"Endometrial remodelling and regeneration in reproductive disorders satellite symposium 2009"

Pasquale FLORIO, PhD, MD

The clinical soundness of basic research on activin A in endometriosis and endometrial cancer

University of Siena, Siena, Italy

Activin A and related-proteins

Activin A and related proteins: endometrial products
Activin A and follistatin: actions on an in-vitro model of human peritoneum invasion

Monolayer of confluent peritoneal mesothelial cells grown in a Matrigel invasion assay

Epithelial endometrial cells

Activin A and related proteins in the eutopic endometrium of patients with endometriosis

Despite the histological similarity of eutopic endometrium in women with and without endometriosis, differences in growth factor profiles have been demonstrated and are believed to be involved in the pathogenesis of infertility related to endometriosis.
Actin A and decidualization of the human endometrium

Activin A is associated with the release of PRL and activin A

Activin A is a key component of pathways related to endometrial stromal cell decidualization

Activin A and the cross-talk between the endometrium and the embryo at implantation

Decidual transformation of endometrial stromal cells is a prerequisite for human implantation

Activin A as a marker of receptive endometrium

50 women of whom 25 became pregnant after up to three IUI attempts and 25 did not

Activin A as a marker of receptive endometrium

Criterion values and coordinates of the ROC curve activin A

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
<th>+LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.04</td>
<td>76.00</td>
<td>54.9 - 90.6</td>
<td>100.00</td>
<td>86.2 - 100.0</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

Criterion values and coordinates of the ROC curve endometrial thickness

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
<th>+LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;6</td>
<td>68.00</td>
<td>46.5 - 85.0</td>
<td>48.00</td>
<td>27.8 - 68.7</td>
<td>1.31</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Activin A receptors antagonist: ebaf

- protein of 370 amino acids;
- member of the TGF-ß family;
- similarity with lefty-A (important determinant of vertebrate development such as left-right asymmetry)
- binds the activin receptor type II B (ACTRIIB)

% Similarity

<table>
<thead>
<tr>
<th>Protein</th>
<th>% Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ebaf</td>
<td>100</td>
</tr>
<tr>
<td>TGF-ß1</td>
<td>65</td>
</tr>
<tr>
<td>activin ßA</td>
<td>65</td>
</tr>
<tr>
<td>activin ßB</td>
<td>63</td>
</tr>
</tbody>
</table>

Activin A receptors antagonist: ebaf in the human endometrium

endometrial bleeding

the mRNA of ebaf is strongly expressed in all endometria irrespective of whether the endometrium was in the proliferative or secretory phase of the menstrual cycle

Dysregulated expression of ebaf, a novel molecular defect in the endometria of patients with infertility - Northern blot analysis

Tabibzadeh et al., JCE&M 2000
Dysregulated expression of ebafebaf, a novel molecular defect in the endometria of patients with infertility - Western blot analysis/immunohistochemistry.

Tabibzadeh et al., JCE&M 2000

Disrupted cyclicity of activin A expression in eutopic endometrium


Activin A antagonists: nodal & cripto in eutopic endometrium

Shen M, J Clin Invest. 2003

A new role for Cripto as an inhibitor of activin A signaling

Reprod Sci 2009, in press
Proposed markers of endometriosis

<table>
<thead>
<tr>
<th>Proposed markers of endometriosis</th>
<th>Sensitivities and specificities</th>
<th></th>
<th>Sensitivities and specificities</th>
</tr>
</thead>
<tbody>
<tr>
<td>- large range of variability</td>
<td>- not ever specific to endometriosis</td>
<td>- not ever specific to endometriosis</td>
<td></td>
</tr>
</tbody>
</table>

Follistatin and endometriosis

✓ to quantify the concentration of follistatin and CA-125 in the serum of women with ovarian endometrioma and other benign cysts
✓ to investigate the use of follistatin as a marker in the differential diagnosis of benign ovarian cysts

Group A
- ovarian endometriomas (n=52)

Group B
- benign ovarian cysts (n=52)
  - serous (n=17)
  - mucinous (n=18)
  - dermoid (n=7)
  - hemor. corpora lutea cysts (n=10)

Group C
- extra-ovarian endometriosis
  - (stage I or II pelvic endometriosis, n=11)

Group D
- Healthy controls (n=27) (laparoscopic tubal sterilization)
High serum follistatin levels in women with endometriosis

Follistatin detected 48/52 cases of endometrioma (92% sensitivity) with the cutoff 1433 pg/ml, which corresponds to 96% specificity.

In contrast, CA-125 detected only 44% of the endometriomas with 90% specificity.

