] describe in detail the protocols for creation of adequate cycles. All protocols used in these studies were approved by the Institutional Animal Care and Use Committee.

### **Tissue Collection and RNA Preparation**

Tissue was collected by endometriectomy at hysterotomy as previously described [26,28]. Tissue harvesting was performed prior to insertion or removal of implants from 3 individual animals for each of days 13, 21, and 23 of the menstrual cycle, and stored at -80 °C until further processing. Total RNA was isolated and poly(A)<sup>+</sup>-enriched RNA was prepared using oligo d(T) spin columns. The integrity of all total mRNA samples was examined by visual inspection of intact 28S and 18S ribosomal RNA bands on agarose gels prior to further processing. Samples were treated with DNase I to ensure the removal of traces of genomic DNA.

### **Synthesis and amplification of cDNA populations**

One microgram of poly(A)<sup>+</sup> RNA from three animals (n = 3) for each time point (days 13, 21, and 23) was pooled just prior to cDNA synthesis (a total of 3 µg per time point). The Superscript™ Choice System (Life

Technologies, Rockville, MD), was used for cDNA synthesis using the same procedure as previously described [21].

### Microarray hybridization

For probe preparation, 2 micrograms of day 13 cDNA and one microgram each of days 21 and 23 cDNA (pooled) were converted into biotinylated cRNA by *in vitro* transcription (IVT) using the ENZO BioArray High Yield RNA T7 Transcription Labelling Kit (ENZO, Farmingdale, NY). After a 37°C incubation step for 6 h, the labelled cRNA was cleaned up by phenol/chloroform extraction and ethanol precipitation, and quantitated spectrophotometrically by absorbance at 260 nm. Typically, each IVT reaction yielded ~50 µg. A total of five probe sets for both day 13 and days 21–23 were prepared (N = 5) for hybridization to Affymetrix Human Genome U95A oligonucleotide microarrays (Affymetrix, Santa Clara, CA), corresponding to 12,686 human genes and expressed sequence tags (EST's). 15 micrograms of cRNA was chemically fragmented in 5X fragmentation buffer (200 mM Tris, pH 8.1; 500 mM KOAc; 150 mM MgOAc) and was added to a hybridization cocktail containing 50, 1.5, 5.0, 25 and 100 pM respectively of prokaryotic gene oligonucleotide hybridization controls B2, *bioB*, *bioC*, *bioD* and *cre* (biotin synthesis pathway genes of *E.coli*, [Affymetrix, Santa Clara, CA]), 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 100 mM MES, 1 M [Na<sup>+</sup>], 20 mM EDTA and 0.01% Tween. Arrays were hybridized for 16 h on an Affymetrix GeneChip fluidics station at the University of Massachusetts Medical School Genomics Core Facility. Washing, fluorescent staining and scanning for data generation for subsequent analysis were conducted in the Genomics Core Facility at UMass Medical School.

### Statistical analysis

Analysis of gene expression data from Affymetrix GeneChip® experiments was performed at the Bioinformatics facility at UMass Medical School, in a two step process:

1. In the first step, model based expression estimates were made using the method of Li and Wong [29]. These estimates were expressed as logs with base 2.
2. In the second step, differentially expressed genes were identified by performing Analysis of Variance (ANOVA) for mixed models [30] on all genes with the series of microarrays as a random factor and with treatment as a fixed factor.

The genes were then sorted in ascending order by the p-value of the treatment effect from the ANOVA. The subset of differentially expressed genes were those genes with a p-value less than or equal to the p-value for a gene with a False Discovery Rate (FDR) [31] of 5% (analogous to a significance level of 0.05). Estimates of the effects expressed

as fold changes were made by taking the antilog base 2 of the treatment effect estimates from the Mixed Model ANOVA. Model based expression estimates based on the Li and Wong method were made using the dChip software package [29]. Analysis of Variance for Mixed Models were made using PROC MIXED in the SAS Statistical Analysis Software Package [32-34]. Post ANOVA analyses including analysis for FDR and fold change were made using Microsoft Excel. Genes that are differentially expressed >2-fold with an FDR <5% are listed in Tables 1 and 2.

