Evidence for Endometrial Stem/Progenitor cells

Caroline E Gargett
The Ritchie Centre
Monash Institute of Medical Research
Monash University Department Obstetrics and Gynaecology
Presentation Plan

- Endometrial regeneration, the stem cell hypothesis and gynaecological disease

- Evidence for Stem/progenitor cell activity in human and mouse endometrium

- Markers of endometrial stem/progenitor cells

- Origin of endometrial stem/progenitor cells

- Endometrial cancer and evidence for cancer stem-like cells

- Endometrial Stem/progenitor cells in endometriosis
Regenerative Capacity of Human Endometrium

Cycling endometrium

- Lumen
- Stromal glands
- Functionalis
- Basalis

Menstrual cycle
- Parturition
- Resection
- Postmenopausal

Gargett et al 2008
The endometrial basalis contains a small population of epithelial stem cells and stromal stem cells.
Endometrial Stem Cell Disorders

Endometrial Cancer
Mutated stem/progenitor cell → tumor
responsible for progression, metastasis, recurrence

Endometriosis
Normal stem/progenitor cell shed into peritoneal cavity → ectopic implant

Adenomyosis
Normal stem/progenitor cells, abnormal niche, inappropriate differentiation → ectopic growth, SMC hyperplasia

Asherman’s Syndrome, Ablation
Damage/loss of normal stem/progenitor cells

Inadequate endometrium for IVF
Diminished activity of normal stem/progenitor cells
Adult Stem Cells - Properties

There are no specific markers for adult stem cells

Rare cells in tissues
Undifferentiated

Self renew
Differentiation capacity
High proliferative potential
Quiescent

Clonogenic in vitro (CFU)

Chan et al 2004
CFU Activity of Human Endometrial Cells

- CFU are present in inactive endometrium
- CFU activity similar for proliferative and secretory stages

Stem/Progenitor Cell Activity in Normal Human Endometrium (CFU)

Self renewal

High proliferative potential

Differentiation

Gargett et al. 2009
Human Endometrial Stem/Progenitor Cell Activity in vivo

Reconstruction of human endometrial like tissue from transplanted human endometrial cells

Hormone-dependent functional changes of reconstructed endometrium

Masuda H. et.al. 2007
Side Population (SP) Cells in Human Endometrium

- Endometrial SP cells are heterogeneous
  - Endothelial, Epithelial, Stromal cells
- Express ABCG2/Brpc1, telomerase, OCT-4, c-KIT
- Clonogenic
- Differentiate
- Reconstruct endometrial tissue components

Kato K et al 2007
Tsuji S et al 2008
Cervello I et al, 2010
H Masuda et al, 2010
Where are Endometrial Stem/Progenitor Cells Located?

The search for endometrial stem/progenitor cell markers

- Luminal epithelium
- Glandular epithelium
- Stroma
- Basalis
- Myometrium

Putative epithelial S/P cell
Putative stromal S/P cell

Gargett, 2007
**Mouse Endometrial Epithelial and Stromal Stem/Progenitor Cells** - Label Retaining Cells (LRC)

**Epithelial LRC**
- 3% of epithelial cells
- Luminal epithelium
- ERα- 
- Proliferate in response to E

**Stromal LRC**
- 6% of stromal cells
- Perivascular
- Most ERα-, 16% ERα+, Some proliferate in response to E

**Stromal LRC**
- αSMA+, CD45- 
- Some OCT4+

---

Chan & Gargett 2006

Gargett, 2007

Cervello et al 2007
LRC in Mouse Model of Endometrial Breakdown and Repair

Luminal epithelium

A
B
C
D

Glandular epithelium

G
H
I
J

Kaitu’U-Lino et al, 2010
Differential Epithelial Proliferation During Breakdown and Repair

Kaitu’U-Lino et al, 2010
Location of LRC varies between Endometrial Regeneration and Repair Models

1. Endometrial growth during development
   Luminal epithelial LRC

2. Endometrial repair following breakdown
   Glandular epithelial LRC

Different mechanisms involved between growth and repair following tissue damage
Searching for Human Endometrial Epithelial Stem/Progenitor Cell Markers

Screened with an antibody panel to 30 surface markers

H3D12

• Self renewal
• Differentiation
• In vivo reconstitution

Charmaine Tan
Markers to Isolate Endometrial Mesenchymal Stem Cells

Co-expression of CD146 and PDGFRβ → 8 fold purification of MSC-like cells compared to freshly isolated stromal cells

- clonogenic
- Multipotent
  - adipocytes, SMCs, chondrocytes, osteoblasts
- MSC surface phenotype
- perivascular location

Schwab & Gargett, 2007
Are Endometrial MSC-like cells in the Basalis or Functionalis?

Mesenchymal stem-like cells

luminal epithelium
stroma
glandular epithelium

Endometrial MSC will be shed in menstrual blood

Functionalis

Basalis

Myometrium

blood vessel
Origin of Human Uterine Stem/Progenitor Cells

- Residual Müllerian duct fetal stem cells  
  Gargett, Hum Reprod Update, 2007
- Circulating bone marrow (stem) cells
  - HLA or gender mismatch BMT recipients with donor HLA type or male glands, stroma

Human

- monoclonal glands

Mouse

- CD45+ Y+ leukocyte CK+Y+ epithelial cell

Conditional expression of EGFP in CD45 expressing cells using CD45/Cr−Z/EG mouse (n=1)

