Integrated Genomic Characterization of Endometrial Carcinoma

Endometrial Disease and Analysis Working Groups

and

The Cancer Genome Atlas Research Network
Washington
Washington
Endometrial Cancer Origins

To lymph nodes

To omentum

The Cancer Genome Atlas
Types of Endometrial cancer

Endometrioid (low grade)  Serous (high grade)  Serous vs Endometrioid

- More solid
- Less glandular
- Higher grade nuclei
- Greater N:C ratio
- Loss of polarity
Poor Inter-Observer Reproducibility in the Diagnosis of High-Grade Endometrial Carcinoma
C. Blake Gilks MD, Esther Oliva MD, Robert A. Soslow MD

- In press – Am J Surg Path
- In 20 of 56 (35.8%) cases [high-grade endometrial carcinoma] there was a major disagreement

<table>
<thead>
<tr>
<th>Grading System/Parameter</th>
<th>Intraobserver Reproducibility (kappa value)</th>
<th>Interobserver Reproducibility (kappa value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New grade</td>
<td>0.8</td>
<td>0.76</td>
</tr>
<tr>
<td>FIGO grade</td>
<td>0.73</td>
<td>0.61</td>
</tr>
<tr>
<td>(three-tiered, 1 vs 2 vs 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGO grade</td>
<td>0.90</td>
<td>0.71</td>
</tr>
<tr>
<td>(two-tiered, 1 and 2 vs 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binary grade</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Architectural score</td>
<td>0.7</td>
<td>0.50</td>
</tr>
<tr>
<td>Nuclear grading (three-tiered)</td>
<td>0.66</td>
<td>0.33</td>
</tr>
<tr>
<td>Nuclear grading (two-tiered)</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>Mitotic activity (two categories)</td>
<td>0.75</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Endometrial Classification

- **Type I (85%)**
  - Endometrioid, prototype
  - Younger
  - Obese
  - Unopposed estrogen
  - Hyperplasia precursor
  - Usually confined to the uterus
  - Favorable outcome

- **Type II (15%)**
  - Serous, prototype
  - Older
  - Thin
  - Atrophic endometrium or endometrial polyp as precursor
  - More often metastatic at diagnosis
  - Worse prognosis
## Mutation Spectrum

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Prevalence in type I (%)</th>
<th>Prevalence in type II (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA mutation</td>
<td>~30</td>
<td>~20</td>
</tr>
<tr>
<td>Exon 9</td>
<td>7–15.5</td>
<td>0</td>
</tr>
<tr>
<td>Exon 20</td>
<td>10–34</td>
<td>21</td>
</tr>
<tr>
<td>PIK3CA amplification</td>
<td>2–14</td>
<td>46</td>
</tr>
<tr>
<td>KRAS2 mutation</td>
<td>11–26</td>
<td>2</td>
</tr>
<tr>
<td>AKT mutation</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>PTEN loss of function</td>
<td>83</td>
<td>5</td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td>20–45</td>
<td>0–5</td>
</tr>
<tr>
<td>Nuclear accumulation of β-catenin</td>
<td>18–47</td>
<td>0</td>
</tr>
<tr>
<td>E-cadherin loss</td>
<td>5–50</td>
<td>62–87</td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>~20</td>
<td>~90</td>
</tr>
<tr>
<td>Loss of function of p16</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>HER2 overexpression</td>
<td>3–10</td>
<td>32</td>
</tr>
<tr>
<td>HER2 amplification</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>FGFR2 mutations</td>
<td>12–16</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10.

Early stage vs late stage outcomes

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>2009 FIGO surgical stage</th>
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</thead>
<tbody>
<tr>
<td>Mixed epithelial carcinoma</td>
<td></td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td>IA</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>1,128</td>
</tr>
<tr>
<td>Endometrioid grade 1</td>
<td>182</td>
</tr>
<tr>
<td>Endometrioid grade 2</td>
<td>345</td>
</tr>
<tr>
<td>Endometrioid grade 3</td>
<td>272</td>
</tr>
<tr>
<td>Serous</td>
<td>258</td>
</tr>
<tr>
<td>Mixed Epithelial</td>
<td>117</td>
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<tr>
<td>2009 FIGO surgical stage IA</td>
<td>69.6</td>
</tr>
<tr>
<td>2009 FIGO surgical stage IB</td>
<td>12.6</td>
</tr>
<tr>
<td>2009 FIGO surgical stage II</td>
<td>65</td>
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<tr>
<td>2009 FIGO surgical stage IIIA</td>
<td>42</td>
</tr>
<tr>
<td>2009 FIGO surgical stage IIIIC1</td>
<td>77</td>
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<tr>
<td>2009 FIGO surgical stage IIIIC2</td>
<td>66</td>
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<tr>
<td>2009 FIGO surgical stage IVB</td>
<td>39</td>
</tr>
<tr>
<td>2009 FIGO surgical stage III</td>
<td>55</td>
</tr>
<tr>
<td>2009 FIGO surgical stage IV</td>
<td>405</td>
</tr>
<tr>
<td>Recurrent/Progression</td>
<td>349</td>
</tr>
</tbody>
</table>

