Transformation of Endometrioma to Ovarian Cancer

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R Botschorischvili ++
N Bourdel
C Houlle
AS Gremeau
S Curinier
S Campagne

Dept Gynecologic Surgery
## Old Studies

Borgfeld Andolf (Acta Obst Gynecol Scand 2004)

<table>
<thead>
<tr>
<th>Age</th>
<th>Ovarian cyst</th>
<th>Functional cyst</th>
<th>Endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CI 95%</td>
<td>OR</td>
</tr>
<tr>
<td>10-29</td>
<td>2.23</td>
<td>1.29-3.86</td>
<td>1.76</td>
</tr>
<tr>
<td>30-49</td>
<td>0.83</td>
<td>0.6-1.16</td>
<td>0.86</td>
</tr>
<tr>
<td>50+</td>
<td>0.44</td>
<td>0.25-0.77</td>
<td>-</td>
</tr>
</tbody>
</table>
Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies


Summary
Background Endometriosis is a risk factor for epithelial ovarian cancer; however, whether this risk extends to all invasive histological subtypes or borderline tumours is not clear. We undertook an international collaborative study to assess the association between endometriosis and histological subtypes of ovarian cancer.

Table 3: Association between history of endometriosis and the histological subtypes of ovarian cancer

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Crude OR (95% CI)</th>
<th>p value</th>
<th>Stratified only OR (95% CI)</th>
<th>p value</th>
<th>Stratified and adjusted OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive</td>
<td>1.49 (1.34–1.65)</td>
<td>&lt;0.0001</td>
<td>1.53 (1.37–1.70)</td>
<td>&lt;0.0001</td>
<td>1.46 (1.31–1.63)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clear cell</td>
<td>3.73 (3.04–4.58)</td>
<td>&lt;0.0001</td>
<td>3.44 (2.78–4.27)</td>
<td>&lt;0.0001</td>
<td>3.05 (2.43–3.84)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>2.32 (1.94–2.78)</td>
<td>&lt;0.0001</td>
<td>2.20 (1.82–2.66)</td>
<td>&lt;0.0001</td>
<td>2.04 (1.67–2.48)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1.09 (0.76–1.58)</td>
<td>0.63</td>
<td>1.04 (0.71–1.51)</td>
<td>0.86</td>
<td>1.02 (0.69–1.50)</td>
<td>0.93</td>
</tr>
<tr>
<td>High-grade</td>
<td>1.11 (0.96–1.29)</td>
<td>0.16</td>
<td>1.16 (1.00–1.35)</td>
<td>0.056</td>
<td>1.13 (0.97–1.32)</td>
<td>0.13</td>
</tr>
<tr>
<td>Low-grade</td>
<td>2.02 (1.38–2.97)</td>
<td>&lt;0.0001</td>
<td>2.22 (1.48–3.31)</td>
<td>&lt;0.0001</td>
<td>2.11 (1.39–3.20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serous</td>
<td>1.26 (1.05–1.50)</td>
<td>0.012</td>
<td>1.19 (0.99–1.43)</td>
<td>0.062</td>
<td>1.12 (0.93–1.35)</td>
<td>0.24</td>
</tr>
<tr>
<td>Borderline</td>
<td>1.27 (0.97–1.67)</td>
<td>0.078</td>
<td>1.19 (0.90–1.57)</td>
<td>0.23</td>
<td>1.12 (0.84–1.48)</td>
<td>0.45</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1.31 (1.05–1.63)</td>
<td>0.015</td>
<td>1.28 (1.02–1.61)</td>
<td>0.034</td>
<td>1.20 (0.95–1.52)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

OR=odds ratio. *Stratified by age (5 year categories), ethnic origin (non-Hispanic white, Hispanic white, black, Asian, and other). †Stratified by age (5 year categories), ethnic origin (non-Hispanic white, Hispanic white, black, Asian, and other), and adjusted for duration of oral contraceptive use (never, <2 years, 2–4.99 years, 5–9.99 years, ≥10 years), and parity (0, 1, 2, 3, ≥4 children).
In the pooled analysis, reported a history of endometriosis

- 738 (9.3%) of 7911 women with invasive epithelial ovarian cancer
- 168 (8.8%) of 1907 with borderline ovarian cancer
- 818 (6.2%) of 13 226 controls
- 136 (20.2%) of 674 women with clear-cell
- 169 (13.9%) of 1220 with endometrioid
- 31 (6.0%) of 516 with mucinous,
- 261 (7.1%) of 3659 with high-grade serous
- 31 (9.2%) of 336 with low-grade serous subtypes of invasive ovarian cancer

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Invasive</th>
<th>Clear cell</th>
<th>Endometrioid</th>
<th>Invasive Serous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>818/13226</td>
<td>738/7911</td>
<td>136/674</td>
<td>169/1220</td>
<td>31/336</td>
</tr>
<tr>
<td>(6.2%)</td>
<td>(9.3%)†</td>
<td>(1.31–1.63)</td>
<td>(2.43–3.84)</td>
<td>(1.67–2.48)</td>
<td>(9.2%)†</td>
</tr>
</tbody>
</table>

†: Confidence interval.
Conclusions

Our findings suggest that the association of a history of endometriosis with increased risk of ovarian cancer is only apparent for invasive low-grade serous, clear-cell, and endometrioid subtypes.
Epidemiology of Endometriosis may be changing ....
Clinical Characteristics of Patients in Japan with Ovarian Cancer Presumably Arising from Ovarian Endometrioma

Fuminori Taniguchi\textsuperscript{a} Tasuku Harada\textsuperscript{a} Hiroshi Kobayashi\textsuperscript{b} Kunihiko Hayashi\textsuperscript{c} Mikio Momoeda\textsuperscript{d} Naoki Terakawa\textsuperscript{a}

\textsuperscript{a}Department of Obstetrics and Gynecology, Tottori University Faculty of Medicine, Yonago, and \textsuperscript{b}Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, \textsuperscript{c}Department of Laboratory Science and Environment Health Sciences, Gunma University School of Health Sciences, Maebashi, and \textsuperscript{d}Department of Women's General Medicine, St. Luke General Hospital, Tokyo, Japan
Clinical Characteristics of Patients in Japan with Ovarian Cancer Presumably Arising from Ovarian Endometrioma

Taniguchi F.a · Harada T.a · Kobayashi H.b · Hayashi K.c · Momoeda M.d · Terakawa N.a

- **Patients**: Thirty-three patients diagnosed with ovarian cancer presumably arising from endometrioma were recruited retrospectively. These patients had been followed for at least 2 years after the ovarian endometrioma diagnosis, then continued to be followed after they had been found to have malignant transformation.

- **Results**: The average age of the patients was $47.7 \pm 9.3$ years; 75.7% were premenopausal at the time of diagnosis of ovarian cancer.

- The average latent period from the diagnosis of endometrioma to that of malignant transformation was $8.3 \pm 4.6$ years (range: 2–17 years).

- Among the 33 patients, all cases developed in the ipsilateral ovary, in 6 cases after a cystectomy.

- 28 patients were diagnosed with stage I ovarian cancer, and major histotypes were clear cell in 23 cases and endometrioid in 8.

