Delineating the prime mover action of progesterone for endometrial receptivity in primates

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Progestrone is essential for endometrial receptivity in primates. It is now evident that embryo-derived signal influences implantation stage endometrium under progesterone dominance, and collectively results in endometrial receptivity to implanting blastocyst. Previously, a few studies were performed using global gene profiling based on microarray technology to identify changes in gene expression between early luteal phase and mid luteal phase endometrium, however, the issue of combinatorial regulation by progesterone-dependent regulation and by embryo-derived signal on transcripts profiles during endometrial differentiation toward receptivity for blastocyst implantation in primates has not been addressed. The present review summarizes a few issues, specifically that of transforming growth factor β-tumour necrosis factor α (TGFβ-TNFα) pathways and signal transducer and activator of transcription (STAT) signalling system related to luteal phase progesterone action on endometrial receptivity in terms of its transcriptomic expression using a potent antiprogestin (mifepristone) in conception cycles of the rhesus monkey as a non-human primate model.

Key words Endometrium - mifepristone - receptivity - Rhesus monkey - transcriptomics

Introduction

Progestrone is essential for endometrial receptivity and pregnancy maintenance in the human and non-human primates. As shown in Table I, there are a few reports on the molecular characteristics of the ‘window’ of implantation in which high density cDNA microarray screening was performed for global gene profiling to essentially identify changes in gene expression between early luteal (pre-receptive) and mid luteal (receptive) phases in primate and human endometrium to elucidate steroidal regulation of endometrial physiology towards receptivity. There are two studies describing the differential display of genomic expression in human endometrium in response to high affinity progesterone antagonist (mifepristone) during the period of progesterone dominance in vitro and in vivo. However, it is now clearly evident that a dialogue between embryo and endometrium under progesterone dominance results in complex interaction of genomic expressions that collectively manifests endometrial receptivity to an implanting blastocyst. The potential effect of human chorionic gonadotropin (hCG), a putative preimplantation stage embryo-derived signal on transcript profiles in implantation...
stage baboon endometrium has been demonstrated\textsuperscript{13}. All of the above-mentioned studies, except two\textsuperscript{10,13} adopted an inductive approach based on genome wide expression arrays. We, on the contrary, delineated the transcripts profile during endometrial differentiation for ‘receptivity’ under the dual control of steroidal regulation and embryo-derived signal using a deductive approach\textsuperscript{18}. To this effect, we have compared transcript profiles for known 409 genes between ‘receptive’, and mifepristone-induced desynchronized ‘non-receptive’ stage monkey endometrial samples from mated, potential conception cycles, using cDNA arrays containing sequence-verified clones known to be important in endometrial function\textsuperscript{18}. In the present review, we discuss a few issues related to luteal phase progesterone action on endometrial receptivity using mifepristone as a potent antiprogestin in terms of its transcriptomic expression in fecund cycles of rhesus monkeys as a non-human primate model. The significance of transforming growth factor β-tumour necrosis factor α (TGFβ-TNFα) pathways and signal transducer and activator of transcription (STAT) signaling system under the receptivity-hostility paradigm of endometrial preparation for blastocyst implantation has been specifically addressed.

### Transcriptomics of receptive endometrium of conception cycle

A large number of gene products (31 out of 54) showed changes within five fold, while relatively less (23) number of genes showed more than five fold changes in both days 4 (10 out of 26 gene products) and 6 (13 out of 28 gene products) post-ovulation endometrium\textsuperscript{18}. Further analysis revealed a total of 26 genes (20 increased and 6 decreased) on day four, and 28 genes (25 increased and 3 decreased) on day 6 after fertilization displayed significant differential expression in endometrium as a result of mifepristone treatment on day 2 after ovulation in proven fertile cycles\textsuperscript{18}. The relative abundance of transcripts of nine gene products (\textit{Jun B}, \textit{KDR}, Leptin receptor, matrix metalloprotease 9 (\textit{MMP 9}), ribosomal protein s7 (\textit{Rsp7}), ribosomal protein s9 (\textit{Rsp9}), \textit{STAT3}, \textit{Tastin}, TGF beta1) was higher in endometrial samples on both LH 7-9 phases.

