The molecular genetics of endometrial cancer

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Introduction

• Classification of endometrial carcinoma
• Morphologic shortcomings of light microscopy
• Molecular genetics of endometrial carcinoma
• Application of genetic studies to diagnostics
• Remaining important diagnostic issues
• Mouse model to further explore diagnostic and treatment possibilities
Type I vs. II (Bokhman 1983)

Type I

- Unopposed estrogen (hyperplasia)
- Pre- and perimenopausal (mean age 59 years)
- Low to moderate grade, minimal myometrial invasion
- Good prognosis

Type II

- Lack of unopposed estrogen (atrophy)
- Postmenopausal (mean age in late 60s)
- High grade, often with metastases
- Poor prognosis (cause a disproportionate number of deaths)
Endometrial Tumorigenesis

Estrogen

NI epithelium

SH → CH → CAH → Endometrioid Ca

Atrophy

EIC → Serous Ca
Endometrial Hyperplasia

- Proliferative Endometrium
- Simple Hyperplasia
- Complex Hyperplasia
- Complex Atypical Hyperplasia
Uterine Endometrioid Carcinoma (UEC)

Complex Atypical Hyperplasia

Grade 1 UEC

Grade 2 UEC

Grade 3 UEC
Uterine Serous Carcinoma (USC)

Atrophic Endometrium
Endometrial Intraepithelial Carcinoma (EIC)
Serous Carcinoma
Serous Carcinoma
Endometrial Tumorigenesis

Estrogen

SH → CH → CAH → Endometrioid Ca

Atrophy → EIC → Serous Ca

1. Complex hyperplasia vs Complex atypical hyperplasia
   3% vs 25% risk of carcinoma

2. Complex atypical hyperplasia vs Carcinoma
   Hormone Rx vs TAH in younger women

3. Complex atypical hyperplasia vs EIC
   TAH vs TAH with staging

4. UEC vs USC
   TAH with limited staging vs staging and chemoRx
Molecular Genetics

- **PTEN mutational analysis**
  Exon specific PCR with direct sequencing
- **Microsatellite Instability**
  7 anonymous loci
- **KRAS mutational analysis**
  Oligonucleotide hybridization
- **TP53 mutational analysis**
  Exon specific PCR with direct sequencing
**Comparison of molecular genetic alterations between UEC and USC**

<table>
<thead>
<tr>
<th></th>
<th>UEC</th>
<th>USC</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTEN</strong></td>
<td>62%</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>MI</strong></td>
<td>28%</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>K-ras</strong></td>
<td>26%</td>
<td>2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>p53</strong></td>
<td>17%</td>
<td>93%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* 2-sided Fisher’s exact test
Clinical Utility of Molecular Markers

PTEN ? antibodies

MI May identify some HNPCC families

K-ras Studies on prognosis are conflicting

P53 Associated with a poor prognosis, immunostaining is used diagnostically
Molecular genetic alterations

- Supports the notion of two major types of endometrial carcinoma
- Provides some insight into the pathogenesis:
  - Early and late changes?
  - Relationship to one another?
  - Relationship to hormonal influence?
Endometrial Intraepithelial Carcinoma

H&E

p53
Glandular USC

H&E

p53
Endometrial Tumorigenesis

Estrogen

NI epithelium → SH → CH $\xrightarrow{1}$ CAH $\xrightarrow{2}$ Endometrioid Ca

Atrophy → EIC → Serous Ca

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Fundamental Questions

• Are mutations in \textit{PTEN} sufficient for the development of CAH or UEC?

• What is the relationship of \textit{PTEN} mutations and MI in the development of CAH and UEC?
Endometrial Tumorigenesis

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NI epithelium

PTEN

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MI

k-ras

p53

Atrophy

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p53
Endometrial Tumorigenesis

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Mouse model of UEC

Pten Knockout Mouse

- *PTEN* most commonly mutated gene in UEC
- Deletion of exon 5 (contains phosphatase domain)
- Genetic background: C57B6/129sJ
- Homozygous deletion: Embryonic lethal
- Heterozygous deletion: Variety of abnormalities including endometrial neoplasia
$Pten^{++}$ 32 weeks

$Pten^{+-}$ 32 weeks

$Pten^{+-}$ 40 weeks

CAH in human tissue
Morphologic variants of mouse carcinomas

Mucinous

Squamous
### Endometrial Lesions in *Pten* Heterozygous Mice

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>n</th>
<th>% of mice with lesions</th>
<th>No. (% of mice with invasive carcinoma)</th>
<th>No. of lesions per mouse (mean±SD)</th>
<th>LOH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>7</td>
<td>71.4%</td>
<td>0</td>
<td>1.14±1.07</td>
<td>NA</td>
</tr>
<tr>
<td>24</td>
<td>9</td>
<td>88.9%</td>
<td>0</td>
<td>9.78±5.91</td>
<td>30</td>
</tr>
<tr>
<td>32</td>
<td>9</td>
<td>100%</td>
<td>0</td>
<td>18.56±8.57</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>100%</td>
<td>2 (25%)</td>
<td>28.75±15.34</td>
<td>60</td>
</tr>
</tbody>
</table>
Immunohistochemical Analysis of Endometrial Lesions