- Before surgery for cancer, mural nodules within the endometriomas were detected in 32 patients, and 1 patient had a small 3-mm nodule. In 30 patients, the diameter of the tumor doubled in size 6 months prior to the diagnosis of malignant transformation.

- **Conclusions**: To detect malignant transformation of ovarian endometrioma early and precisely, the clinician should determine the existence of a mural nodule and assess the rapid growth of the endometrioma.

Gynecol Obstet Investigation 2014
We probably should think twice when proposing a non-surgical approach to patients diagnosed with ovarian endometriomas.
Conclusion

« The hormonal ablative treatment of endometriosis may increase the risk of malignant transformation in the endometriotic implants by causing a negative selection and increasing the rate of dyskaryosis and loss of heterozygosity. »
Endometriosis and Its Treatment with Danazol or Lupron in Relation to Ovarian Cancer

Carrie M. Cottreau, Roberta B. Ness,1 Francesmary Modugno, Glenn O. Allen, and Marc T. Goodman
University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania [C. M. C., R. B. N., F. M., G. O. A.];
University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania [F. M., G. O. A.]; and Cancer Research Center, University of Hawaii, Honolulu, Hawaii [M. T. G.]

<table>
<thead>
<tr>
<th>Medication</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
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<tbody>
<tr>
<td>All women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danazol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither medication</td>
<td>1354</td>
<td>1962</td>
<td>3.1 (1.2–8.3)</td>
<td>3.2 (1.2–8.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupron or nafarelin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither medication</td>
<td>1354</td>
<td>1962</td>
<td>0.9 (0.4–2.2)</td>
<td>1.0 (0.4–2.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women with endometriosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danazol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither medication</td>
<td>101</td>
<td>113</td>
<td>2.9 (1.0–8.5)</td>
<td>2.9 (1.0–8.5)</td>
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<tr>
<td>Yes</td>
<td>13</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupron or nafarelin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither medication</td>
<td>101</td>
<td>113</td>
<td>1.3 (0.6–4.4)</td>
<td>1.4 (0.5–4.1)</td>
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<tr>
<td>Yes</td>
<td>9</td>
<td>8</td>
<td></td>
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</table>

a Adjusted for age and gravidity as continuous variables and oral contraceptive use, and family history of ovarian cancer use as dichotomous variables.
Atypical Endometriosis

- A Precursor of Malignancy

Lagrenade and Silverberg Hum Pathol 1988

We present five cases in which ovarian neoplasms (three clear cell carcinomas, two endometrioid carcinomas) were associated--and in four cases contiguous--with atypical glandular epithelial changes in endometriosis. This association has not been reported previously in the ovary, but four cases of extragonadal malignant tumors have been noted to occur with or after atypical endometriosis. We propose that a diagnosis of atypical endometriosis be followed by careful long-term observation of the patient to detect possible subsequent development of neoplasia.
Epithelial Abnormalities in Cystic Ovarian Endometriosis

Federico Prefumo, M.D.,* A Federica Todeschini, M.D.,† Ezio Fulcheri, M.D.,† and Pier Luigi Venturini, M.D.*

*Unità Operativa di Ostetricia e Ginecologia, Istituto “G. Gaslini”, Università di Genova, Genova, Italy; and †Sezione di Anatomia e Istologia Patologica, Dipartimento di Chirurgia e Methodologie Integrate, Università di Genova, Genova, Italy

**EPITHELIAL ABNORMALITIES IN ENDOMETRIOMAS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n)</th>
<th>Percentage (95% confidence interval)</th>
<th>Bilateral</th>
<th>Right</th>
<th>Left, n (%)*</th>
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</thead>
<tbody>
<tr>
<td>Metaplasia</td>
<td>41</td>
<td>12.1 (8.8–16.0)</td>
<td>5</td>
<td>11</td>
<td>25 (69%)</td>
</tr>
<tr>
<td>Squamous</td>
<td>6</td>
<td>1.8 (0.7–3.8)</td>
<td>1</td>
<td>1</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Ciliated</td>
<td>6</td>
<td>1.8 (0.7–3.8)</td>
<td>1</td>
<td>1</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>18</td>
<td>5.3 (3.2–8.3)</td>
<td>2</td>
<td>5</td>
<td>11 (69%)</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>19</td>
<td>5.6 (3.4–8.6)</td>
<td>1</td>
<td>4</td>
<td>13 (76%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1</td>
<td>0.3 (0.01–1.6)</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>32</td>
<td>9.4 (6.5–13.1)</td>
<td>2</td>
<td>9</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Simple</td>
<td>22</td>
<td>6.5 (4.1–9.7)</td>
<td>2</td>
<td>7</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>Complex</td>
<td>10</td>
<td>2.9 (1.4–5.4)</td>
<td>0</td>
<td>2</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>Atypia</td>
<td>20</td>
<td>5.9 (3.6–9.0)</td>
<td>2</td>
<td>7</td>
<td>11 (61%)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>14</td>
<td>4.1 (2.3–6.8)</td>
<td>0</td>
<td>7</td>
<td>7 (50%)</td>
</tr>
</tbody>
</table>

* Percentage of left abnormalities is calculated for unilateral cases only.
Atypia and complex hyperplasia are much more common in patients with cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Endometrioid carcinoma (n = 14)</th>
<th>Others (n = 325)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaplasia</td>
<td>2 (14%)</td>
<td>39 (12%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>8 (57%)</td>
<td>24 (7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Simple</td>
<td>1 (7%)</td>
<td>21 (6.5%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Complex</td>
<td>7 (50%)</td>
<td>3 (0.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Atypia</td>
<td>14 (100%)</td>
<td>6 (1.8%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Chi-square.

* Fisher’s exact test.
FIG. 1. Patient age in cases of cystic ovarian endometriosis with normal epithelial lining (n = 273), metaplasia (n = 43), hyperplasia (n = 24), atypia (n = 20), or endometrioid carcinoma arising in endometriosis (n = 14). The median, interquartile range, and extremes are shown. The differences between groups are statistically significant (P < 0.001, Kruskal–Wallis test).

FIG. 2. Patient age in cases of hyperplasia and atypia associated (gray boxes) or not associated (white boxes) with endometrioid carcinoma arising in endometriosis. The median, interquartile range, and extremes are shown. The differences between groups are statistically significant (hyperplasia P = 0.007, atypia P = 0.009, Mann–Whitney U test).
Ovarian atypical endometriosis: its close association with malignant epithelial tumours

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Date of submission 5 September 1995
Accepted for publication 2 September 1996

Ovarian atypical endometriosis: its close association with malignant epithelial tumours

The incidence of ovarian atypical endometriosis and its association with malignant epithelial tumours in a consecutive series of cases during the period 1987 to 1995 were studied. Atypical glandular changes were observed in four (1.7%) of 255 ovarian endometriosis cases and one patient with ovarian atypical endometriosis developed subsequent endometrioid carcinoma in the abdominal wall. Fifty-four (24.1%) of the 224 ovarian cancers were associated with ovarian endometriosis; 21 with typical and 33 with atypical endometriosis. Clear cell carcinomas and endometrioid carcinomas were most frequently associated with endometriosis, with 54% (27 of 50 cases) and 41.9% (13 of 31), respectively. Atypical endometriosis was found in 18 clear cell carcinomas, in seven endometrioid carcinomas, in four serous carcinomas, in three mucinous borderline tumours, and in one serous borderline tumour. In 13 cases, the atypical endometriosis was in contiguity with malignant epithelial tumours. We consider that atypical endometriosis possesses a precancerous potential or is most frequently associated with clear cell and endometrioid carcinomas. Close screening of cellular atypia or hyperplasia in ovarian endometriosis and careful long-term follow-up of patients with atypical endometriosis is required.

