Molecular Pathology of Ovarian Carcinoma with Morphological Correlation

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Key Issues

• The major histotypes of ovarian carcinomas have distinctive, albeit partially overlapping, molecular signatures

• Genetic alterations in ovarian carcinomas deregulate specific cell signaling pathways

• Ovarian cancer treatment will likely evolve to include drugs that inhibit the signaling pathways known to be activated in a given tumor ("personalized medicine")
Major Types of Ovarian Carcinoma

- **Serous** (50%)
- **Mucinous** (10%)
- **Endometrioid** (20%)
- **Clear cell** (10%)
Treatment Guidelines for Ovarian Carcinoma

- Standard therapy is surgical debulking followed by chemotherapy (carboplatin + paclitaxel)
- In contrast to endometrial carcinoma, Rx is NOT histotype dependent
- Treatment of recurrent/drug-resistant disease remains a major challenge

References:
1) National Comprehensive Cancer Network, NCCN Clinical Practice Guidelines in Oncology, 2006
2) RF Ozols, Challenges for chemotherapy in ovarian cancer. Ann Oncol 17(Supp 5), 2006
On the horizon...

• “Personalized” medicine using drugs that target specific molecular defects in tumor cells

• Ovarian carcinomas have characteristic genetic alterations, but the frequency with which a given gene is mutated varies substantially with:
  – Histologic type
  – Tumor grade

• What role will pathologists play in determining the specific molecular defects in ovarian cancer cells?
Major Types of Ovarian Carcinoma: Characteristic Genetic Alterations (Selected)

- Serous (*p53*)
- Mucinous (*K-RAS*)
- Endometrioid (*CTNNB1, PTEN, K-RAS, p53*)
- Clear cell (?)

Reference:
1) Bell DA. Origins and Molecular Pathology of Ovarian Cancer. Mod Pathol, 18:S19-32, 2005
What are we learning about ovarian cancer?
Gene Expression Profiling of Ovarian Carcinomas

- Affymetrix oligonucleotide microarrays
- U133A array: approximately 22,000 probe sets (14,500 genes)
- **Data Processing**: quantile normalization to adjust for differences in probe intensity across different chips
- **Statistical Analyses**: Principal component analysis
First two principal components for 103 human samples, all probe-sets, log-transformed data

Clear Cell (N=8)
Endometrioid (N=37)
Mucinous (N=13)
Serous (N=41)
Normal (N=4)
Ovarian Endometrioid Adenocarcinoma (OEA) Tumor Progression Model

Genetic Alterations:
- Tumor suppressor genes: \( PTEN, \ p53 \)
- DNA mismatch repair genes: \( MSH2, \ MSH6, \ MLH1, \ MLH3 \)
- Oncogenes: \( K-RAS, \ CTNNB1/\beta\text{-catenin} \)
• Ovarian carcinomas arise through a multi-step process in which clonal selection acts on cells with somatic mutations and altered gene expression to allow outgrowth of progeny with increasingly aggressive growth properties.

• The genes mutated in cancer frequently encode proteins that function in conserved signaling pathways.
Wnt signaling plays major roles in:
- Cell proliferation
- Differentiation
- Morphogenesis

β-catenin plays a central role in the signal transduction pathway to the nucleus (canonical pathway)

The Wnt signaling pathway is frequently deregulated in cancers
Wnt Signaling Overview
Wnt/β-catenin/Tcf Pathway Defects
Ovarian Endometrioid Adenocarcinomas (OEAs)

- 72 primary OEAs collected (CHTN, UM, Kumamoto U.)
- All OEAs evaluated for mutations in CTNNB1 (β-cat) exon 3

Results
- Missense mutations found in 18 OEAs (25%)
- OEAs with CTNNB1 mutations show nuclear accumulation of β-cat by immunohistochemical staining
Other Wnt/β-catenin/Tcf Pathway Defects in Ovarian Endometrioid Adenocarcinomas: **APC**

- **CTNNB1** or **APC** mutations present in 26% of OEAs
WNT PATHWAY DEFECTS: CORRELATION WITH LOW TUMOR GRADE AND STAGE