### Table 1. Summary of differential expression studies in endometrial receptivity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subject (No. of gene targets)</th>
<th>Experimental design (sample size)</th>
</tr>
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<tbody>
<tr>
<td>Kao \textsuperscript{6}</td>
<td>Normally cycling human (~12,000)</td>
<td>Differential array analysis between late proliferative phase, cycle days 8-10 (n=4) and mid secretory phase LH 8-10 (n=7)</td>
</tr>
<tr>
<td>Carson \textsuperscript{7}</td>
<td>Fertile human (~12,000)</td>
<td>Differential array analysis between early luteal LH 2-4 (n=3) and mid luteal LH 7-9 (n=3) phases</td>
</tr>
<tr>
<td>Borthwick \textsuperscript{8}</td>
<td>Normally cycling human (~60,000)</td>
<td>Differential array analysis between proliferative phase, cycle days 9-11 (n=5) and secretory phase LH 6-8 (n=5)</td>
</tr>
<tr>
<td>Riesewijk \textsuperscript{9}</td>
<td>Normally cycling (~12,000)</td>
<td>Differential array analysis between paired samples (n=5) collected during pre-receptive LH 2 and receptive LH 7 phases</td>
</tr>
<tr>
<td>Catalano \textsuperscript{10}</td>
<td>Human endometrial explant culture (1000)</td>
<td>Differential array analysis between mid-secretory phase (n=5) endometrial explants treated with 10\textsuperscript{6} M estradiol plus 10\textsuperscript{7} M medroxy-progesterone acetate and 10\textsuperscript{9} M estradiol 10\textsuperscript{7} M medroxy-progesterone acetate plus 10\textsuperscript{4} M RU486</td>
</tr>
<tr>
<td>Ace and Okuliz\textsuperscript{11}</td>
<td>Ovariectomized hormone simulated rhesus monkey (~12,000)</td>
<td>Differential array analysis of normal proliferative, day 13 (n=3) and mid secretory days 21(n=3) and day 23 (n=3) endometrial samples</td>
</tr>
<tr>
<td>Talbi \textsuperscript{12}</td>
<td>Normally cycling human (~55000)</td>
<td>Differential array analysis of samples collected during mid-late proliferative (n=5), early (n=3), mid (n=8) and late (n=6) secretory phases of cycle</td>
</tr>
<tr>
<td>Sherwin \textsuperscript{13}</td>
<td>Adult female baboons (custom made; 8000)</td>
<td>Differential array analysis of LH+10 samples with either no treatment (control; n=2) or rhCG (1.25 IU/h) treatment (n=2) during days 5-10 after ovulation</td>
</tr>
<tr>
<td>Catalano \textsuperscript{14}</td>
<td>Normally cycling human (custom made;16000)</td>
<td>Differential array analysis of LH+8 endometrial samples with no treatment (control; n=15) and with mifepristone (200 mg) treatment at either 6 h (n=5) or 24 h (n=4)</td>
</tr>
</tbody>
</table>

LH, luteinizing hormone
3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor] showed decreased expression. Similarly, a different set of fourteen gene products showed changes only in day 6 samples, twelve of these (CD44, cyclooxygenase 1 (COX1), DLX4, E74-like factor 1 (ELF1), interlukin 6 (IL6), JAK1, JUN, MIF, MMP10, PGFR, Thioredoxin, 3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor] showed decreased expression. Similarly, a different set of fourteen gene products showed changes only in day 6 samples, twelve of these (CD44, cyclooxygenase 1 (COX1), DLX4, E74-like factor 1 (ELF1), interlukin 6 (IL6), JAK1, JUN, MIF, MMP10, PGFR, Thioredoxin, 3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor] showed decreased expression. Similarly, a different set of fourteen gene products showed changes only in day 6 samples, twelve of these (CD44, cyclooxygenase 1 (COX1), DLX4, E74-like factor 1 (ELF1), interlukin 6 (IL6), JAK1, JUN, MIF, MMP10, PGFR, Thioredoxin, 3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor] showed decreased expression. Similarly, a different set of fourteen gene products showed changes only in day 6 samples, twelve of these (CD44, cyclooxygenase 1 (COX1), DLX4, E74-like factor 1 (ELF1), interlukin 6 (IL6), JAK1, JUN, MIF, MMP10, PGFR, Thioredoxin, 3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor] showed decreased expression. Similarly, a different set of fourteen gene products showed changes only in day 6 samples, twelve of these (CD44, cyclooxygenase 1 (COX1), DLX4, E74-like factor 1 (ELF1), interlukin 6 (IL6), JAK1, JUN, MIF, MMP10, PGFR, Thioredoxin, 3β-HSD, Integrin-α, Keratan sulphotransferase1, matrix metalloprotease 3 (MMP3), STAT-PIASγ, Tissue factor/Thromboplastin and Wnt inhibitor factor were increased and two of these [erythroblastic leukemia viral oncogene B (ErB3) and Prolactin receptor]...
TNFa,) were increased and two (17, 20-desmolase, MUC1) were decreased following mifepristone treatment18.