Keywords: atypical endometriosis, ovarian tumour
### Table 1. Ovarian malignant epithelial tumours and endometriosis

<table>
<thead>
<tr>
<th>Type</th>
<th>Endometriosis (%)</th>
<th>Typical endometriosis</th>
<th>Atypical endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell Ca</td>
<td>50</td>
<td>27 (54)</td>
<td>9</td>
</tr>
<tr>
<td>Serous Ca</td>
<td>63</td>
<td>6 (9.5)</td>
<td>2</td>
</tr>
<tr>
<td>Endometrioid Ca</td>
<td>31</td>
<td>13 (41.9)</td>
<td>6</td>
</tr>
<tr>
<td>Mucinous Ca</td>
<td>35</td>
<td>2 (5.7)</td>
<td>2</td>
</tr>
<tr>
<td>SBT</td>
<td>17</td>
<td>1 (5.9)</td>
<td>0</td>
</tr>
<tr>
<td>MBT</td>
<td>25</td>
<td>3 (12)</td>
<td>0</td>
</tr>
<tr>
<td>MMMT</td>
<td>2</td>
<td>1 (50)</td>
<td>1</td>
</tr>
<tr>
<td>Adenosarcoma</td>
<td>1</td>
<td>1 (100)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>224</strong></td>
<td><strong>54 (24.1)</strong></td>
<td><strong>21</strong></td>
</tr>
</tbody>
</table>

Ca, carcinoma; SBT, serous borderline tumour; MBT, mucinous borderline tumour; MMMT, malignant mixed müllerian tumour.

In 37 cases with endometriosis, 33 showed typical endometriosis and 29 had atypical endometriosis (25 cases had both).

The transition from typical endometriosis to atypical endometriosis was observed in 22 cases, and the transition from atypical endometriosis to carcinoma, in 23 cases.

Only one case showed a direct transition from typical endometriosis to carcinoma. The range of endometriosis was focal and adjacent to the carcinoma in most cases.

**Conclusions.** Ovarian carcinomas, especially clear cell and endometrioid adenocarcinomas, are highly associated with endometriosis. Atypical endometriosis shows proliferation activity intermediate to those of typical endometriosis and ovarian carcinoma, suggesting it is a precancerous status.
Endometriosis as the origin of Endometrioid carcinoma?

Which mechanisms?
Genetic alterations in Endometriotic tissue

Endometriosis is a monoclonal disease (7 studies)

Resolution of clonal origins for endometriotic lesions using laser capture microdissection and the human androgen receptor (HUMARA) assay

Result(s): Thirty-eight specimens were polymorphic and thus informative. Most specimens were monoclonal, as determined by the HUMARA assay. In four specimens of multifocal lesions, polyclonality was detected, but upon more refined microdissections and further analyses, we found that each focus was monoclonal individually.

Conclusion(s): Previously reported polyclonality is very likely to be attributed to the pooling of multifocal lesions or contamination of normal tissues. These results suggest that endometriotic lesions were monoclonal in origin, and in the case of multifocal lesions, each focus originates monoclonally; hence, different foci have independent origins. The monoclonality of endometriotic lesions suggests that they may carry neoplastic potentials, and the apparent independent origins of multifocal lesions suggest that reconstruction of individual lesion histories may help us to understand the initiation and progression of endometriosis. (Fertil Steril® 2003; 79(Suppl 1):710–7. ©2003 by American Society for Reproductive Medicine.)
Does endometriosis really have premalignant potential? A clonal analysis of laser-microdissected tissue

Doris Mayr, Gudrun Amann, Christina Siefert, Joachim Diebold, Birgit Anderegg*

Department of Pathology, Ludwig-Maximilians University Munich, D-80337 Munich, Germany

The technique used was different, is the polyclonality explained by the pooling of epithelial cells from different lesions?

Figure 5. Hypothetical pathogenesis of endometriosis. Monoclonal endometriotic lesions might develop by a single endometrial cell growing in a monoclonal fashion and, subsequently, relocating toward distant sites (A). Alternatively, a single endometrial cell might first spread to a distant site or organ, establish a monoclonal population there, and retain the potential to spread further (B). In stark contrast, we propose here a “polyclonal model,” which is characterized by an originally polyclonal endometrial population from which polyclonal cell groups relocate to distant sites and organs where they establish polyclonal endometriotic foci (C). This model leaves the possibility of a subsequent hypothetical step in which the polyclonal lesions might become monoclonal in a distinct further step, possibly leading to malignancy.
LOH: Loss of Heterozygosity

- LOH commonly indicates regions of tumor suppression gene inactivation
Eleven of the 40 (27.5%) endometriosis cases demonstrated LOH at one or more markers on chromosome arms 9p, 11q, and 22q as summarized in Table 3. Three cases showed LOH on chromosome 9p only, two on 11q only, and one on 22q only. The remaining five cases showed LOH on two or more of these chromosomes.
We examined 14 cases of endometriosis synchronous with ovarian cancer for loss of heterozygosity on 12 chromosome arms, X chromosome inactivation, and TP53 mutation to determine whether they shared genetic alterations.

In all four of the cases where the carcinoma had arisen within endometriosis and in five of the seven cases where the carcinoma was adjacent to the endometriosis, common genetic lesions were detected, consistent with a common lineage.

Common LOH events were detected in 9/11 of ovarian endometriosis adjacent to ovarian carcinoma.
LOH: Loss of Heterozygosity

- Prowse et al, in 4 endometrioid and 6 clear cell cancer with coexisting endometriosis, found 22 common LOH events
- Obata et al frequent LOH in atypical endometriosis
- Goumenou et al LOH in 36.4% of endometriosis foci close to malignant transformation
- 3 fold increase in LOH incidence in Stage II, suggesting that LOH accumulates with more severe disease.
- Sato et al identified LOH in 10q23.3 (location of the tumor suppressor gene PTEN) in
  - 56% of endometrial cyst (somatic mutation of PTEN in 20.6 of endometrial cyst)
  - 42 % of endometrioid carcinoma (somatic mutation of PTEN in 20 of endometrioid carcinoma))
  - and 27.3% of clear cell cancer. (somatic mutation of PTEN in 8.3% of clear cell carcinoma)
Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—Shifting the paradigm

Robert J. Kurman MD*, Ie-Ming Shih MD, PhD

Division of Gynecologic Pathology, Departments of Pathology, Gynecology and Obstetrics and Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

- Type I
  - Low grade serous
  - Mucinous
  - Low grade endometrioid
  - Clear cell

- Type II
  - High grade serous
  - High grade endometrioid ??
  - Carcinosarcomas
  - Undifferentiated
Thus, if retrograde menstruation accounts for most cases of endometriosis, it is logical to assume that endometrioid and clear cell tumors develop from endometrial tissue (mullerian derived) that implanted on the ovary and therefore the ovary is involved secondarily.