Serum follistatin measurement in the diagnosis of endometriosis

Serum follistatin levels in endometrioma vs. no ovarian cyst

Serum follistatin levels in endometrioma vs. other benign ovarian cysts

<table>
<thead>
<tr>
<th></th>
<th>Endometrioma</th>
<th>CA-125 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>4 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Postoperative</td>
<td>4 (5)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

JCE&M, 1998
Activin A in human endometrial adenocarcinoma

Follistatin & FLRG in human endometrial adenocarcinoma

Inhibin α subunit & betaglycan in human endometrial adenocarcinoma

Otani et al., Gynecol Oncol. 2001

Florio et al., Eur J Endocrinol. 2004

Worbs et al., Oncol Rep. 2007

Florio et al., Eur J Endocrinol. 2005
Nodal, cripto and endometrial cancer

Shen M, J Clin Invest. 2003

A new role for Cripto as an inhibitor of activin A signaling

Activin and related proteins in and endometrial cancer

Activin A is a regulator of endometrial cancer cell growth

Activin A inhibits endometrial cancer cell line growth & proliferation

Estradiol promotes resistance to the growth-inhibiting effects of activin through the effect on Bcl-2 levels
Activin A and tumorigenesis: growth inhibition and induction of apoptosis

Immortalized ovarian surface epithelium cell line (IOSE-29) to study adenocarcinoma tumorigenesis.

Activin caused a dose-dependent inhibition of cell proliferation. Cotreatment with follistatin (100 ng/mL) blocked the effects of activin A.

Activin caused increased DNA fragmentation in a dose-dependent manner.

Activin A inhibits IOSE-29 cell line growth & proliferation.
Activin A induces apoptosis increasing DNA fragmentation.

Choi et al., J Clin Endocrinol Metab 2001

Activin A and endometrial cancer

From basic research to clinical applications.

Biochemical screening of endometrial cancer: activin A

Case-control study in patients (n=110) with post-menopausal uterine bleeding.

Control group (n=52): endometrial polyps (n=39), atrophic endometrium (n=16).
Study group (n=58): endometrioid adenocarcinoma (FIGO stage 1A: 24; stage 1B: 18; 1C: 13).

Measurement of serum concentrations of activin A by ELISA.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control Group</th>
<th>Study Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menarche</td>
<td>12.27 ± 1.09</td>
<td>12.27 ± 1.09</td>
<td>0.62</td>
</tr>
<tr>
<td>Fertility</td>
<td>40.56 ± 5.03</td>
<td>40.56 ± 5.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Parity</td>
<td>1.98 ± 1.19</td>
<td>1.98 ± 1.19</td>
<td>0.47</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>63.03 ± 5.28</td>
<td>67.69 ± 8.99</td>
<td>0.001</td>
</tr>
</tbody>
</table>

BMI: 26.6 ± 2.16 (n=26/55); 27.05 ± 4.71 (n=6/55). Diabetes: 26/55 (n=3/55); 21/55 (n=2/55). Hypertension: 11/55 (n=3/55); 15/55 (n=2/55).

Measurement of serum concentrations of activin A by ELISA.

Transvaginal ultrasonography, operative hysteroscopy with histology, major surgery.
Biochemical screening of endometrial cancer: activin A

Criterion values and coordinates of the ROC curve for activin A

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
<th>+LR</th>
<th>95% CI</th>
<th>-LR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.346 *</td>
<td>81.82</td>
<td>69.1 - 90.9</td>
<td>90.91</td>
<td>80.0 - 96.9</td>
<td>9.00</td>
<td>7.7 - 10.5</td>
<td>0.20</td>
<td>0.07 - 0.5</td>
</tr>
</tbody>
</table>

Criterion values and coordinates of the ROC curve for endometrial thickness

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
<th>+LR</th>
<th>95% CI</th>
<th>-LR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;12 *</td>
<td>54.55</td>
<td>40.6 - 68.0</td>
<td>92.73</td>
<td>82.4 - 97.9</td>
<td>7.50</td>
<td>5.8 - 9.7</td>
<td>0.49</td>
<td>0.2 - 1.3</td>
</tr>
</tbody>
</table>

Activin A in endometriosis and endometrial cancer

- putative functions
- expression
- clinical usefulness
- molecular interactions

Activin A in endometriosis and endometrial cancer: inhibin showed minimal to moderate expression in well-differentiated endometrial cancer tissue, associated with histological grading, surgical staging, lymph node status and diabetes. Loss of inhibin expression resulted in a poorer survival.