### Semi-quantitative RT-PCR

Specific amplimers were designed for SLPI and WFDC2 (see below) and used in PCR reactions with six endometrial cycle day-specific cDNA populations (day 13, 14, 17, 21, 23, and 26)[21] as template to confirm their expression patterns. 40 ng of template were analyzed in 100 µl reactions containing 0.5 µM fragment-specific primers, 0.25 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 1x buffer and 2 units taq polymerase in a thermal cycler (94°C, 1 minute; 48–55°C, 1 minute; 72°C, 2 minutes). PCR reactions were done for 24–45 cycles to determine the linear range of amplification for each primer set. Products were analyzed by agarose gel electrophoresis and comparative evaluation was performed by densitometric analysis (QuantiScan, Biosoft Inc.) of photographed gels. Amplimer sequences were as follows:

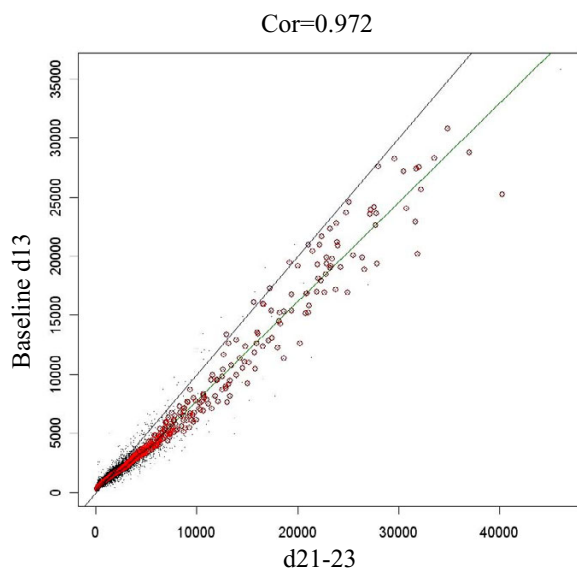
SLPI: 5'-AGCTTAACGAGGGAGGAAGC-3' & 5'-AAAG-GACCTGGACCACACAG-3' (30 cycles); WFDC2: 5'-CGGCTTCACCCTAGTCTCAG & 5'-ATTTCATCT-GGCCAGGACAC (25 cycles).

## Results

### Microarray hybridization data analysis

A common problem when assaying the expression of thousands of transcripts in complex organs is biological and experimental variability. Variation in gene expression can arise not only from cellular heterogeneity of individual samples and genetic differences among the population, but also by mechanisms independent of genetics under identical conditions (experimental variation). In order to reduce such variability encountered in microarray analysis, cRNA from three Rhesus monkeys in the proliferative phase (day 13; n = 3) and six monkeys in secretory phase (day 21; n = 3 and 23; n = 3) were separately pooled and used as probes on human U95 oligonucleotide arrays and a total of five hybridizations were performed for both probe sets (n = 5).

The data were analyzed as detailed in *Methods* and a minimal change of 2-fold was adopted to select up- and down-regulated genes. As can be seen in the scatter plot of normalized data (Figure 1), the vast majority of genes displayed <2-fold change of expression between proliferative



**Figure 1**  
**Normalization plot of microarray data** Normalization plot from a representative analysis of changes in gene expression between day 13 (proliferative, y axis) and days 21–23 (secretory, x axis) endometrium. The blue line is the diagonal  $y = x$ , the red circles are the probes selected in the "invariant set" and the green curve indicates the running median normalization curve based on the "invariant set".

and secretory phases. Many of these genes have previously been shown to exhibit little or no differential expression during the menstrual cycle. Using a FDR/P value of <5.0% to identify statistical significance between the two groups of data, 39 genes exhibited up-regulation and 69 genes exhibited down-regulation during the shift from a proliferative to secretory endometrium.

Tables 1 and 2 list the genes that we found up- or down-regulated respectively in the secretory phase. Their potential endometrial function and associated biological pathway were assessed by searches of LocusLink (NCBI), NETAFFX (Affymetrix), KEGG and BioCarta databases and gene ontology/annotation tools. Of the significantly regulated genes, we identified six genes previously shown by our laboratory and other investigators (see table 1 for references) to be up-regulated directly or indirectly by progesterone in human secretory phase endometrium: secretoglobin (uteroglobin), histone 2A, polo-like kinase (PLK), spermidine/spermine acetyltransferase 2 (SAT2), secretory leukocyte protease inhibitor (SLPI) and metallothionein 1G (MT1G). We also identified nine genes previously shown (see table 2 for references) to be down-regulated in secretory versus proliferative phase human

endometrium: transforming growth factor beta-induced (TGFB1 or BIGH3), matrix metalloproteinase 11 (stromelysin 3), proenkephalin (PENK), cysteine/glycine-rich protein 2 (CSRP2), collagen type VII alpha 1 (COL7A1), secreted frizzled-related protein 4 (SFRP4), progesterone receptor membrane component 1 (PGRMC1), chemokine (C-X-C) ligand 12 (CXCL12) and biglycan (BGN).