Of further interest has been the observation that the eutopic endometrium in women with endometriosis exhibits intrinsic molecular abnormalities, including activation of oncogenic pathways. (Bulun 2009)

Kurman 2010
Tubal ligation and the risk of ovarian cancer: review and meta-analysis

D. Cibula 1, M. Widschwendter 2, O. Málek 3, and L. Dusek 3

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Figure 5. Relative risk of ovarian cancer after tubal ligation. Invasive analysis of subgroups by histology (serous, mucinous, endometrioid). Subtotal and overall pooled estimates are supported by t-statistics and statistical tests for heterogeneity. Grey boxes represent weight of individual studies in overall meta-analysis.

Table VI Risk of OC after TL: final outcomes of performed meta-analyses.

<table>
<thead>
<tr>
<th>Analysis/outcome</th>
<th>Outcome of meta-analysis RRs relative risk</th>
<th>Comparison of subgroups (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Main analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC after TL/all studies/</td>
<td>0.69 (0.64–0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OC after TL/strict selection/</td>
<td>0.66 (0.60–0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OC after TL/extended selection/</td>
<td>0.74 (0.66–0.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subgroups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years since TL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>0.69 (0.51–0.93)</td>
<td>0.015</td>
</tr>
<tr>
<td>5–9</td>
<td>0.82 (0.65–1.05)</td>
<td>0.113</td>
</tr>
<tr>
<td>10–14</td>
<td>0.65 (0.44–0.97)</td>
<td>0.034</td>
</tr>
<tr>
<td>15–19</td>
<td>0.88 (0.58–1.35)</td>
<td>0.573</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive</td>
<td>0.68 (0.61–0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Borderline</td>
<td>0.86 (0.67–1.10)</td>
<td>0.227</td>
</tr>
<tr>
<td>Histology of Invasive OC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>0.73 (0.63–0.85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mucinous</td>
<td>0.92 (0.66–1.30)</td>
<td>0.653</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>0.40 (0.30–0.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BRCA1/2 mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>0.69 (0.53–0.89)</td>
<td>0.004</td>
</tr>
<tr>
<td>BRCA2</td>
<td>0.73 (0.42–1.24)</td>
<td>0.243</td>
</tr>
</tbody>
</table>

1CI: confidence interval.
2Statistically significant heterogeneity was observed within one of the subgroups.
3RR for endometrioid tumors is significantly lower (P < 0.001) than RR for serous and mucinous tumors.
Clear cell adenocarcinoma

ARID1A inactivating mutation of this tumor suppression gene
Functional Studies about the Loss of ARID1A Expression In Vitro and In Vivo

- Although the subject of intensive study, the exact function and role of ARID1A as a tumor suppressor remains far from being elucidated.

- In vitro studies have suggested different roles for ARID1A to exert its tumor suppressive action, which are mainly through proliferation, differentiation and apoptosis.

- ARID1A regulates hundreds of different genes through the SWI/SNF chromatin remodeling complex.

- Furthermore, it is likely that ARID1A mutations have divergent effects, depending on different cell and tumor types in which they are present, probably also depending on the mutational landscape in different cancer types. As a result, functional studies of ARID1A present a substantial scientific challenge.
**ABSTRACT**

**BACKGROUND**

Ovarian clear-cell and endometrioid carcinomas may arise from endometriosis, but the molecular events involved in this transformation have not been described.

**METHODS**

We sequenced the whole transcriptomes of 18 ovarian clear-cell carcinomas and 1 ovarian clear-cell carcinoma cell line and found somatic mutations in ARID1A (the AT-rich interactive domain 1A [SWI-like] gene) in 6 of the samples. ARID1A encodes BAF250a, a key component of the SWI–SNF chromatin remodeling complex. We sequenced ARID1A in an additional 210 ovarian carcinomas and a second ovarian clear-cell carcinoma cell line and measured BAF250a expression by means of immunohistochemical analysis in an additional 455 ovarian carcinomas.

**RESULTS**

ARID1A mutations were seen in 55 of 119 ovarian clear-cell carcinomas (46%), 10 of 33 endometrioid carcinomas (30%), and none of the 76 high-grade serous ovarian carcinomas. Seventeen carcinomas had two somatic mutations each. Loss of the BAF250a protein correlated strongly with the ovarian clear-cell carcinoma and endometrioid carcinoma subtypes and the presence of ARID1A mutations. In two patients, ARID1A mutations and loss of BAF250a expression were evident in the tumor and contiguous atypical endometriosis but not in distant endometriotic lesions.

**CONCLUSIONS**

These data implicate ARID1A as a tumor-suppressor gene frequently disrupted in ovarian clear-cell and endometrioid carcinomas. Since ARID1A mutation and loss of BAF250a can be seen in the preneoplastic lesions, we speculate that this is an early event in the transformation of endometriosis into cancer. (Funded by the British Columbia Cancer Foundation and the Vancouver General Hospital–University of British Columbia Hospital Foundation.)
The mechanism by which somatic mutations in ARID1A enable the progression of benign endometriosis to carcinoma is unclear.

ANALYSIS OF ARID1A IN ENDOMETRIOSIS ASSOCIATED WITH OVARIAN CANCER

Two patients with ovarian clear-cell carcinomas (samples CCC13 and CCC23) carrying ARID1A mutations had contiguous atypical endometriosis (Fig. 3, and Fig. 3 in the Supplementary Appendix). For one of the two patients, the specimen was heterozygous for an ARID1A truncating mutation (G6139T [E2047*]) in exon 20. This mutation was also found in 17 of 42 clones derived from atypical endometriosis but in none of 52 clones from a distant endometriotic lesion (P<0.001 by Fisher’s exact test) (Fig. 3C). Epithelial samples of both the atypical and normal underlying epithelium.
Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma

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Received July 5, 2012; Accepted August 3, 2012; Epub September 5, 2012; Published September 15, 2012

Table 4. Co-expression patterns of biomarkers in ovarian endometriosis and CCC

<table>
<thead>
<tr>
<th>biomarkers</th>
<th>BAF250a-/HNF-1b+ % (N)</th>
<th>BAF250a-/ER- % (N)</th>
<th>HNF-1b+/ER- % (N)</th>
<th>BAF250a-/HNF-1b+/ER- % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign endometriosis (N=36)</td>
<td>8.3% (3/36)</td>
<td>8.3% (3/36)</td>
<td>0% (0/36)</td>
<td>0% (0/36)</td>
</tr>
<tr>
<td>Atypical endometriosis (N=13)</td>
<td>23.1% (3/13)</td>
<td>23.1% (3/13)</td>
<td>46.2% (6/13)</td>
<td>23.1% (3/13)</td>
</tr>
<tr>
<td>CCC (N=26)</td>
<td>53.8%* (14/26)</td>
<td>46.2%* (12/26)</td>
<td>76.9%* (20/26)</td>
<td>42.3%* (11/26)</td>
</tr>
</tbody>
</table>

*p<0.01 vs. benign endometriosis (Fisher’s exact test).