GOG-209, unpublished data, courtesy of D. Miller

**Fig 2.**Cumulative incidence of recurrence by randomly assigned treatment group. (**) Deaths prior to recurrence.

*J Clin Oncol 30:695-700. © 2012*
Endometrial Tissue Requirements

- Primary, newly diagnosed, untreated, endometrial cancer
- Tissue specimen from the endometrium or uterus
- One of three general histologic subtypes as designated by DWG
  - Grade 1 or 2 endometrioid, grade 3 endometrioid, serous

Figure 1. Case composition in selected states. Values obtained from various GOG protocols.
### Sample Characteristics

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>373</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean, years (STD)</td>
<td>63 (11)</td>
</tr>
<tr>
<td>Range</td>
<td>31-90</td>
</tr>
<tr>
<td>Recurrent Disease</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72 (19.3%)</td>
</tr>
<tr>
<td>No</td>
<td>279 (74.8%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>22 (5.9%)</td>
</tr>
<tr>
<td>Vital Status</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>332 (89%)</td>
</tr>
<tr>
<td>Dead</td>
<td>39 (10.5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (0.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>EndoGr1</th>
<th>EndoGr2</th>
<th>EndoGr3</th>
<th>MixedGr3</th>
<th>SerousGr3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>78 (89%)</td>
<td>83 (79%)</td>
<td>70 (63%)</td>
<td>6 (46%)</td>
<td>17 (32%)</td>
<td>254 (69%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>3 (3%)</td>
<td>9 (9%)</td>
<td>6 (5%)</td>
<td>2 (15%)</td>
<td>5 (9%)</td>
<td>25 (7%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>7 (8%)</td>
<td>12 (11%)</td>
<td>26 (23%)</td>
<td>4 (31%)</td>
<td>25 (47%)</td>
<td>74 (20%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>(0%)</td>
<td>1 (1%)</td>
<td>9 (8%)</td>
<td>1 (8%)</td>
<td>6 (11%)</td>
<td>17 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (100%)</td>
<td>105 (100%)</td>
<td>111 (100%)</td>
<td>13 (100%)</td>
<td>53 (100%)</td>
<td>370 (100%)</td>
</tr>
</tbody>
</table>
### Data summary

<table>
<thead>
<tr>
<th>Assay</th>
<th>Number of endometrial patient specimens</th>
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<tbody>
<tr>
<td>Exome sequencing</td>
<td>248 pairs</td>
</tr>
<tr>
<td>Whole genome sequencing</td>
<td>107 pairs</td>
</tr>
<tr>
<td>RNA sequencing</td>
<td>333</td>
</tr>
<tr>
<td>miRNA sequencing</td>
<td>367</td>
</tr>
<tr>
<td>DNA methylation (Infinium HM450)</td>
<td>256</td>
</tr>
<tr>
<td>DNA methylation (Infinium HM27)</td>
<td>117</td>
</tr>
<tr>
<td>DNA copy number (Affymetrix SNP6.0)</td>
<td>363 pairs</td>
</tr>
<tr>
<td>Reverse phase protein arrays</td>
<td>293</td>
</tr>
</tbody>
</table>
DATA

CANCERGENOME.NIH.GOV
Somatic Copy Number Alterations
More genomic instability as tumors become less differentiated

Endometrioid Grade 1 or 2

Endometrioid Grade 3

Serous

Andrew Cherniack, Broad

Loss
Gain

The Cancer Genome Atlas
Copy number alteration clusters

Andrew Cherniack, Broad

24% of high-grade endometrioid tumors cluster with serous tumors (serous-like)