#### Confirmation of microarray data by RT-PCR

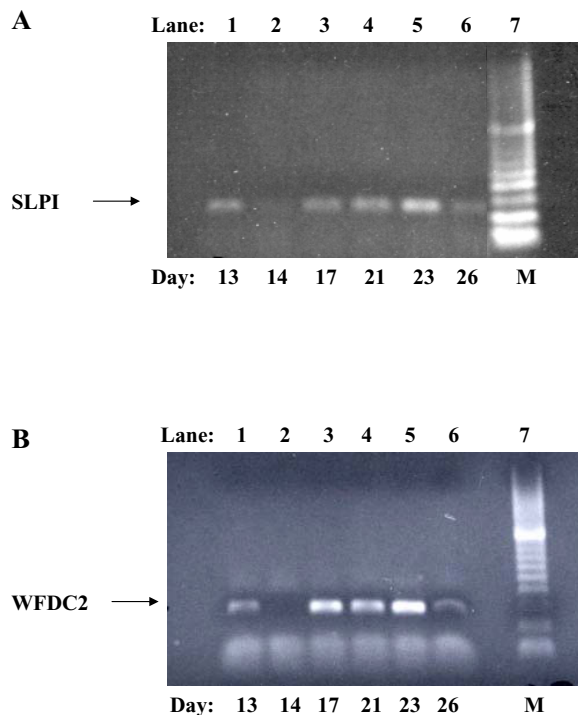
We have previously shown by differential display and RT-PCR that one of the genes up-regulated during the secretory phase in the microarray analysis, SLPI, was expressed in a temporally restricted manner during the expected window of implantation (days 17–23) [21]. We have expanded these data to include temporal endometrial cDNA populations from days 13–26 and further confirm the elevated expression of SLPI during days 21–23 compared to day 13 (Figure 2). Another gene that was highly up-regulated (27.5-fold) in the microarrays, WAP four-disulfide core domain 2 (WFDC2), is also a member of the same family as SLPI (Whey Acidic Proteins). We selected this gene in addition for semi-quantitative RT-PCR analysis using our temporal endometrial cDNA populations as template for validation of the microarray procedure. Figure 2 shows that indeed, WFDC2 expression is predominantly up-regulated between day 13 and days 17–23 of the secretory phase. These results are consistent with the microarray data and demonstrate the temporal expression of the above two genes, i.e. coincident and restricted to the window of implantation.

#### Discussion

Advances in gene expression profiling aided by high throughput microarray screening of thousands of genes has allowed a relatively unbiased approach toward identifying genes, gene families and signalling pathways that are candidates for endometrial receptivity. The availability of this technology makes it possible to investigate global changes in gene expression that accompany the transition from a proliferative to secretory endometrium and facilitates the identification of molecular mechanisms underlying implantation and the expression signature of genes during the window of receptivity. These studies provide an avenue for design of diagnostic tests for endometrial disorders and for targeted drug discovery for the treatment of implantation-based infertility and endometrial-based contraception. In these studies we have used microarray gene expression profiling to identify genes/gene networks that are differentially expressed in the proliferative versus secretory endometrium of the Rhesus monkey.

#### Genes up-regulated during secretory phase

Secretory leukocyte protease inhibitor (SLPI): Our laboratory has previously shown by differential display and RT-PCR that SLPI was expressed in a temporally restricted



**Figure 2**  
**RT-PCR of selected genes** Expression of selected gene fragments SLPI (218 bp, panel A) and WFDC2 (266 bp, panel B) in temporal cDNA populations of Rhesus endometrium by semi-quantitative RT-PCR. cDNA Populations used in the microarray analysis were day 13 (proliferative phase, lane 1) and days 21–23 (secretory phase, lanes 4 and 5). Lane 7 is 100 bp marker (M).

