Stem cells in endometriosis: pathogenetic factors and target for new medical treatments?

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PATHOGENESIS OF ENDOMETRIOSIS

RETROGRADE MENSTRUAL FLUX
Simpson, Am J Obstet Gynecol 1927

IMMUNE TOLERANCE
Khan, Am J Reprod Immunol 2008

ENDOMETRIAL STEM CELLS

EPIGENETIC DISREGULATION
Baranov, Obstet Gynecol Reprod Biol 2015

COMBINED PATHOGENETIC THEORY
Endometrial regeneration occurs cyclically 400 times during reproductive life.

It depends on **endometrial stem cells (ESC)**, that were found mainly in the basalis layer, but also in the functionalis, shedding layer.

ESC have been isolated and characterized in animals and humans.
MESENCHYMAL STEM CELLS (MSC)

MSC are rare, undifferentiated cells with specific **functional ability**
ESC HAVE MESENCHYMAL NATURE (E-MSC)

**Immunofluorescence**
Expression of stromal markers (vimentin) and no expression of epithelial markers (cytokeratin)

**FACS analysis**
Expression of Mesenchymal cell markers CD44, CD105 and CD73
E-MSC were found in the normal endometrium (Ctrl-MSC), but also in endometriotic tissue (Ecto-MSC) and in the endometrium of women with endometriosis (Euto-MSC).

<table>
<thead>
<tr>
<th>CD</th>
<th>Ecto</th>
<th>Euto</th>
<th>Ctrl</th>
<th>MSC</th>
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<tr>
<td>CD146</td>
<td>12%</td>
<td>10%</td>
<td>13%</td>
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<tr>
<td>CD 105</td>
<td>90%</td>
<td>95%</td>
<td>98%</td>
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<td>CD 73</td>
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<td>SSEA 4</td>
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<td>70%</td>
<td>59%</td>
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</tbody>
</table>
Ecto, Euto and Ctrl ESC are M-ESC

Differentiation to osteoblasts
Alizarin red staining +

Differentiation to epithelium after 14 days
Cytokeratin staining +, E-cadherin staining -

Differentiation to endothelium after 14 days
Von Willebrand factor staining +
Ecto, Euto and Ctrl ESC are M-ESC

Acquisition of epithelial markers and estrogen receptors (ER) after 14 days in differentiating medium

Immunofluorescence
Ecto, Euto and Ctrl ESC are M-ESC

Expression of CD31 after direct coculture with HUVEC cells (48 h)
Immunofluorescence

Organization in capillary-like structure
3D digital reconstruction
A. Proliferation
DNA synthesis assay (BrdU)

B.C. Migration and invasion
Matrigel assay

D. VEGF release in culture medium
Multiplex cytokine assay

E.F. Expression of VEGF and Hypoxia-inducible factor (HIF) genes
RT-PCR analysis of mRNAs
E-MSC-INDUCED «in vivo» ANGIOGENESIS

Neoangiogenesis in implants of Ecto-MSC and Euto-MSC in SCID mice

A. After 30 days new blood vessels formation (vWF expression)
C. New blood vessels connecting with mice vessels (RBC inside)
PATHOGENESIS OF ENDOMETRIOSIS: a novel combined hypothesis

UTERUS

Retrograde flux

Euto-MSC

PERITONEAL CAVITY

Immunological dysfunction (tolerance)

Environmental-linked (e.g. hypoxia) epigenetic changes

Euto-MSC ➔ Ecto-MSC

↑ Proliferation  ↑ Invasion  ➔ ↑ Neo-angiogenesis

↑ Migration

Endometriosis
PATHOGENESIS OF ENDOMETRIOSIS: a novel combined hypothesis

UTERUS

Retrograde flux

Amenorrhea

Euto-MSC

Drug-induced hypoE

PERITONEAL CAVITY

Immunological dysfunction (tolerance)

Environmental-linked (e.g. hypoxia) epigenetic changes

Euto-MSC → Ecto-MSC

↑Proliferation

↑Invasion

↑Neu-angiogenesis

↑Migration

Endometriosis

?
**E-MSC INSENSITIVITY TO ESTROGENS**

Lack of ER-1alpha expression
Immunohistochemistry

Lack of E2-induced proliferation
Immunohistochemistry
Euto- and Ecto-MSC express dopamine receptor 2, targeted by Cabergoline

Cabergoline reduces neoangiogenesis and endometrial implants in mice

Effect of antiangiogenic treatment on peritoneal endometriosis-associated nerve fibers

Cabergoline reduces VEGF release by endometrial implants in mice
EFFECTS OF CABERGOLINE TREATMENT ON HUMAN Euto and Ecto-MSC

Cabergoline reduces endothelial differentiation of E-MSC without affecting tubular organization
FACS analysis of CD31 expression before (basal) or after (Ctr)
48h direct coculture with HUVEC cells
PATHOGENESIS OF ENDOMETRIOSIS: a novel combined hypothesis