1q amplification

The Cancer Genome Atlas
GISTIC focal peaks

CN Cluster 1

CN Cluster 2

CN Cluster 3

CN Cluster 4
Mutations in select genes

- PTEN mutations are uncommon in Serous cases and very common in low grade Endometrioid cases
- TP53 mutations are uncommon in low grade Endometrioid cases and very common in serous cases
- PIK3CA mutations are distributed across histology and grade
- Higher frequencies than previous reports may be due to more comprehensive sequencing methods

<table>
<thead>
<tr>
<th>HistologyGrade</th>
<th>PTEN (Freq)</th>
<th>TP53 (Freq)</th>
<th>PIK3CA (Freq)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>EndoGr1</td>
<td>62 (0.83)</td>
<td>3 (0.04)</td>
<td>43 (0.57)</td>
<td>75</td>
</tr>
<tr>
<td>EndoGr2</td>
<td>62 (0.82)</td>
<td>9 (0.12)</td>
<td>38 (0.5)</td>
<td>76</td>
</tr>
<tr>
<td>EndoGr3</td>
<td>35 (0.71)</td>
<td>17 (0.35)</td>
<td>30 (0.61)</td>
<td>49</td>
</tr>
<tr>
<td>SerousGr3</td>
<td>1 (0.02)</td>
<td>39 (0.89)</td>
<td>19 (0.43)</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>160 (0.66)</td>
<td>68 (0.28)</td>
<td>130 (0.53)</td>
<td>244</td>
</tr>
</tbody>
</table>

Cyriac Kandoth and Li Ding, WashU
Mutation spectrum

- MSI, MLH1 meth, Few SCNA, high mutation rate
- MSS, Few SCNA, very high mutation rate
- MSS, Few SCNA, Low mutation rate
- MSS, Many SCNA, Low mutation rate

Cyriac Kandoth and Li Ding, WashU
Niki Schultz, Nils Weinhold, MSKCC
MSS, Few SCNA, very high mutation rate, different mutation spectrum (excess transversions), Universal POLE mutations, 13 of 17 (76%) with hotspot mutations, similar findings seen in CRC.
Progression free survival

Serous – poorest PFS
No difference between MSI and MSS groups
No events in small POLE group
Mutation spectrum

- All endometrioid, PTEN mutations, few TP53 mutations
- All endometrioid, PTEN mutations, no TP53 mutations
- TP53 mutations, few PTEN mutations, high grade tumors, serous and some endometrioid

- Cyriac Kandoth and Li Ding, WashU
- Niki Schultz, Nils Weinhold, MSKCC
SMGs
Unusual serous case

High mutation rate

No SCNA

No TP53 mutation

Serous histology
cBio patient portal

- Serous case, no TP53 mutation, no SCNA, high mutation rate
- Doesn’t make sense
Path c/w endometrioid histology, ?MSH6 mutation

Micropapillae
MicroRNA sequencing

Supplemental Figure 1
Methylation

PURITY ESTIMATE
MSI STATUS
MUTATION RATE
MLH1 HYPERMETHYLATION
TP53 MUTATION
MICRO RNA EXPRESSION
MRNA EXPRESSION
COPY NUMBER
HISTOLOGY
DNA METHYLATION CLUSTER

785 MOST VARIABLE CPG LOCI
789 X-LINKED CPG LOCI

NORMAL ENDOMETRIUM (N=27)
SEROUS AND ENDOMETRIOID ENDOMETRIAL TUMORS (N=373)
Gene expression clusters

- Mitotic cluster contains serous and serous-like cases
- Hormonal cluster contains samples with greater ER/PR expression
- Immunoresponsive cluster contains immune activated genes

Wei Zhang and Yuexin Liu, MDACC
Supervised RPPA

- DNA repair and proliferative genes in the RNASeq mitotic cluster
- High ER, PR, AR in the hormonal RNASeq subtype
- STAT3 activation and elevated LKB1, LCK and annexin in the immunoreactive RNASeq subtype.
Endometrial Proteomics Atlas

TCGA 210 samples

210 Antibodies

Median Centered Unsupervised Hierarchical Cluster

Signaling

Doris Siwak

Pink signaling on ER/PR/AR on
Green serous proliferative erbB2, ErbB3
Blue dark Signaling off ER/AR/PR off
Red MAPK selective on reactive
Blue light Collagen, caveolin and VEGFR on metabolism off