manner in Rhesus endometrium during the expected window of implantation [21], in agreement with its up-regulation during the secretory phase described in this microarray study. SLPI is a neutrophil elastase inhibitor which also has antibacterial and anti-inflammatory properties. King et al [35] showed that endometrial expression was menstrual cycle dependent with increased secretion in the secretory phase and the main site of SLPI synthesis in endometrium and decidua was found to be the glandular epithelium. Furthermore, progesterone treatment increases expression of SLPI mRNA and protein and this up-regulation is attenuated in the presence of the anti-progestogen, RU486 [36]. An antibiotic role for SLPI in the endometrium and decidua during implantation and pregnancy would be consistent with the expression profile and localization of SLPI. This is in agreement with a previous microarray profiling study in humans where SLPI expression increased in secretory endometrium and par-

ticularly in receptive (day LH+7) compared to pre-receptive (day LH+2) endometria [19]. SLPI has also been shown to be involved in the control of tissue remodelling (epithelialization) and wound healing pathways in SLPI-null mice [37,38]. Because increased SLPI expression coincides with the window of endometrial receptivity it has been postulated that it may have a role in implantation, although the existence of viable SLPI-null mice suggests it is not required for fertility in mice. SLPI belongs to a family of 14 WAP proteins (Whey Acidic Proteins) that have duplicated on chromosome 20 over evolution [39]. Interestingly, one gene that was highly up-regulated (27.5-fold) in the microarrays, WAP four-disulfide core domain 2 (WFDC2), is also a member of the SLPI family of secretory proteins and its expression profile was confirmed by RT-PCR in the current study. Although there is little literature on WFDC2, it is interesting to speculate that one or more of these uncharacterized family members could provide functional redundancy regarding endometrial development in the female reproductive tract. Our data on SLPI and WFDC2 (figure 2) confirm the microarray analysis and provide an important avenue for future studies on their potentially important gene family in endometrial function.

**Secretoglobin/uteroglobin:** Uteroglobin is a progesterone binding protein, a member of the antiinflammin gene family and possibly a novel cytokine. Initially, uteroglobin was identified as the major protein of rabbit uterine secretion during the phase of preimplantation. Muller-Schottle et al [40] show that secretory uteroglobin is found in endometrial tissue homogenates in highest levels of expression during the early luteal phase (days 15–19). In turn, uteroglobin is released into the uterine lumen in peak amounts during the receptive phase of the menstrual cycle (mid-luteal phase, days 20–23). Immunohistochemical studies match with these results, as uteroglobin is located during the early and mid-luteal phase in the apical compartments of endometrial gland cells. These observations strongly suggest an involvement of uteroglobin in endometrial preparations for implantation. While uteroglobin appears as one of the most extensively studied proteins, particularly its physico-chemical properties, including its crystal structure and its gene, the true physiological role of this protein still remains to be unravelled [40].

**Histone 2A:** Beier-Hellwig et al [41] identified several individual proteins that appear as characteristic markers for the receptive stage of the luteal phase, including histone H2A. In order to identify the endocrine dependency of the proteins which significantly contribute to the "receptive stage pattern," patients were treated with the progesterone antagonist RU 486 at day LH +2. The assessment 4 days later revealed diminished and missing bands

of the H2A-, H2B-, and H3-histones. These results demonstrate progesterone-dependent components of the endometrium at the receptive stage, which can be used as useful markers for an improved precision in luteal phase diagnostics.

**Polo-like kinase:** The enzyme, polo-like kinase (PLK), is a mammalian serine/threonine kinase involved in cell cycle regulation. Takai et al [42] showed that PLK staining was detected in the basement membrane of many endometrial glands, stromal cells, and some endothelial cells. The number of PLK-positive endometrial gland cells was significantly higher in the late proliferative phase and the early secretory phase than in the early proliferative phase or the late secretory phase. PLK expression seemed to be correlated with the expression of Ki-67 and proliferating cell nuclear antigen (PCNA) in many endometrial glands and stromal cells particularly in the late proliferative phase, reflecting a role of PLK in cellular proliferation. Nevertheless, in the early secretory phase, at which point the expression of Ki-67 and PCNA decreased in endometrial glands, PLK was strongly expressed. This finding suggests that PLK may have some post-mitotic functions in certain specialized cell types. Although the highest expression of PLK was observed in the late proliferative and the early secretory phases, the expression drastically decreased in the late secretory phase. These findings, taken together, indicate that the expression of PLK in normal endometrium fluctuates over the course of the menstrual cycle, suggesting in turn that PLK is associated with hormone-dependent cellular proliferation.

**Spermidine/spermine N<sup>1</sup>-acetyltransferase:** Using the mRNA differential display methodology, Green et al [43] identified spermidine/spermine N<sup>1</sup>-acetyltransferase (SSAT or SAT) as a porcine endometrial gene whose expression is maximal at peri-implantation and which is induced by conceptus-derived factors and by progesterone. SSAT, by acetylating the naturally occurring polyamines (PA) spermine and spermidine, typically functions as a cell growth inhibitor. SSAT, through its control of intracellular PA levels, likely plays a modulatory role in the establishment of an optimal uterine environment for successful embryo attachment [44]. SSAT was also up-regulated in the secretory phase in an independent microarray study [20].