UTERUS

- Retrograde flux
- Amenorrhea
- Euto-MSC

Drug-induced hypoE

PERITONEAL CAVITY

- Immuneological dysfunction (tolerance)
- Environmental-linked (e.g. hypoxia) epigenetic changes
- Euto-MSC → Ecto-MSC
- ↑ Proliferation
- ↑ Invasion
- ↓ Neo-angiogenesis
- ↑ Migration

Cabergoline

Endometriosis
Sorafenib has dose-dependent anti-proliferative effect on E-MSC
Proliferation (DNA synthesis) assay (BrdU)
**ANTI-MIGRATION EFFECT OF SORAFENIB**

**Ezrin:** cytoskeletal protein involved in migration and cellular plasticity, active when phosphorilated (**Ezrin-P**)

*Sorafenib has dose-dependent effect reducing Ezrin activation*

*Western Blot: Ezrin and Ezrin-P expression in E-MSC*
SORAFENIB EFFECTS ON PRO-ANGIOGENIC FACTORS PRODUCED BY E-MSC

 Reduction of VEGF mRNA expression
 RT-PCR

 Reduction of HIF1-alpha expression
 Western blot analysis
Reduced density of micro-vessels  
CD31 staining

Preservation of primordial follicles (*)  
Hematoxylin-eosin staining

Treatment with TK-inhibitors lowers neoangiogenesis in rat endometriosis implants without detectable toxic effects on primordial follicles
PATHOGENESIS OF ENDOMETRIOSIS: a novel combined hypothesis

UTERUS

Retrograde flux

Immunological dysfunction (tolerance)

Environmental-linked (e.g. hypoxia) epigenetic changes

PERITONEAL CAVITY

Ecto-MSC → Euto-MSC

↓ Proliferation, migration, invasion, neo-angiogenesis

Drug-induced hypoE

TK inhibitors, Cabergoline

Endometriosis

Amenorrhea

Euto-MSC

Cabergoline

TK inhibitors
Original Article
Vascular endothelial growth factor receptor-2 inhibitor cediranib causes regression of endometriotic lesions in a rat model

Fang Liu1,2,3, Li Wang1,2,3, Xian-Xia Zhang1,2,3, Shu-Yun Min1,2,3, Yi-Xuan Liu1,2,3, Zhi Zuo1,2,3, Zhi-Xing Jin1,2,3, Zhi-Ling Zhu1,2,3

Endometrial implant volume

Micro-vessel density in controls (A) and treated rats (B)

CD31 staining
Stem Cells with mesenchymal phenotype, named E-MSC, may be isolated from endometriosis implants and from the endometrium of women with or without the disease.

Endometrial E-MSC reach the peritoneal cavity (retrograde menstruation) and then change their functional properties in response to the ectopic environment, becoming Ecto-MSC.

As a consequence, they increase their attitude to proliferate, migrate and invade the peritoneum, as well as their pro-angiogenic properties, promoting the initiation, growth and maintenance of endometriosis implants.
Ecto-MSCs have **no estrogen receptors** and are not affected by estrogen-depleting treatments.

**Cabergoline** can reduce Ecto-MSC endothelial differentiation, limiting neo-angiogenesis in the early stages.

**TK inhibitors** (e.g. **Sorafenib**) inhibit Ecto-MSC proliferation and migration, as well as neo-angiogenesis. Their effects in vivo have been investigated in the mouse model.

These novel treatment options should be tested in vivo on reliable animal models of endometriosis (e.g. primates) in order to understand if they may be effective in humans.
Sorafenib inhibits growth, migration, and angiogenic potential of ectopic endometrial mesenchymal stem cells derived from patients with endometriosis

Aldo Moggio, M.Sc.,* Giulia Pittatore, M.D.,† Paola Gassoni, M.D., Ph.D.,‡ Gian Luigi Marchino, M.D.,§ Alberto Revelli, M.D., Ph.D.,∥ and Benedetta Bussolati, M.D., Ph.D.□

* Department of Internal Medicine, Molecular Biotechnology Center; † Physiopathology of Reproduction and IVF Unit, Department of Gynecological and Obstetrical Sciences; and □ Department of Biomedical Sciences and Human Oncology, University of Torino, Torino, Italy

Endometrial Adult/Progenitor Stem Cells: Pathogenetic Theory and New Antiangiogenic Approach for Endometriosis Therapy

G. Pittatore, MD, A. Moggio, BiolSci, C. Benedetto, MD, PhD, B. Bussolati, MD, PhD, and A. Revelli, MD, PhD

Angiogenic properties of endometrial mesenchymal stromal cells in endothelial co-culture: an in vitro model of endometriosis

Canosa S, Moggio A, Brossa A, Pittatore G, Marchino GL, Leoncini S, Benedetto C, Revelli A, Bussolati B
THANKS!