In Vitro Models in Endometriosis Research

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ESHRE Campus Course, Leuven 2009
General R&D process

Lead Finding
1. Target
2. Lead
3. Lead Optimization
4. Selection phase

Lead Optimization
5. Phase I
   - Safety/tolerability
6. Phase II
   - Proof-of-concept
7. Phase III
   - Efficacy

Discovery

Prioritization over projects

Research

Development

Full Development and Launch

Registration Marketing

Early clinical development
**Target discovery**

**Target identification**
- Internal Research
- Literature Research - Collaborations
- Bioinformatics (external databases)
- Research on physiology / disease (models)

**In vivo validation**
- Tissue distribution
- Transgenic animals
- Knockout animals
- Human genetics

**Overexpression**
- Transfection
- Transduction

**Functional interference**
- siRNA
- Blocking antibodies
- Antisense
- Induce mutations
<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>In vitro efficacy in screening assay</td>
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<tr>
<td>1b</td>
<td>In vitro efficacy in biologically relevant assay</td>
</tr>
<tr>
<td>2a</td>
<td>PK, In vivo efficacy</td>
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<tr>
<td>2b</td>
<td>In vivo efficacy in therapeutic/predictive model, PK/PD</td>
</tr>
<tr>
<td>Selection phase</td>
<td>PK dog/monkey, bioavailability, safety, tolerability</td>
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“Priorities for endometriosis research: recommendations from an international consensus workshop”

- Xth World Congress on Endometriosis, Melbourne, Australia
- Moderator Peter Rogers, Monash University
- Problem:
  - Disease with high social and healthcare impact
  - No non-invasive diagnostic tools available
  - Research is under-funded
  - Current therapies are not satisfactory: poor efficacy, high relapse rate
  - Practical difficulties to investigate this complex disease
  - Lack of novel targets and drugs
“Priorities for endometriosis research: recommendations from an international consensus workshop“

• Recommendations:
  – A better understanding of the role of the eutopic endometrium in the establishment and continuation of endometriosis is required
  – Develop non-invasive diagnostic tools
  – Biomarkers are required
  – Appropriate in vitro and therapeutic models are needed for studying different aspects of endometriosis pathophysiology

What are appropriate in vitro models?
Endometriosis

Pathogenesis
In vitro models to study endometriosis

– Endometrial cells/tissue from women without endometriosis
– Endometrial cells/tissue from lesions/cysts
– Immortalized cells from lesions/cysts
How should we employ them?

Focus on particular biological processes that contribute to the total complexity of the disease, i.e.:

- adhesion
- angiogenesis
- proliferation/apoptosis
- progesterone resistance
- estrogen metabolism

Create conditions mimicking in vivo situation by combining primary cells/tissues with other cellular/tissue components, i.e.:

- menstrual endometrium
- (Activated) macrophages, lymphocytes
- ECM components
- peritoneum/mesothelium
- chick chorioallantois membrane model (model for peritoneum)
Primary cell/tissue cultures from endometrium (from women without endometriosis)
Endometrium function in part dictated by paracrine, juxtacrine and matricrine interactions

(E. Marbaix; Punyadeera et al., 2004 JSBMB)
Primary cultures of endometrial stromal cells are steroid-hormone responsive

Regulation of prolactin production by SPRMS in primary endometrial stromal cells
Responsiveness of primary stromal cells to steroid hormones reduces in time

Prolactin production in stromal culture in different passages (n=2) treated with Org 2058 and DHT

- P0
- P1
- P3
- P6

control  P

prolactin (mU/L)
NRs in stroma determine proliferative response

Recombination and renal capsule transplantation

(Cunha et al., 2004 Arch Histol Cytol)
Chick chorioallantoic membrane model

This only works when transplanting tissue fragments

(Nap et al., 2004)
Short term explant cultures human endometrium are responsive to estrogen

(Punyadeera et al., 2004 JSBMB)
Effect of progestins/SPRMs on MMP activation in cultured explants of pre-menopausal endometrium

3 day incubation

No hormone

P+E

P+E+AP

Silver-stained reticulin (collagen) fibers
Effect of progestins/SPRM s on MMP activation in cultured explants of post-menopausal endometrium

Gelatin zymography  8%,  day 3
Endometriosis Pathogenesis
Preferably shed menstrual endometrium should be used

Integrin expression on shed menstrual endometrium

(Koks et al., 2000 Mol Hum reprod)
Coculture menstrual endometrium and peritoneum

(Groothuis et al., 1999; Koks et al., 2000)
Coculture menstrual endometrium and ECM

Adhesion of menstrual endometrium to extracellular matrix is largely mediated by integrin alpha(6)beta(1) and laminin interaction.