#### **Genes down-regulated during the secretory phase**

**Betaig-H3 (BIGH3 or TGFBI):** TGFBI is a transforming growth factor  $\beta$ -induced extracellular matrix protein with cell adhesion-promoting activity [45]. Carson et al [18] found that TGFBI expression decreased by 92% by the microarray analysis and >80% by Northern blot analysis during the early to mid-luteal transition. This is in agreement with our results that show a large decrease in expres-

sion during the secretory phase (24-fold). In a second microarray study, Borthwick et al [20] also found that BIGH3 was down regulated in secretory phase endometrium.

**Matrix metalloproteinase 11 (stromelysin 3 or MMP-11):** Matrix metalloproteinases are a highly regulated family of enzymes that together can degrade most components of the extracellular matrix. Using in situ hybridization, Rodgers et al [46] show that transcripts for stromelysin-2 were only detected in late secretory and menstrual endometrium, while those for matrilysin and stromelysin-3 were consistently detected in proliferative endometrium. In addition, endometrial expression of matrix metalloproteinase (MMP)-3, MMP-7 and MMP-11 occurs during menstrual breakdown and subsequent estrogen-mediated growth, but not during the secretory phase, and these enzymes are suppressed by progesterone treatment [47]. These data indicate that matrix metalloproteinases are expressed in cell-type, tissue, and reproductive cycle-specific patterns, consistent with regulation by steroid hormones, and with specific roles in the complex tissue growth and remodeling processes occurring in the endometrium during the reproductive cycle. In two other microarray studies expression of stromelysin 3 was also found to be decreased in the secretory phase [18,20].

**Proenkephalin (PENK):** Using Northern blots Low et al [48] detected a strong band of proenkephalin mRNA of 1.3 kilobases almost exclusively in the proliferative endometrium from monkeys in the follicular phase of the cycle. No proenkephalin mRNA was detected in secretory endometrium obtained from monkeys in the luteal phase. Furthermore, proenkephalin mRNA was regulated by 17 beta-estradiol in the endometrium and this effect of estradiol was antagonized by progesterone. Borthwick et al [20] and Carson et al [18] also found decreased expression of PENK during the secretory phase using microarray analysis.

**Secreted frizzled-related protein 4 (SFRP4 or FrpHE):** Microarray analysis has previously shown that FrpHE is down-regulated during the window of implantation [11,18,20]. FrpHE is an inhibitor of Wnt signaling [49] and FrpHE inhibits Wnt action by competitive binding to Wnt ligand(s). Wnt7A (-/-) null mice are infertile and have complete absence of uterine glands and a reduction in mesenchymally derived uterine stroma [50]. It is possible that the Wnt family may play a role in epithelial-embryo and/or epithelial-stromal interactions and thus in uterine receptivity. By in situ hybridization, Abu-Jawdeh et al [51] have determined that frpHE is expressed by mesenchymal cells but not by epithelial cells. The expression of frpHE is modulated during the endometrial cycle: it is expressed in the stroma of proliferative endometrium and

not significantly detectable in secretory or menstrual endometrium, suggesting that frpHE is under hormonal regulation. The data also indicates that frpHE functions as a regulator of the Wnt-frizzled signaling pathway and is involved in endometrial physiology. In an independent study, FrpHE expression was detected in stromal cells, with no evidence of expression by glandular cells. The pattern of expression was dependent on the phase of the menstrual cycle, with abundant expression in proliferative endometrium, whereas secretory endometrium showed little or no signal, consistent with RT-PCR and quantitative PCR data [52].

Other genes that were down-regulated in the secretory phase in this study and in independent microarray analyses by other investigators were: cysteine/glycine-rich protein 2 (CSRP2) [11,19]; collagen type VII alpha 1 (COL7A1) [18]; progesterone receptor membrane component 1 (PGRMC1 or Hpr6) [11,53]; chemokine C-X-C ligand 12 (CXCL12) [18], and biglycan (BGN) [11].