<table>
<thead>
<tr>
<th></th>
<th>Grade 1</th>
<th>Grade 2 or 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-cat or APC mut</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>β-cat or APC wt</td>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>54</td>
</tr>
</tbody>
</table>

p = 1.2 X 10^{-6} (Fisher’s exact)

<table>
<thead>
<tr>
<th></th>
<th>Stage 1 or 2</th>
<th>Stage 3 or 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-cat or APC mut</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>β-cat or APC wt</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>28</td>
</tr>
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</table>

p = 1.5 X 10^{-5}
Modified from: DA Altomare and JR Testa (*Oncogene*, 2005)
### Mutational analysis of PTEN (n=72) and corresponding mutations of CTNNB1 and K-RAS in OEAs

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>PTEN mutation (exons 1-9)</th>
<th>CTNNB1 mutation (exon 3)</th>
<th>K-RAS mutation (codons 12 and 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE-13T</td>
<td>del T, exon 4, frameshift</td>
<td>TCT→TGT</td>
<td>None</td>
</tr>
<tr>
<td>OE-19T</td>
<td>GAG→TAG, exon 1, nonsense</td>
<td>TCT→TAT</td>
<td>None</td>
</tr>
<tr>
<td>OE-31T</td>
<td>TAT→AAT, exon 5 (Tyr→Asn)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>OE-48T</td>
<td>ACT→CCT, exon 5 (Thr→Pro)</td>
<td>GGA→GAA</td>
<td>None</td>
</tr>
<tr>
<td>OE-54T</td>
<td>ACG→AGG, exon 5 (Thr→Arg)</td>
<td>GAC→GCC</td>
<td>None</td>
</tr>
<tr>
<td>OE-55T</td>
<td>del ACTT, exon 8, frameshift</td>
<td>TCT→TTT</td>
<td>None</td>
</tr>
<tr>
<td>OE-63T</td>
<td>CAG→TAG, exon 6, nonsense</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>OE-75T</td>
<td>GAT→GGT, exon 5 (Gly→Asp)</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

### Mutational analysis of PIK3CA (n=72) and corresponding mutations of PTEN and CTNNB1 in OEAs

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>PIK3CA mutation</th>
<th>PTEN mutation</th>
<th>CTNNB1 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE-21T</td>
<td>H1047R, exon 20</td>
<td>None</td>
<td>GGA→GAA (Gly34Glu)</td>
</tr>
<tr>
<td>OE-31T</td>
<td>H1047R, exon 20</td>
<td>TAT→AAT, exon 5 (Tyr→Asn)</td>
<td>None</td>
</tr>
<tr>
<td>OE-55T</td>
<td>E542K, exon 9</td>
<td>del ACTT, exon 8, frameshift</td>
<td>TCT→TTT (Ser37Phe)</td>
</tr>
<tr>
<td>OE-71T</td>
<td>E542K, exon 9</td>
<td>None</td>
<td>GGA→GAA (Gly34Glu)</td>
</tr>
<tr>
<td>OE-75T</td>
<td>H1047R, exon 20</td>
<td>GAT→GGT, exon 5 (Gly→Asp)</td>
<td>None</td>
</tr>
</tbody>
</table>
Mutations in the Wnt/\(\beta\)-cat/Tcf and PI3K/Pten/Akt Pathways Frequently Co-Occur in OEAs

Correlation of *PTEN* and/or *PIK3CA* mutation with Wnt/\(\beta\)-cat/Tcf pathway defects in OEAs

<table>
<thead>
<tr>
<th></th>
<th>Wnt/(\beta)-cat/Tcf pathway DEFECTIVE</th>
<th>Wnt/(\beta)-cat/Tcf pathway INTACT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>PTEN</em> or <em>PIK3CA</em> mutation</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Wild type <em>PTEN</em> and <em>PIK3CA</em></td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>53</td>
</tr>
</tbody>
</table>

p=.0024 two-sided Fisher’s exact test
First two principal components for 99 tumors, all probe-sets, log-transformed data.

- Green = CTNNB1 mut
- Blue = APC mut
- P = Pten mut
- 3 = PIK3CA mut
First two principal components for 99 tumors, all probe-sets, log-transformed data.