(Koks et al., 200 Mol Hum Reprod)
Endometrium also adheres to mesothelial surface

(Witz et al., 2002 Fertil Steril)
Coculture menstrual endometrium and mesothelial cells

Binding is mediated by CD44 – hyaluronic acid interaction

(Dechaud et al., 2001 Fertil Steril)
Shed endometrium contains viable cells that express MMPs and TIMPs

(Koks et al., 2000 Fertil Steril)
Chick chorioallantoic membrane model

Antegradely shed menstrual endometrium forms lesions in CAM

(Nap et al., 2004)
Chick chorioallantois membrane model

Endometrium is angiogenic

(Maas et al., 2001; Nap et al., 2005)
Chick chorioallantois membrane model

Angiostatic therapy inhibits lesion formation in the CAM

(Nap et al., 2005)
Summary

• Endometrium tissue is suitable to study basic processes involved in the early pathogenesis of endometriosis

• The tissue context is critical, particularly with regard to steroid hormone responsiveness and implantation

• Endometrium tissue is not likely suitable for the generation of therapeutic models, many endometriosis-related characteristics are lacking
  – i.e. changes in cell endometrial physiology, altered immune system
Primary cell/tissue cultures from lesions/cysts
Stromal cell cultures from ovarian cysts

(Noble et al., 1997 JCEM)
Bulun hypothesis

- ERβ and SF1 are upregulated in ovarian endometriosis (demethylation)
- COX-2 is induced and consequently PGE2 and aromatase production

Not validated by others; only shown in stromal cells from ovarian cysts

(Bulun, 2009, NEJM)
**Explant cultures endometriosis tissue**

- One publication: Sharpe-K et al., Fertil Steril 1993
- Proteomics study which led to the discovery of the endometriosis-specific haptoglobin isoform

*(Bulun, 2009, NEJM)*
**Xenografts with explants of endometriotic tissue**

**Human primary xenografts:**
- Tissue from ovarian cysts and peritoneal endometriosis is transplanted in pockets made in abdominal wall
- OVX RAG-2γ(c) Knockout mice, E2 and P4 pellets, to support four menstrual cycles

(Greenberg and Slayden, AJOG 2004)
Immortalised cells from lesions/cysts
**Examples**

SV40 T-antigen transformed epithelial cells from peritoneal endometriosis *(Akoum et al., 1999 Am J Pathol)*
- Nuclear receptor status unknown, many chromosomal aberrations

hTERT-immortalized endometriotic stromal cells *(Annunziata et al., 2009 Fertil Steril)*
- No details on lesion type/location, no characterization of cell line
SV40 T-antigen transformed epithelial and stromal cell lines from peritoneal endometriosis

(Banu et al., Fertil Steril, 2008)
SV40 T-antigen transformed epithelial and stromal cell lines from peritoneal endometriosis

(Banu et al., Fertil Steril, 2008)
**COX2 overexpression in endometriosis**

Neg control  
Ectopic  
Eutopic patient  
Eutopic control  

Banu et al., 2008
Prostaglandin receptor overexpression in endometriosis

Ectopic

Eutopic patient

Eutopic control

Neg control

Banu et al., 2008
Prostaglandins regulate cell survival in endometriosis

A

B

Schering-Plough

9/7/2009

41
Conclusions

• The choice of in vitro model for target validation and to demonstrate mechanism of action, depends on the target and the biological mechanism of interest

• There is a need for more studies using explant cultures of endometriotic tissue as well as for more cell lines derived from endometriotic tissues

• Models should be validated with reference drugs
In vitro assays in LO

- Factors dictating selection assays HO and LO are different
- Reproducibility may be compromised
  - biological variation
  - variation expression target
- Biological relevance
- Biological connection in vitro assays and therapeutic models
  - tissue context
- Predict clinical efficacy
  - demand for returning therapeutically relevant data
  - check/search for biomarkers