Although gene microarrays have the potential to shed light on cellular processes by identifying groups of genes that appear to be differentially expressed, this "function by association" approach has potential caveats in the view of some investigators [54]. Some genes known to be involved in a particular pathway can be missed whereas other apparently unrelated genes exhibit expression profiles that are strikingly similar to known pathways. Also, there is little consensus about how to interpret the gene expression patterns of hypothetical genes, genes of unknown function or transcripts identified only by expressed sequence tags. Because statistical significance does not always correlate with biological significance, we have focused our attention on highly up- and down-regulated genes whose expression profiles are consistent with our previous studies and the literature and/or are known components of cellular pathways. When identified genes can be assigned to specific pathways, we can subsequently study the expression profiles of functionally related genes by PCR and other techniques in our cDNA populations.

## Conclusions

The observation that more genes are down-regulated in secretory endometrium than up-regulated has been noted previously by Kao et al [11]. It is speculated that this is due to a combination of both loss of E action (E up-regulated genes in the proliferative phase) and direct or indirect down regulation of genes by P in the secretory phase. Although there is a degree of overlap in differentially expressed genes between different microarray data sets in the literature, they are by no means similar. Some genes expected to change in expression from a proliferative to secretory endometrium were not found to change in our analysis. A direct comparison between different studies is

difficult for several reasons. Not only are there disparities in study designs, but also different software and statistics were used to analyze the hybridization data. Also, because the endometrium is a complex organ composed of many compartments and cell types, gene products expressed in multiple cell types may be differentially regulated at levels that are undetectable in whole tissue analysis. In future studies, we propose to address this potential shortcoming by focusing on each cell type separately using laser capture microdissection prior to mRNA isolation as previously described by our laboratory [55].

This unbiased quantitative analysis of a large number of genes during endometrial development has provided results that agree with previous findings and has also identified several novel genes not previously known to be expressed in the endometrium or to be steroid responsive. Interpretation of these data with regard to their precise functional roles in endometrial maturation must await future studies.

## Additional material

### Additional file 1

Table 1. Genes up-regulated during the secretory phase

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1477-7827-2-54-S1.doc>]

### Additional file 2

Table 2. Genes down-regulated during the secretory phase

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1477-7827-2-54-S2.doc>]

## Acknowledgements

The authors thank Phyllis Spatrick for Affymetrics GeneChip® processing, and Stephen Baker and David Lapointe for statistical analysis of microarray data (Bioinformatics/Genomics Core Facility, UMMS, supported in part by the Diabetes Endocrinology Research Center, DK-32520, NIH). This work was supported in part by NICHD grant HD 31620 (WCO).

## References

1. Bartelmez GW, Corner GW, Hartman CG: **Cyclic changes in the endometrium of the rhesus monkey (*Macaca mulatta*)**. *Contrib Embryol* 1951, **34**:99-144.
2. Ferenczy A, Bergeron C: **Histology of the human endometrium: From birth to senescence**. *Ann NY Acad Sci* 1991, **622**:6-27.
3. Gurpide E, Tseng L: **Induction of human endometrial oestradiol dehydrogenase by progestins**. *Endocrinology* 1975, **97**:825-833.
4. Clarke CL, Sutherland RL: **Progesterone regulation of cellular proliferation**. *Endocr Rev* 1990, **11**:266-301.
5. Fay TN, Grudzinskas JG: **Human endometrial peptides: A review of their potential role in implantation and placentation**. *Hum Reprod* 1991, **6**:1311-1326.
6. Bartelmez GW: **The phases of the menstrual cycle and their interpretation in terms of the pregnancy cycle**. *Am J Obstet Gynecol* 1957, **74**:931-955.