Serous

Not ER Gata3 IGFBP2 PR BCL2

Rab25 SetD2 Met Snail Rad51 Casp9 BiD

Gordon Mills, MDACC
PARADIGM

Cluster 3: MYC activation, low TP53 pathway activation due to mutation

Cluster 5: MYC and FOXA1/ER activity, TP53 intact

Cluster 1: Low MYC, high WNT signalling c/w CTNNB1 mutation in the MSS/Low SCNA/Low mutations group

Christina Yau and Chris Benz, Buck Institute
RAS/CTNNB1 pathway - MEMo

The diagram illustrates the RAS/CTNNB1 pathway and its components, including RTK/RAS/beta-catenin, proliferation, and SOX17 mutations. The pathway shows interactions and alterations in genes such as FGFR2, ERBB2, SOX17, CTNNB1, and FBXW7. The diagram also highlights 70% altered genes with hypermutated (71%), endometrioid low mutation rate (82%), and serous-like (50%) categories. SOX17 mutations are presented with missense variants A96G and S403I, which are associated with high mobility group domain and C-terminal transactivation domain binding to CTNNB1.
PI3K/AKT – most active in endometrial cancer

b. PI3K pathway

- **PIK3CA**
  - 55% 53% 47%
  - Proliferation, cell survival, translation

- **PIK3R1**
  - 40% 34% 13%

84% altered

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Homozygous deletion</th>
<th>Somatic mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypermutated (95%)</td>
<td>Endometrioid low mutation rate (92%)</td>
<td>Serous-like (60%)</td>
</tr>
</tbody>
</table>

- **PTEN**
- **PIK3R1**
- **PIK3CA**

### Genes

- **PIK3CA**
- **PIK3R1**

### Protein Domains

- **SH3_2**
- **RhoGAP**
- **SH2**

### Mutation Sites

- **R348**
PTEN mutations

LUSC – no PTEN hotspots

GBM – 10% at R130

UCEC – 38% at R130
PIK3CA

Breast – few x2 mutations

Colon – 10% in x2

UCEC – 25% in x2 (endometrioid, not serous)
SuperClusters

Rehan Akbani, MDACC
Do uterine serous, ovarian serous, and basal like breast carcinomas have a common molecular phenotype?

Case study – Cross-tumor comparisons: the power of TCGA
Multiplatform molecular similarities among ovarian serous, uterine serous, basal like breast

Andrew Cherniack, Hui Shen, Wei Zhang, Chris Benz, Peter Laird, Yuexin Liu, Christina Yau
Mutation frequencies vary across tumors
Case study - Answer

Do uterine serous, ovarian serous, and basal like breast carcinomas have a common molecular phenotype? Mostly

Genomic similarities are likely due to shared TP53 mutations, but it is possible that ovarian serous and uterine serous do have a common site of origin and differentiate according to microenvironment.
Summary

- Recurrent POLE mutations identified and associated with altered mutation spectrum and very high mutation rate
- PI3K/AKT pathway most activated in endometrial – ramifications for targeted inhibition, unique mutation spectra among genes
- Novel genomic stratification may complement or supplant histologic subtyping
  - Has immediate impact on current schizophrenic approaches to adjuvant treatment after hysterectomy
  - Warrants re-design of clinical trials with stratification or separation of subtypes
- In the era of ‘precision medicine’ these finding will help to bring targeted agents to the clinic in a rational manner
TCGA versus cigarettes
Announcement

- Endometrial Disease / Analysis Working Group meeting
- Today, Wednesday, 5pm – 7pm in Salon II
- Punch list
Acknowledgements

The Cancer Genome Atlas Research Network

Endometrial Tissue Source Sites

TCGA/NCI
Kenna Shaw
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Yuexin Liu
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Christina Yau
Josh Stuart

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Ari Kahn
Margi Sheth
Gordon Robertson
Andy Chu
Shauwo Meng
Nils Weinhold
Giovanni Ciriello

MSKCC
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Chris Sander
Agnes Viale
Niki Schultz
Ethan Cerami
Ronglai Shen
Narciso Olvera
Fanny Dao
Faina Bogomolniy
Robert Soslow

Others
Paul Goodfellow
Russell Broadus
Andrew Berchuck
Beth Karlan
Marc Goodman
David Mutch
Daphne Bell
Sean Dowdy
Boris Winterhoff
Jenny Lester

cancergenome.nih.gov
An AACR Special Conference on
ADVANCES IN OVARIAN CANCER RESEARCH: FROM CONCEPT TO CLINIC

September 18-21, 2013
J.W. Marriott Marquis, Miami, FL

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www.aacr.org/meetingcalendar