New leads in transcriptomics of receptive endometrium

Table II shows the common gene products appeared to be regulated by progesterone in previous reports including our study6-14,18. Table II also shows a new group of 28 gene products that were affected in implantation stage endometrium following early luteal phase administration of anti-progestin mifepristone18 and those were not reported to be affected by progesterone in earlier array-based studies using the model of comparative study between pre-receptive and receptive endometrium6-14. We have validated our cDNA array based data for nine gene products using TaqMan validation; of the nine gene products, seven (CD 44, CSF-1, DLX4, Enigma, KDR, Leptin receptor, and MIF) appeared to be novel candidates, while two (MUC1 and Uteroglobin) have been reported earlier11,12. While previous reports indicated that wnt-associated pathways were involved in progesterone action on endometrial differentiation12, changes in transcripts profiles of homeobox genes like distal less homeobox 4 (DLX4) and enigma, as well as, of transcription and translation related gene products like JunB, c-Jun, Elf1, and ribosomal proteins in implantation stage endometrium following mifepristone treatment18 have not been reported earlier. It is assumed that endometrial receptivity in the primate is associated with categorical reprogramming in endometrial transcriptomics as observed in human endometrial fibroblasts in vitro19, human placental villous cytotrophoblasts in vitro20, and mouse decidual cells31. Thus, the elucidation of the time course pattern of endometrial receptivity associated transcriptomic networks will be beneficial to clinical sciences for improvising strategies to assist the establishment of pregnancy.

Functional correlates

General: The results of our study in the rhesus monkey18 have substantiated earlier reports indicating that endometrial cyclooxygenase, prostaglandin receptor, matrix metalloproteinases, tissue necrosis factor, prolactin receptor and mucin are regulated in mid-luteal phase endometrium by progesterone22-33. On the other hand, it is notable that relative abundance of transcripts for colony stimulating factor 1 (CSF1), interleukin 6 (IL-6), leptin receptor, and kinase insert domain receptor (KDR) were actually up-regulated in response to mifepristone treatment, contrary to the expected changes based on earlier reports14-40. Collectively, the Enrichment Analysis by Process Maps and Process Networks using Metacore platform (GeneGo, St. Joseph, MI, USA) revealed that the group of 40 genes showing differential expression in endometrium regulated by progesterone action computed upon cell cycle, differentiation and development, immune response and inflammation. Of the all the 40 genes that showed differential expression on inhibition of progesterone action in implantation stage endometrium, two gene products, namely TGFβ1 and STAT3, appear intriguing for disparate reasons as discussed below.

TGFβ: Transforming growth factor beta (TGFβ) has reportedly been observed in a good number of genome-wide expression array studies6-8, 11,12 to be involved in the process of luteal phase endometrial differentiation under progesterone dominance. TGFβ denotes a family of structurally related, dimeric protein that controls proliferation, apoptosis, cellular differentiation, angiogenesis, tissue remodelling and repair, immune responses and other functions depending on cell types, physiological states and its concentration and availability of its receptors51,52. Our observation of increased TGFβ1 expression along with higher expression MMPs and TNF in mifepristone treated endometrium corroborates well with the earlier reports that (i) progesterone mediates a balance of MMPs and TNF in endometrium involving paracrine action of TGFβ43-45 and thus it plays an important role in the process of endometrial receptivity and blastocyst implantation46,47, and (ii) a very high level of TGFβ may suppress gestational maturation of endometrium48-50. Furthermore, we have observed that the protein expression for these mediators was higher in anti-progestin (mifepristone) treated monkey endometrium as compared to control endometrium. Thus, it appears that higher expression of TNFα and TGFβ may mediate endometrial hostility in mifepristone treated fecund cycle through a putative model function as shown in the Figure.

STAT3 signaling: STAT3 activation in endometrium was reportedly associated with endometrial receptivity to implantation in rodents51-53. Furthermore, Janus Kinase 1 (JAK1) at the transcript level in human luteal phase endometrial explant cultures following mifepristone administration was reduced and immunopositive protein levels of JAK1 was higher in stroma and luminal epithelium of luteal phase endometrium as compared to other stages of menstrual cycle19. Thus, the observed relative higher abundance of transcripts
Changes in gene expression during 18 Smads. Gene expression profiling of human receptivity. Molecular phenotyping of human endometrium. The effect of RU486 on the gene expression changes are triggered in luteal phase endometrium treatment are unclear, it is likely that inflammatory signals for increased expression of pSTAT3 in vascular compartment following mifepristone treatment resulted in higher pSTAT3 in vascular compartments on days 4 and 6 after ovulation, while mifepristone treatment were depressed in stromal and were consistently and markedly higher in epithelial compartments on days LH+2 versus LH+7 by mifepristone induced vasocentric JAK1-STAT3 pathway54,55, thereby rendering endometrial hostility to blastocyst implantation. This observation corroborated well with earlier observation that early luteal phase administration of mifepristone resulted in significant changes in mid-luteal phase endometrial vascular physiology associated with phenotypic hostility of endometrium towards blastocyst implantation56.

In conclusion, we propose that TGFβ-TNFα pathways and STAT signaling system are important effectors in mediating the combinatorial regulation of luteal phase progesterone action on endometrial receptivity in fecund cycle.

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