Further increased to 42.3% in CCC. Therefore, the molecular alterations accumulate in a stepwise manner along the transformation process from benign endometriosis through atypical endometriosis to CCC. These data suggest that a portion of benign ovarian endometriosis has already undergone genetic alterations that lead to aberrant protein expression, possibly conferring a higher risk for malignant transformation.
Table 1. Studies that investigated AT-rich interacting domain containing protein 1A (*ARID1A*) mutations and protein expression in ovarian cancer with sequencing methods and by immunohistochemistry (IHC). OCCC, ovarian clear cell carcinomas; EnOC, endometrioid ovarian carcinomas.

<table>
<thead>
<tr>
<th>Authors, year of publication</th>
<th>Ovarian carcinoma subtypes</th>
<th>Loss of ARID1A protein expression</th>
<th>ARID1A mutations by sequencing methods</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones et al., 2010</td>
<td>42 OCCC</td>
<td>-</td>
<td>57% somatic ARID1A mutations in a total of 42 OCCC</td>
<td>[2]</td>
</tr>
<tr>
<td>18 OCCC tumor samples and 1 OCCC cell line (whole transcriptome) — discovery cohort</td>
<td>Loss of ARID1A protein expression correlated strongly with the presence of ARID1A mutations in the mutation discovery and validation cohort.</td>
<td>Somatic ARID1A mutations (3 nonsense, 2 insertion/deletion, 1 missense and 1 gene rearrangement) in the discovery cohort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiegand et al., 2010</td>
<td>210 ovarian carcinomas and a second OCCC cell line (<em>ARID1A</em> sequencing); mutation validation cohort</td>
<td>ARID1A mutations in 55 of 119 OCCC (46%), 10 of 33 EnOC (30%) and none of the 76 high-grade serous ovarian carcinomas.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>455 ovarian carcinomas (IHC validation cohort)</td>
<td>Loss of ARID1A protein expression in 55 (42%) of 132 OCCC, 39 (31%) of 125 EnOC, and 12 (6%) of 198 high-grade serous ovarian carcinomas.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maeda et al., 2010</td>
<td>OCCC</td>
<td>Negative ARID1A expression in 88 of 149 (59%) OCCC tumor samples by IHC</td>
<td>Sequencing of 12 OCCC tumor samples; 9 samples with ARID1A mutations and 3 with wild-type expression</td>
<td>[99]</td>
</tr>
<tr>
<td>Guan et al., 2011</td>
<td>serous and mucinous OC</td>
<td>No loss of ARID1A expression in 221 high-grade serous, 15 low-grade serous, and 36 mucinous ovarian carcinomas</td>
<td>No ARID1A mutations detected in 32 high-grade serous, 19 low-grade serous and 5 mucinous ovarian carcinomas</td>
<td>[88]</td>
</tr>
<tr>
<td>Katagiri et al., 2011</td>
<td>OCCC</td>
<td>Loss of ARID1A expression in 9 (15%) of 60 OCCC</td>
<td>-</td>
<td>[100]</td>
</tr>
<tr>
<td>Yamamoto et al., 2012</td>
<td>OCCC</td>
<td>Loss of ARID1A expression in 23 (55%) of 42 OCCC</td>
<td>-</td>
<td>[101]</td>
</tr>
<tr>
<td>Yamamoto et al., 2012</td>
<td>90 cases of primary OCCC (including 42 previously examined)</td>
<td>Loss of ARID1A expression in 44% of 90 OCCC samples</td>
<td>-</td>
<td>[102]</td>
</tr>
<tr>
<td>Lowery et al., 2012</td>
<td>212 OCCC and EnOC</td>
<td>Loss of ARID1A expression in 34 (41%) of 82 OCCC and 62 (48%) of 130 EnOC</td>
<td>-</td>
<td>[103]</td>
</tr>
<tr>
<td>Samartzis et al., 2012</td>
<td>136 ovarian cancer samples as study control (23 OCCC, 28 EnOC, 63 serous ovarian carcinomas, 15 mucinous ovarian carcinomas)</td>
<td>Loss of ARID1A expression in 5 (22%) of 23 OCCC, 13 (46%) of 28 EnOC, 7 (11%) of 63 serous ovarian carcinomas, 4 (27%) of 15 mucinous ovarian carcinomas</td>
<td>-</td>
<td>[104]</td>
</tr>
</tbody>
</table>
2.3. Loss of ARID1A Expression in Endometriosis

Mutations of ARID1A have been demonstrated in atypical endometriosis that, in contrast to the adjacent OCCC tissue, was negative for HNF-1β and retained estrogen receptor expression. This indicates that ARID1A mutations are an early event in the pathogenesis of endometriosis-associated ovarian carcinomas. In contrast to tumor-adjacent atypical endometriosis, no mutations or loss of ARID1A expression were found in the distal non-atypical endometriotic tissue of the same patients [1].

This observation sustains the theory of ARID1A being a tumor suppressor in which loss of expression occurs in cell clones that are undergoing a process of precancerous alteration. However, it remains controversial at which stage of pathogenesis ARID1A mutations occur in endometriosis, i.e., if they are limited to atypical endometriosis or if they already occur in a low-frequent manner in non-atypical endometriosis or at the early transition stage from non-atypical to atypical endometriosis.

To date, ARID1A sequencing studies are lacking in non-carcinoma-related endometriosis, probably due to the fact that the occurrence of ARID1A mutations is expected to be low in endometriosis (considering that the relative risk to developing ovarian cancer during a lifetime is approximately 1.5%) and that ARID1A sequencing studies are technically challenging, due to the large size of the gene and the random distribution of the mutations along the gene. Nevertheless, immunohistochemical data from different studies indicated that loss of ARID1A expression is also observable in rare cases of non-atypical endometriosis, especially in endometriotic cysts of the ovary, also referred to as endomtriomas [101,104,106]. An overview of the studies that investigated ARID1A expression in endometriosis with or without relation to ovarian carcinomas is given in Table 2.

Despite the good correlation between the immunohistochemical negative ARID1A expression and its mutations (cf. Section 2.4.), it is not definitely clarified if these observations are the result of ARID1A mutations or epigenetic regulation. Sequencing analyses and further functional studies are warranted to elucidate the exact time point of the occurrence of ARID1A mutations and their role in (atypical) endometriosis.
• Clear cell adenocarcinoma

• PIK3CA a tumor suppressor gene involved in the PI3K/PTEN signaling pathway) in about 20%

PIK3CA mutation is an early event in the development of endometriosis-associated ovarian clear cell adenocarcinoma

Sohei Yamamoto, Hitoshi Tsuda, Masashi Takano, Keiichi Iwaya, Seiichi Tamai and Osamu Matsubara

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2 Obstetrics and Gynecology, National Defence Medical College, Saitama, Japan
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4 Pathology and Clinical Laboratory Division, National Cancer Centre Hospital, Tokyo, Japan

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Activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway supports multiple mechanisms responsible for cancer progression, including proliferation, inhibition of apoptosis, cell adhesion and transformation.

In endometriosis, it has been shown that the PI3K/AKT pathway regulates FOXO1 protein levels, a member of the forkhead-box O family and the decidua-specific gene IGF binding protein-1 (IGFBP-1), which are both involved in the decidualization of endometrial cells.