7. Maslar IA: **The progesterational endometrium.** *Sem Reprod Endo* 1988, **6**:115-128.
8. Navot D, Bergh PA, Williams M, Garrisi GJ, Guzman I, Sandler B, Fox J, Schreiner-Engel P, Hofmann GE, Grunfeld L: **An insight into early reproductive processes through the in vivo model of ovum donation.** *J Clin Endocrinol Metab* 1991, **72**:408-414.
9. Savouret JF, Muchardt C, Quesne M, Mantel A, De The H, Milgrom E: **Regulation of the progesterone receptor functions.** *Annals of the New York Academy of Sciences* 1998, **839**:138-142.
10. Conneely OM, Lydon JP: **Progesterone receptors in reproduction: functional impact of the A and B isoforms.** *Steroids* 2000, **65**:571-577.
11. Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor RN, Lessey BA, Giudice LC: **Global gene profiling in human endometrium during the window of implantation.** *Endocrinology* 2002, **143**:2119-2138.
12. Salamonsen LA, Nie G, Findlay JK: **Newly identified endometrial genes of importance for implantation.** *J Reprod Immunol* 2002, **53**:215-225.
13. Lessey BA: **Adhesion molecules and implantation.** *J Reprod Immunol* 2002, **55**:101-112.
14. Martin J, Dominguez F, Avila S, Castrillo JL, Remohi J, Pellicer A, Simon C: **Human endometrial receptivity: gene regulation.** *J Reprod Immunol* 2002, **55**:131-139.
15. Paria BC, Reese J, Das SK, Dey SK: **Deciphering the cross-talk of implantation: advances and challenges.** *Science* 2002, **296**:2185-2188.
16. Puri CP, Katkam RR, Sachdeva G, Patil V, Manjramkar DD, Kholkute SD: **Endometrial contraception: modulation of molecular determinants of uterine receptivity.** *Steroids* 2000, **65**:783-794.
17. Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K: **Embryo implantation.** *Dev Biol* 2000, **223**:217-237.
18. Carson DD, Lagow E, Thathiah A, Al-Shami R, Farach-Carson MC, Vermon M, Yuan L, Fritz M, Lessey B: **Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening.** *Mol Hum Reprod* 2002, **8**:871-879.
19. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S, Simon C: **Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology.** *Mol Hum Reprod* 2003, **9**:253-264.
20. Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirney R, Phillips SC, Smith SK: **Determination of the transcript profile of human endometrium.** *Mol Hum Reprod* 2003, **9**:19-33.
21. Okulicz WC, Ace CI: **Temporal regulation of gene expression during the expected window of receptivity in the Rhesus monkey endometrium.** *Biology of Reproduction* 2003, **69**:1593-1599.
22. Chismar JD, Mondala T, Fox HS, Roberts E, Langford D, Masliah E, Salomon DR, Head SR: **Analysis of result variability from high-density oligonucleotide arrays comparing same-species and cross-species hybridizations.** *BioTechniques* 2002, **33**:516-520, 522.
23. Hodgen GD: **Surrogate embryo transfer combined with estrogen-progesterone therapy in monkeys. Implantation, gestation, and delivery without ovaries.** *JAMA* 1983, **250**:2167-2171.
24. Longcope C, Bourget C, Meciak PA, Okulicz WC, McCracken JA, Hoberg LM, Padykula HA: **Estrogen dynamics in the female rhesus monkey.** *Biol Reprod* 1988, **39**:561-565.
25. Okulicz WC, Savasta AM, Hoberg LM, Longcope C: **Biochemical and immunohistochemical analyses of estrogen and progesterone receptors in the rhesus monkey uterus during the proliferative and secretory phases of artificial menstrual cycles.** *Fertil Steril* 1990, **53**:913-920.
26. Okulicz WC, Balsamo M, Tast J: **Progesterone regulation of endometrial estrogen receptor and cell proliferation during the late proliferative and secretory phase in artificial menstrual cycles in the rhesus monkey.** *Biol Reprod* 1993, **49**:24-32.
27. Okulicz WC, Ace CI, Longcope C, Tast J: **Analysis of differential gene regulation in adequate versus inadequate secretory-phase endometrial complementary deoxyribonucleic acid populations from the rhesus monkey.** *Endocrinology* 1996, **137**:4844-4850.
28. Ace CI, Balsamo M, Le LT, Okulicz WC: **Isolation of progesterone-dependent complementary deoxyribonucleic acid fragments from rhesus monkey endometrium by sequential subtractive hybridization and polymerase chain reaction amplification.** *Endocrinology* 1994, **134**:1305-1309.
29. Li C, Wong WH: **Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection.** *Proceedings of the National Academy of Science* 2001, **98**:31-36.
30. McLean RA, Sanders WL, Stroup WW: **A unified approach to mixed linear models.** *American Statistician* 1991, **45**:54-64.
31. Benjamini Y, Hochberg Y: **Controlling the false discovery rate: a practical and powerful approach to multiple testing.** *Journal of the Royal Statistical Society* 1995, **57**:287-300.
