Identification of novel endometrial targets for contraception using microarray technology

Sebastian Mirkin, MD
The Jones Institute for Reproductive Medicine
Eastern Virginia Medical School

Window of Implantation

The transition of the early to the mid luteal phase is associated with significant changes in endometrial gene expression

Hypothesis

Humans have an estimated 35,000 genes; the functions of more than half of them are unknown

The Human Genome Project

Only about 2% of the human genome contains genes, which are the instructions for making proteins

Specific Aim

To identify potential new targets for contraception comparing endometrial gene expression in the early versus mid luteal phase (day 16 vs. day 21) of the natural cycle.
Materials and Methods

Subject Population

18 healthy volunteers were subjected to a timed endometrial biopsy:

i. Natural cycles, day 16 (n = 9)
ii. Natural cycles, day 21 (n = 9)

Materials and Methods

Early Versus Mid Luteal Phase During Normal Menstrual Cycles

urinary LH surge

Day 16
Day 21

Gene expression profile was performed using Affymetrix microarrays
Affymetrix contains ~30,000 genes/ESTs
Validation of concordant genes performed using one step Real Time RT-PCR.

Materials and Methods

Microarray Analysis

• Gene expression profile was performed using Affymetrix microarrays
• Affymetrix contains ~30,000 genes/ESTs
• Validation of concordant genes performed using one step Real Time RT-PCR.

Affymetrix Chip Experimental Procedure

Green = Undersuppressed
Red = Oversuppressed
Yellow = Equivalent Expression
114 genes (61 upregulated and 53 downregulated) were found to be differentially expressed in the endometrium during the window of implantation, when compared to the early luteal phase.

### Distribution of Gene Changes Among Different Functional Classes

<table>
<thead>
<tr>
<th>Functional class</th>
<th>% of subset upregulated</th>
<th>% of subset downregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell adhesion proteins and cell surface proteins</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Immuno-modulators</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>Metabolic enzymes</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Other functions</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Unknown or ESTs</td>
<td>20</td>
<td>29</td>
</tr>
</tbody>
</table>

### Comparative results between genes up- and down-regulated in 3 different studies

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Occurrence of the ERE and PRE like sequences</th>
<th>Genes differentially regulated during the window of implantation</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several differences were found when comparing gene expression in early vs. mid luteal phase of the natural cycle. Some of these genes are congruent with previous reports.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This genes can be potential target for the development of non-hormonal contraceptives.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoing functional studies will provide proof of the principle that these genes are indeed contraceptive targets.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>