Overactivation of PI3K/AKT led to reduced decidualization in primary endometriotic stromal cells issuing from endometriomas.

Both inhibition of PI3K and AKT led to increasing nuclear FOXO1 and IGFBP1 levels in response to treatment with medroxyprogesterone acetate and dibutyryl cAMP, supporting evidence that the increased PI3K/AKT pathway is involved in the reduced decidual response in endometriosis.

It may indicate that the PI3K/AKT pathway is involved in processes supporting the effects of progesterone resistance, a well-described characteristic of endometriosis.

Therefore, small molecule inhibitors may be preclinically investigated as a therapeutic option, especially in overcoming progesterone resistance in endometriosis.
PI3K/AKT Pathway Alterations in OCCC and EnOC

- Activating mutations in *PIK3CA* encoding p110 the catalytic subunit of PI3K, have been described to occur in 33%–40% of OCCC. Activation of the PI3K/AKT pathway by loss of PTEN expression has been found in 40% of OCCC [130]. Finally, *AKT2* amplification was observed in 14% of OCCC [131].

- Nevertheless, preclinical and clinical phase-I studies have suggested that inhibition of this pathway may help to overcome resistance to chemotherapy in ovarian cancer
Figure 1. The mutation profile of TP53, KRAS, BRAF, PIK3CA, PTEN, and CTNNB1 in different histological types of ovarian epithelial neoplasms. The frequency of individual mutations is shown in the bar chart in various types of ovarian carcinoma including high-grade (HG) serous carcinoma, low-grade (LG) serous carcinoma, clear cell carcinoma, endometrioid carcinoma (EMCA), and mucinous carcinoma. The mutation frequency is estimated from several studies based on a sizable sample size. The frequency of PIK3CA mutation in clear cell carcinoma is based on the current study showing 46% in purified tumors and cell lines. The mutation frequency of PTEN and CTNNB1 has not been determined (ND) in low-grade serous carcinomas.
PIK3CA mutation is an early event in the development of endometriosis–associated ovarian clear cell adenocarcinoma

Sohei Yamamoto, Hitoshi Tsuda, Masashi Takano, Keichi Ilwaya, Seichi Tamae and Osamu Matsubara

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Abstract

Clear cell adenocarcinoma (CCA), a highly lethal histological subtype of ovarian carcinoma, is a type of human cancer with a high frequency of activating mutations in the PIK3CA gene. In this study, we aimed to determine how these mutations contribute to tumour development of CCAs. Exons 9 and 20 of the PIK3CA gene were analysed by direct genomic DNA sequencing of 23 CCAs with synchronous putative precursor lesions (ie endometriosis adjacent to carcinoma, with or without cytological atypia) and their mutational statuses were compared. Somatic mutations of the PIK3CA gene were detected in 10/23 (43%) carcinomas and in all cases the type of mutation was H1047R in the kinase domain. The identical H1047R mutation was also detected in the coexisting endometriotic epithelium, adjacent to the CCAs, in nine of ten (90%) cases. Moreover, in six of the nine lesions, the H1047R mutation was identified even in the endometrioses lacking cytological atypia. These findings provide evidence that mutations of the PIK3CA gene occur in the putative precursor lesions of CCA, strongly suggesting that they are very early events in tumorigenesis, probably initiating the malignant transformation of endometriosis. A specific kinase inhibitor to mutated PIK3CA may potentially be an effective therapeutic reagent against these carcinomas.

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PIK3CA mutation is an early event in the development of endometriosis-associated ovarian clear cell adenocarcinoma

Sohei Yamamoto, Hitoshi Tsuta, Masashi Takano, Keichi Iwaya, Seiichi Tamai and Osamu Matsubara

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Figure 2. Sequencing chromatograms for PIK3CA exon 20 in three representative cases. Arrows, peaks of the $A \rightarrow G$ substitution at nucleotide 3140, resulting in the H1047R mutation. Left panels (case 5) show a H1047R mutation in all three components: non-atypical and atypical endometrioses and the corresponding clear-cell adenocarcinomas (CCAs). Middle panels (case 12) show a H1047R mutation in both the atypical endometriosis and the corresponding CCA component, but the non-atypical endometriotic lesion in this case shows no mutation in exon 20. Right panels (case 8) show a H1047R mutation in both the CCA and non-atypical endometriosis adjacent to carcinoma, but the endometriosis distant from carcinoma shows no mutation in exon 20. Morphological distinction is not possible between mutation-harbouning (left inset) and mutation-lacking (right inset) endometriosis.
PIK3CA Mutations in Endometriosis-Associated Ovarian Cancer and Endometriosis
Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations

Sohei Yamamoto¹, Hitoshi Tsuda², Masashi Takano³, Seiichi Tamai⁴ and Osamu Matsubara⁵

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Among the precursor lesions adjacent to the 23 ARID1A-deficient carcinomas, 86% of the non-atypical endometriosis (12 of 14) and 100% of the atypical endometriosis (14 of 14), benign (3 of 3), and borderline (6 of 6) clear-cell adenofibroma components were found to be ARID1A deficient.

In contrast, in the 19 patients with ARID1A-intact carcinomas, all of the adjacent precursor lesions retained ARID1A expression regardless of their types and cytological atypia.

Among the 42 clear-cell carcinomas, somatic mutations of PIK3CA were detected in 17 (40%) tumors and majority (71%) of these were ARID1A-deficient carcinomas.

These results suggest that loss of ARID1A protein expression occurs as a very early event in ovarian clear-cell carcinoma development, similar to the pattern of PIK3CA mutation recently reported by our group, and frequently coexists (not mutually exclusive) with PIK3CA mutations.

Table 2 Comparison between the ARID1A immunoreactivity and PIK3CA mutations in ovarian clear-cell carcinomas

<table>
<thead>
<tr>
<th>PIK3CA mutations</th>
<th>Number of cases (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARID1A</td>
<td></td>
</tr>
<tr>
<td>In exon 9 or 20</td>
<td>Deficient</td>
<td>12 (71)</td>
</tr>
<tr>
<td>Present (n = 17)</td>
<td>Intact</td>
<td>5 (29)</td>
</tr>
<tr>
<td>Absent (n = 25)</td>
<td></td>
<td>11 (44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 (56)</td>
</tr>
</tbody>
</table>

0.083
Evidence for Cooperative Mechanisms between ARID1A and the PI3K/AKT Pathway
• Guan B et al (presented at a meeting not published…)

• This shows that ARID1A inactivation in itself is not sufficient to initiate tumor development and requires a second hit, possibly consistent with an alteration in the PI3K/PTEN/AKT pathway to lead to carcinogenic transformation [158].

• These observations are exciting for at least two important reasons.

• Firstly, it may explain the observation of ARID1A expression in non-atypical endometriosis made by several groups [101,104,106]; loss of ARID1A expression may be an early molecular event in these cases that might increase the overall risk of developing endometriosis-associated cancer, but in itself is not sufficient to initiate cancerogenesis.