32. Littell RC, Milliken GA, Stroup WW, Wolfinger RD: *The SAS system for mixed models.* Carey, N.C., SAS Institute Inc; 1996.
33. **The MIXED Procedure.** SAS Institute, Inc.; 1997:571-702.
34. Littell R, Wolfinger R: **Mixed model analysis of data with the SAS system.** . Walt Disney World Dolphin - Salon V. 8-12-1995.; 1995:1-93.
35. King AE, Critchley HOD, Kelly RW: **Presence of secretory leukocyte protease inhibitor in human endometrium and first trimester decidua suggests an antibacterial protective role.** *Molecular Human Reproduction* 2000, **6**:191-196.
36. King AE, Morgan K, Sallenave JM, Kelly RW: **Differential regulation of secretory leukocyte protease inhibitor and elafin by progesterone.** *Biochem Biophys Res Commun* 2003, **310**:594-599.
37. Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song XY, Wahl SM: **Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing.** *Nature Med* 2000, **6**:1147-1153.
38. Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, Lacomis L, Erdjument-Bromage H, Tempst P, Wright CD, Ding A: **Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair.** *Cell* 2002, **111**:867-878.
39. Clauss A, Lilja H, Lundwall A: **A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein.** *Biochem J* 2002, **368**:233-242.
40. Muller-Schottle F, Classen-Linke I, Alfer J, Krusche C, Beier-Hellwig K, Sterzik K, Beier HM: **Expression of uteroglobin in the human endometrium.** *Mol Hum Reprod* 1999, **5**:1155-1161.
41. Beier-Hellwig K, Sterzik K, Bonn B, Hilmes U, Bygdeman M, Gemzell-Danielsson K, Beier HM: **Hormone regulation and hormone antagonist effects on protein patterns of human endometrial secretion during receptivity.** *Ann N Y Acad Sci* 1994, **734**:143-156.
42. Takai N, Miyazaki T, Miyakawa I, Hamanaka R: **Polo-like kinase expression in normal human endometrium during the menstrual cycle.** *Reprod Fertil Dev* 2000, **12**:59-67.
43. Green ML, Blaesser LL, Simmen FA, Simmen RCM: **Molecular cloning of spermidine/spermine N1-acetyltransferase from the periimplantation porcine uterus by messenger ribonucleic acid differential display: Temporal and conceptus-modulated gene expression.** *Endocrinology* 1996, **137**:5447-5455.
44. Rodriguez-Sallaberry C, Simmen FA, Simmen RCM: **Polyamine- and insulin-like growth factor-I-mediated proliferation of porcine uterine endometrial cells: A potential role for spermidine/spermine N1-acetyltransferase during peri-implantation.** *Biology of Reproduction* 2001, **65**:587-594.
45. Kim JE, Kim SJ, Lee BH, Park RV, Kim KS, Kim IS: **Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, betaig-h3.** *J Biol Chem* 2000, **275**:30907-30915.
46. Rodgers WH, Matrisian LM, Giudice LC, Dsupin B, Cannon P, Svitek C, Gorstein F, Osteen KG: **Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones.** *J Clin Invest* 1994, **94**:946-953.
47. Osteen KG, Keller NR, Feltus FA, Melner MH: **Paracrine regulation of matrix metalloproteinase expression in the normal human endometrium.** *Gynecol Obstet Invest* 1999, **48**:2-8.
48. Low KG, Nielsen CP, West NB, Douglass J, Brenner RM, Maslar IA, Melner MH: **Proenkephalin gene expression in the primate uterus: regulation by estradiol in the endometrium.** *Mol Endocrinol* 1989, **3**:852-857.
49. Nusse R: **Developmental biology. Making head or tail of Dickkopf.** *Nature* 2001, **411**:255-256.
50. Miller C, Pavlova A, Sassoon DA: **Differential expression patterns of Wnt genes in the murine female reproductive tract**



- during development and the estrous cycle. *Mech Dev* 1998, **76**:91-99.
51. Abu-Jawdeh G, Comella N, Tomita Y, Brown LF, Tognazzi K, Sokol SY, Kocher O: **Differential expression of frpHE: a novel human stromal protein of the secreted frizzled gene family, during the endometrial cycle and malignancy.** *Lab Invest* 1999, **79**:439-447.
  52. Tulac S, Nayak NR, Kao LC, Van Waes M, Huang J, Lobo S, Germeyer A, Lessey BA, Taylor RN, Suchanek E, Giudice LC: **Identification, characterization, and regulation of the canonical Wnt signaling pathway in human endometrium.** *J Clin Endocrinol Metab* 2003, **88**:3860-3866.
  53. Krebs CJ, Jarvis ED, Chan J, Lydon JP, Ogawa S, Pfaff DW: **A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors.** *PNAS* 2000, **97**:12816-12821.
  54. Quackenbush J: **Genomics. Microarrays--guilt by association.** *Science* 2003, **302**:240-241.
  55. Torres MST, Ace CI, Okulicz WC: **Application of laser microdissection for analysis of gene expression in the rhesus endometrium.** *Biology of Reproduction* 2002, **In Press**.

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