PTEN

P-AKT
Histologic analysis of $Pten^{+/-}/Mlh1^{-/-}$ Mice

16 weeks

Multifocal CAH

14 weeks

Invasive carcinoma
ENDOMETRIAL LESIONS IN 14-18 WEEK MICE

<table>
<thead>
<tr>
<th>$Pten$ geno type</th>
<th>$Mlh1$ geno type</th>
<th>n</th>
<th>No. (%) of mice with lesions</th>
<th>No. (%) of mice with invasive carcinoma</th>
<th>No. of lesions per mouse (mean±SD)</th>
<th>Size of lesion (mm$^2$)</th>
<th>LOH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>-/-</td>
<td>5</td>
<td>5 (100)</td>
<td>2(40)</td>
<td>12.20±9.09</td>
<td>0.98±2.39</td>
<td>60</td>
</tr>
<tr>
<td>+/+</td>
<td>-/-</td>
<td>5</td>
<td>0</td>
<td>0</td>
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MI and Pten LOH
LOH of Pten and additional loci on chromosome 19

[Imagery of gel electrophoresis and Southern blot with DNA markers and bands]
Deletion in Exon 5 in a *Pten+/-/Mlh1-/-* CAH

Normal
Pten Sequence Analysis

Deletion A Exon 8  G to A transition Exon 5
Conclusions

• Mouse model mimics the human disease.

• Pten loss leads to hyperplasia but is not sufficient for invasion.

• DNA mismatch repair deficiency accelerates the phenotype, maybe in part due to increased mutation in the wild type allele of Pten (human disease).

• Objective markers of invasion would have clinical utility (mouse model).
Objective Markers of Invasion

• Use the mouse model to identify markers of invasion.

• Gene expression profiles of CAH vs carcinoma using Affymetrix Mouse Genome 430A

• Arrays were analyzed for differentially expressed genes between 8 CAH and 4 invasive carcinomas and specifically analyzed for those showing significant increased expression in carcinoma.

• Interesting candidates were confirmed by RT-PCR
RT-PCR of Ovgp1

Figure 1. Semi-quantitative RT-PCR for ovarian glycoprotein. Lanes 1, 2 and 3 invasive carcinoma. Lanes 4, 5, and 6 non-invasive lesions.
OGP Immunohistochemistry on human tissue

CAH

UCEC

H&E

OGP
PIK3CA Mutations

- PIK3CA mutations recently identified in endometrioid carcinoma
- PIK3CA is the catalytic subunit of PI3K an enzyme with activity that directly opposes the action of PTEN
- We recently investigated the status of PIK3CA in 44 cases of UEC and CAH 29 cases of CAH
- Mutations were found in 2(7%) of CAH and 17(39%) of UEC
- In contrast PTEN mutations were found in 14(48%) of CAH and 25(57%) of UEC
- PIK3CA mutation may be a marker of invasion
PTEN and PIK3CA Mutations in CAH and UEC

CAH

UEC

PTEN in CAH

PIK3CA in UEC

PTEN in UEC
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<th>CAH 2</th>
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<tr>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
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Atrophy       EIC    Serous Ca

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   Profiling 3% vs 25% risk of carcinoma

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Relationship of Hormones and Genetics

• UEC has been associated with the use of estrogen

• Association of tamoxifen and endometrial cancer remains controversial

• Recent studies have shown that AKT phosphorylates ER alpha in a ligand independent manner

• What is the relationship between PTEN and estrogen pathway
Alterations in hormone status

CD1 Pten het No rx 50 weeks

CD1 Pten het ovx 26 weeks

CD1 wt ovx/estrogen 24 weeks

CD1 Pten het estrogen 26 weeks
Alterations in hormone status

Wild type ovx 32 weeks

Pten het ovx 32 weeks

Pten het ER null 32 weeks
Conclusions

• Loss of Pten can lead to hyperplasia in the absence of estrogen

• Development of endometrial carcinoma is accelerated by estrogen treatment

• ER alpha is not required for Pten related tumor development and lack of ER alpha may be associated with a more aggressive phenotype

• ? Relevance to hormonal therapy for women with PTEN mutation positive endometrial carcinoma?
Summary

- Molecular genetics support the dualistic categorization of endometrial carcinoma

- *PTEN* plays a central role in the endometrial tumorigenesis and the absence of mismatch repair accelerates the process

- Objective markers of invasion (OGP and PIK3CA) may have an impact on management of women with CAH

- The relationship of *PTEN* mutations and hormones may change the approach to hormonal therapy
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