• Secondly, it confirms that there is a close cooperative mechanism between ARID1A mutations and PI3K/AKT pathway alterations that might be of importance for early tumor detection, as well as in a therapeutic context. Interdependency on PI3K/AKT activation of ARID1A mutated tumor clones might be a process that is targetable by small-molecule inhibitors of the PI3K/AKT/mTOR pathway.
The role of PTEN ?
Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer

Daniela M Dinulescu¹,², Tan A Ince³,⁴,⁵, Bradley J Quade⁴,⁵, Sarah A Shafer¹,², Denise Crowley¹,² & Tyler Jacks¹,²

Epithelial ovarian tumors present a complex clinical, diagnostic and therapeutic challenge because of the difficulty of early detection, lack of known precursor lesions and high mortality rates. Endometrioid ovarian carcinomas are frequently associated with endometriosis, but the mechanism for this association remains unknown. Here we present the first genetic models of peritoneal endometriosis and endometrioid ovarian adenocarcinoma in mice, both based on the activation of an oncogenic K-ras allele. In addition, we find that expression of oncogenic K-ras or conditional Pten deletion within the ovarian surface epithelium gives rise to preneoplastic ovarian lesions with an endometrioid glandular morphology. Furthermore, the combination of the two mutations in the ovary leads to the induction of invasive and widely metastatic endometrioid ovarian adenocarcinomas with complete penetrance and a disease latency of only 7 weeks. The ovarian cancer model described in this study recapitulates the specific tumor histomorphology and metastatic potential of the human disease.

Figure 1. (facing page). Making a Mouse Model of Gynecologic Disease.

Dinulescu et al.³ genetically engineered mice to carry latent alleles of active mutant K-ras (left side) or active mutant K-ras and inactive Pten (right side). Injection of an adenviral Cre recombinase construct into the ovarian bursa led to tissue-specific expression of active mutant K-ras, resulting in pelvic endometriosis, or tissue-specific expression of active mutant K-ras and inactivation of Pten, resulting in metastatic endometrioid ovarian carcinoma.
Ovarian and endometrial endometrioid carcinomas have distinct **CTNNB1** and **PTEN** mutation profiles

Melissa K McConechy¹, Jiarrui Ding²,³, Janine Senz¹, Winnie Yang¹, Nataliya Melnyk¹, Alicia A Tone⁴, Leah M Prentice¹, Kimberly C Wiegand¹, Jessica N McAlpine³, Sohrab P Shah²,³, Cheng-Han Lee⁷, Paul J Goodfellow⁷, C Blake Gilks⁸ and David G Huntsman¹,²

**Table 1** Comparison of mutation frequencies in low-grade ovarian endometrioid carcinomas and low-grade endometrial endometrioid carcinomas

<table>
<thead>
<tr>
<th></th>
<th>Low-grade ovarian endometrioid (grades 1 and 2)</th>
<th>Low-grade endometrial endometrioid (grades 1 and 2)</th>
<th>Fisher's exact test (P-value)</th>
<th>Adjusted P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>5 (17%)</td>
<td>185 (67%)</td>
<td>1.08e-07</td>
<td>0.001</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>12 (40%)</td>
<td>107 (39%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ARID1A</td>
<td>9 (30%)</td>
<td>129 (47%)</td>
<td>0.086</td>
<td>0.120</td>
</tr>
<tr>
<td>KRAS</td>
<td>10 (33%)</td>
<td>50 (18%)</td>
<td>0.055</td>
<td>0.120</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>16 (53%)</td>
<td>76 (28%)</td>
<td>0.006</td>
<td><strong>0.020</strong></td>
</tr>
<tr>
<td>PPP2R1A</td>
<td>5 (17%)</td>
<td>19 (7%)</td>
<td>0.071</td>
<td>0.120</td>
</tr>
<tr>
<td>TP53</td>
<td>2 (7%)</td>
<td>28 (10%)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*P-values are adjusted using the Benjamini–Hochberg method. Values in bold are statistically significant.

Additional mutations have been verified by Sanger sequencing post original publication.

**Figure 1** Low-grade ovarian and endometrial endometrioid mutation profiles. (a) Low-grade endometrial endometrioid carcinomas, including grades 1 and 2 (n = 276). (b) Low-grade ovarian endometrioid carcinomas, including grades 1 and 2 (n = 30). Individual columns designate one tumor case, and rows indicate genes. All colored boxes specify a genetic alteration such as missense, truncating, indels, splice site mutations and combinations of these mutations, gray boxes indicate no mutations were identified by sequencing. These colors are specifically shown in the color legend.
Mutations in both *PTEN* and *PIK3CA* can act to maintain constitutively activated PI3K signaling (reviewed in Cully *et al*\textsuperscript{51}). This activation leads to the degradation of GSK3β and thus allows β-catenin nuclear translocation.\textsuperscript{52} The PI3K and Wnt features of these diseases. Mutations in *CTNNB1* and deregulation of the Wnt pathway are well-established pathways in cancer signaling first our study, the majority of *CTNNB1* mutations change the phospho-serine/threonine sites, which affects the ability of GSK3β to phosphorylate β-catenin and signal degradation. Lack of β-catenin phosphorylation results in nuclear accumulation causing expression of cell proliferation and inflammatory genes (reviewed in Klaus and Birchmeier\textsuperscript{49}) (Figure 2). Ovarian carcinomas with Figure 2 PI3K/AKT and WNT signaling pathways. These two signaling pathways show convergence on GSK3β and β-catenin. Genetic alterations caused by mutations in both pathways can result in the transcription of cell growth and proliferation genes. Mutations are indicated by black stars and are found in both ovarian and endometrial endometrioid tumors.
Endometriosis is thought to occur via retrograde menstruation, where endometrial epithelial cells travel from the uterus through the fallopian tubes and can establish as an endometriotic cyst within the ovary. This creates a unique microenvironment where menstruation-like blood and necrotic tissue is trapped within the cyst, resulting in high concentrations of iron in a confined space, causing oxidative stress and a hypoxic environment leading to DNA damage and mutation accumulation. The mutational analysis of a small number of endometriosis
There are 54 genes highly upregulated in cEOC. Among 54 genes highly upregulated in cEOC, 47 genes (87.0%) were associated with the redox-related genes (8, 12, 13) (Table I and Fig. 1). We found that the vast majority of genes that increased in cEOC overlap the oxidative stress-related genes.
• Retrograde menstruation or ovarian hemorrhage carries highly pro-oxidant factors, such as heme and iron, into the peritoneal cavity or ovarian endometrioma.

• Even a histologically normal ectopic endometrium might bear genetic damage caused by oxidative stress.

• DNA damage or loss of heterozygosity (LOH) caused by oxidative stress may be a critical factor in the carcinogenic process.

• These data support the hypothesis that several significant common pathways observed in cEOC overlap the datasets identified in genes involved in oxidative stress and detoxification pathway.
It became apparent that oxidative stress can initiate cell demise by apoptosis, but also prevent cell death by provoking adaptive responses that, in turn, facilitate cell proliferation or angiogenesis, thus contributing to tumor progression.

In the vast majority of endometriotic cells, the context of genetic alterations will shape the role of oxidative stress to affect susceptibility of cells to undergo oxidative stress-induced cell death.