Bratinscsak, A. et al. 2007
Cancer Stem Cells

Initiation, progression, metastasis, drug resistance, recurrence

Tissue hierarchy

Normal tissue

stem cell

progenitor cell

TA cell

Differentiated/Mature Cells

Cancer

Cancer Stem Cell

cancer progenitor cell

Cancer TA cell

tumor cells

chemotherapy

mutations
Cancer Stem Cells in Endometrial Carcinoma

- Clonogenic
- Tumorigenic
- Differentiate in vivo

Transplant 125,000 endometrial cancer cells

Parent tumour
Type 1 Grade 2

EpCAM Vimentin ERα PR PCNA

Hubbard et al, Cancer Res 2009
**Cancer Stem Cells in Endometrial Carcinoma**

- Self renewal in vitro

<table>
<thead>
<tr>
<th>Sample</th>
<th>Subclonings (M, range)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td>2.5 (2,3)</td>
<td>2</td>
</tr>
<tr>
<td>Grade 1</td>
<td>3 (1-5)</td>
<td>11</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3 (3-4)</td>
<td>5</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3.5 (3,4)</td>
<td>2</td>
</tr>
<tr>
<td>Type II</td>
<td>4 (1-4)</td>
<td>3</td>
</tr>
</tbody>
</table>

- Self renewal in vivo

Hubbard et al, Cancer Research 2009
Stem/Progenitor Cells in Menstrual Blood

Cultured cells from menstrual blood contains MSC-like cells

- MSC cell surface phenotype
- Express OCT4, c-KIT, SSEA4 (pluripotency markers), telomerase
- Differentiate in vitro: cardiomyocytes, neural, hepatic, lung cells
- Differentiate in vivo: cardiomyocytes, skeletal muscle

Suggested as a source of cells for regenerative medicine

Endometrial stem/progenitor cells in Endometriosis

Endometrial stem/progenitor cells may

• be shed in menstrual blood

• gain access to the pelvic cavity by retrograde menstruation

• Establish endometriosis lesions in susceptible women

Gargett & Masuda 2010
Summary
Human and Mouse Endometrium contains

Epithelial progenitor cells
• Clonogenic
• Self-renew, high proliferative potential, differentiate
• SP cells
• Label retaining cells, ERα⁻
• Markers unknown

Endometrial MSC-like cells
• Clonogenic
• Self-renew, multipotent
• High proliferative potential
• Perivascular
• Purified in CD146⁺PDGFRβ⁺ stromal fraction
• Shed during menstruation
• Proposed to use in tissue engineering applications

Gargett and Masuda (2010)
<table>
<thead>
<tr>
<th>Stem/Progenitor Cell Type</th>
<th>Possible Commitment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>epithelial cells</td>
</tr>
<tr>
<td>Clonogenic epithelial cells (CFU)</td>
<td>+</td>
</tr>
<tr>
<td>Clonogenic stromal cells (CFU)</td>
<td></td>
</tr>
<tr>
<td>CD146⁺PDGF-Rβ⁺ stromal cells</td>
<td></td>
</tr>
<tr>
<td>Endometrial tissue reconstituting cells</td>
<td>+</td>
</tr>
<tr>
<td>Cultured endometrial stromal cells</td>
<td></td>
</tr>
<tr>
<td>SP cells</td>
<td>+</td>
</tr>
<tr>
<td>Bone-marrow derived cells</td>
<td>+</td>
</tr>
<tr>
<td>Menstrual blood cells</td>
<td></td>
</tr>
<tr>
<td>Endothelial progenitor cells (bone marrow derived)</td>
<td>?</td>
</tr>
<tr>
<td>Mouse LRC</td>
<td>+</td>
</tr>
</tbody>
</table>
**Endometrial Stem/Progenitor Cells: Clinical relevance**

**CSC in Endometrial Cancer**
- Clonogenic, Tumour initiating cells
- Self renew in vitro and in vivo
- Differentiate in vivo
- Derived from epithelial progenitor cells or their progeny
- CD133 may be a marker (Rutella et al 2009)
- Involved in progression, metastasis, recurrence, resistance
- Target for novel treatment

**Normal Endometrial Stem/progenitor Cells may have roles in**
- Endometriosis lesion development
- Adenomyosis
- Generating adequate endometrium for IVF after biopsy/injury

**Potential endometrial stem/progenitor cell therapy**
- MSC for autologous cell-based therapy for pelvic organ prolapse
Unresolved questions in Endometrial Stem/Progenitor Cell Research

1. Markers to identify endometrial epithelial progenitors
2. Relationship between cultured endometrial stromal cells and endometrial MSC (CD146⁺PDGFRβ⁺)?
3. What is the relationship between
   - clonogenic cells
   - SP cells
   - Tissue reconstituting cells
   - LRC?
4. How many endometrial stem/progenitor cell types are there? One or more?
   - ie epithelial, stromal (MSC), endothelial progenitor cells?
5. Endogenous &/or bone marrow origin of endometrial stem/progenitor cells?
Acknowledgements

Endometrial Stem Cell Group
Sonya Hubbard
Hirotaka Masuda
Gayathri Rajaraman
Charmaine Tan
Hong Nguyen
Louie Ye
Isabella Ciurej
Frances Walker
Pam Mamers

Anna Rosamilia
Gareth Weston
Past members
Rachel Chan
Kjiana Schwab
Rachel Zillwood
Erah Anwar
Tu’uhe Kaitu’u-Lino
Jyothsna Rao

Collaborators
Tübingen University
Hans-Jörg Bühring

Monash Medical Centre
Beena Kumar

Massachusetts General Hospital
Bo Rueda
Anne Friel

CSIRO
Keith McLean
Jerome Werkmeister
Sharon Edwards

Funding
RISS
ASCC
NHMRC
Cancer Council Victoria
CASS Foundation
RANZCOG
Helen MacPherson Smith Trust

Tissues
Victorian Cancer Biobank
References

References

Schwab KE, Chan RWS, Gargett CE (2005) Stem cell activity in human endometrial epithelial and stromal cells during the menstrual cycle. Fertility and Sterility 84:1124-1130