However, the remaining endometriotic cells implying the acquisition of resistance to cell death allow adaptation and progression towards malignancy.

Targeting of oxidative stress may be an effective strategy to overcome carcinogenesis and progression of cEOC. In cEOC, a number of defense systems have evolved to combat the accumulation of iron-induced oxidative stress.
P 53 in endometriosis

- Bischoff et al, TP53 frequent in severe and late stage of the disease
- Nezhat et al 9% of benign and 25% of clear cell and endometrioid cancer (while negative in endometrioid cancer without endometriosis)
- Sainz de la Cuesta expression of TP 53 in transition from typical to atypical endometriosis
- Akahane TP53 mutation in 30.8 % of endometriosis associated with clear cell carcinoma
- 3 studies found no mutation in endometriosis without cancer (Vercellini 1994, Jiang X 1996, Bayramoglu H 2001)
- Other studies showed no mutation in clear cell ovarian cancer (Kurman 2010,2011)
Based on our findings and the literature, we support a model whereby persistent HPV infection, especially the high-risk types within an endometriosis lesion, along with an endometriotic cellular signature of genomic instability, steroid hormone overstimulation, chronic inflammation, and immune dysregulation could promote transformation to a malignant state.

It is also interesting to note that HPV genes are positively regulated by estrogen, which is in line with endometriosis as an estrogen-dependent disease.

This is in contrast to control tissues, which do not have these additional endometriotic molecular characteristics; therefore HPV infections are more likely to be cleared by the immune system.

Presently, we are following our HPV-positive group for possible indications of future carcinomas, and it will be important to perform further studies to investigate whether malignant neoplasias arising from endometriosis lesions are specifically connected to HPV infections.

Objective: To investigate whether sexually transmitted viruses or prokaryotes, like human papilloma viruses (HPV), herpes viruses, and Chlamydia trachomatis, are associated with endometriosis lesions.

Design: Sixty-six endometriosis lesions from 56 patients, including 49 peritoneum, 16 ovarian, and one endometrium, were analyzed using polymerase chain reaction–based ELISA and Invader technology. Thirty control tissues including endometrium and peritoneum from patient-matched (n = 13) and patients without endometriosis (n = 13) and one cervical carcinoma were tested for HPV DNA.

Setting: University hospital.

Patient(s): Seventy individual patients with and without endometriosis.

Intervention(s): Laparoscopy or laparotomy was performed, and endometriotic lesions were isolated.

Result(s): Herpes viruses and Chlamydia trachomatis were not detected in endometriosis lesions. High-risk and medium-risk HPV were detected in 11.3% of lesions, corresponding to 13.2% of patients. In addition, 27.5% of control tissues were positive for HPV high and medium risk. One HPV18-positive ovarian endometriosis also associated with an ovarian carcinoma. Associating clinical history with HPV-positive endometriosis and control tissues, all patients had a prior HPV cervical infection.

Conclusion(s): HPV infection in endometriosis lesions including control tissues supports spreading of the virus or HPV-infected endometrial cells via retrograde menstruation. Owing to an association of HPV in carcinomas, we propose that persistent HPV infection of endometriosis lesions could contribute to malignant progression. (Fertil Steril® 2010;93:1778–86. ©2010 by American Society for Reproductive Medicine.)

Key Words: Endometriosis lesions, HPV, HSV, Chlamydia trachomatis, endometriosis-associated carcinoma.
Endometriosis is associated with

- Increased levels of growth factors
  - TNF ……

- Increased levels of cytokines
  - IL1, IL6, IL8 ……..
    - IL1 may up regulate COX 2 expression with increased secretion of PGE2; PGE2 up regulates processes which are characteristic of tumor growth like angiogenesis, proliferation, inhibition of apoptosis

- Increased angiogenesis
Steroid Hormones

- Estrogen are related to the pathogenesis of most gynecologic cancer
- Estrogens seems to be associated with malignant transformation of endometriotic cysts
- Aromatase activity is found in endometriotic tissue while it is absent in normal endometrium (increased E2 production)
- $17\beta$ hydroxysteroid dehydrogenase type 1 is present in endometriotic lesions (increased conversion estrone to estradiol)
- $17\beta$ hydroxysteroid dehydrogenase type 2 is absent in endometriotic lesions (no conversion from estradiol to estrone)
- Estrogen action through ER alpha which is increased in endometriosis whereas ER$\beta$ is not increased
- The presence of the inhibitory progesterone receptor isoform A and the absence of the stimulatory progesterone receptor isoform B and progesterone receptor polymorphism may lead to further escalation of estrogen actions
Why should we understand those mechanisms?

From a therapeutic perspective, our results underscore the importance of carefully separating these different types of ovarian tumors in clinical trials evaluating different types of treatments. The relatively high frequency of PIK3CA mutations holds promise for new therapeutic approaches using small molecule inhibitors targeting PI3K. New PI3K targeting drugs, including GDC-0941, NVP-BEZ235, PI-103, and SF1126, a LY294002 prodrug, have recently been developed and are being evaluated in clinical trials.
In particular, the oncofetal protein IMP3 is epigenetically silenced soon after birth, with little or no detectable protein in normal adult tissues (24).

**Objective:** To determine whether the oncofetal protein IMP3 is detectable in endometriomas with or without histological atypia and whether IMP3 staining can be used as a triage tool to identify foci of atypical endometriosis in doubtful cases.

**Design:** Retrospective study.

**Setting:** Academic department and referral center for endometriosis.

**Patient(s):** A consecutive series of 516 women who underwent excision of 874 endometriomas.

**Intervention(s):** Histological review by three expert pathologists and immunohistochemical staining for IMP3.

**Main Outcome Measure(s):** Test performance of IMP3 immunohistochemistry versus first-round histology.

**Result(s):** The prevalence of atypical endometriosis was 1.7% (95% confidence interval [CI], 0.9%-3.3%) based on the number of women and 1.0% (95% CI, 0.5%-1.9%) based on the number of cysts. Three cases of atypical endometriosis were identified at first-round histological examination. Immunohistochemistry detected seven of the eight cases diagnosed as preneoplastic atypia at second-round histology and one case diagnosed as reactive atypia at second-round histology. The sensitivity of first-round histology was 33.3%, compared with 88.9% of IMP3 immunohistochemistry.

**Conclusion(s):** Immunohistochemical staining for IMP3 expression is a simple, inexpensive, and sensitive test that can be used in routine clinical practice as a triage tool to discriminate between cytological/structural atypia and confounding benign conditions. (Fertil Steril® 2013;99: 1974–9. ©2013 by American Society for Reproductive Medicine.)

**Key Words:** Endometriosis, endometrioma, atypical endometriosis, IMP3

**Discuss:** You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/vercellinip-imp3-biomarker-atypical-endometriosis/
Conclusions

• Evidence comes from atypical endometriosis with histologically proven transition from benign disease to malignancy

• Genetic studies suggest that endometriotic lesions may harbor mutations in genes critical to cancer development

• The frequency increases for cases associated with cancer and these cases support that endometriosis may be a malignant precursor of endometriosis

• These mechanisms may be important when choosing a long term management for our patients

• What are the consequences of long term medical treatments?

• What are the long term consequences of inflammation and retrograde menstruation?