- Green = CTNNB1 mut
- Blue = APC mut
- P = Pten mut
- 3 = PIK3CA mut
The Majority Of TP53 Mutations Are Missense Mutations

Germline:
- Missense: 72%
- Frameshift: 7%
- Nonsense: 8%
- Silent: 0%
- Other: 7%
- Splice: 6%

Somatic:
- Missense: 73%
- Frameshift: 9%
- Nonsense: 7%
- Silent: 5%
- Other: 4%
- Splice: 2%

Missense Mutations are Clustered in the DNA-binding Domain

<table>
<thead>
<tr>
<th>Region</th>
<th>Mut. frequency</th>
<th>Missense mut.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transactivation (1-42; 43-62)</td>
<td>1 %</td>
<td>50.8 %</td>
</tr>
<tr>
<td>Proline-rich (65-97)</td>
<td>2.3 %</td>
<td>45.4 %</td>
</tr>
<tr>
<td>DNA binding (102-292)</td>
<td>80 %</td>
<td>82.1 %</td>
</tr>
<tr>
<td>Oligomerisation (323-356)</td>
<td>3.4 %</td>
<td>36.4 %</td>
</tr>
<tr>
<td>Regulation (363-393)</td>
<td>0.3 %</td>
<td>72.7 %</td>
</tr>
</tbody>
</table>
TP53 Mutations in OEAs: Exons 5-8

- 32 mutations identified (n=72)
  - 81% missense
  - Remainder nonsense or frameshift
- 5 additional tumors showed intense and diffuse nuclear accumulation of p53 protein
  - Presumptive missense mutations outside of region sequenced
### p53 Mutations in OEAs: Association with High Tumor Grade and Stage

<table>
<thead>
<tr>
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<th>Grade 2 or 3</th>
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</thead>
<tbody>
<tr>
<td>Mutant p53</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>15</td>
<td>20</td>
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<td>18</td>
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**p = .0009**

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<tbody>
<tr>
<td>Mutant p53</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>31</td>
<td>4</td>
</tr>
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<td></td>
<td>44</td>
<td>28</td>
</tr>
</tbody>
</table>

**p = 3 X 10^{-6}**
p53 Mutations in OEAs: Negative Association with Wnt/β-Cat and/or PI3K/Pten Pathway Defects

|                   | Wnt/β-cat and/or PI3K/Pten Pathway DEFECT | Wnt/β-cat and PI3K/Pten Pathways INTACT |  
|-------------------|------------------------------------------|----------------------------------------|---
| Mutant p53        | 2                                        | 35                                     | 37
| Wild type p53     | 20                                       | 15                                     | 35
|                   | 22                                       | 50                                     | 72

p = 1.5 X 10^{-6}
First two principal components for 99 tumors, all probe-sets, log-transformed data

Green = CTNNB1 mut
Blue = APC mut
P = Pten mut
3 = PIK3CA mut

OE

First Principal Component
Second Principal Component

p53 mutation
First two principal components for 99 tumors, all probe-sets, log-transformed data.

- Green = CTNNB1 mut
- Blue = APC mut
- P = Pten mut
- 3 = PIK3CA mut

- OE

- p53 mutation
First two principal components for 99 tumors, all probe-sets, log-transformed data.
Conclusions

• The findings support subdivision of ovarian endometrioid adenocarcinomas into two subgroups
  – Low grade OEAs are characterized by frequent Wnt/β-cat/Tcf and PI3K/Pten pathway defects, infrequent p53 mutations, favorable outcome
  – High grade OEAs are characterized by frequent p53 mutations, infrequent Wnt/β-cat/Tcf and PI3K/Pten pathway defects, poorer outcome

• High grade OEAs have a similar gene expression profile to ovarian serous carcinomas (both have frequent p53 mutations)
Why does any of this matter…?
What can pathologists do to help…?

• Current morphological classification provides useful information

• Within a given histotype, specific molecular alterations are associated with tumor grade

• Immunostaining for signaling pathway components, properly interpreted, can substitute for selected mutational analyses
  – Nuclear accumulation of β-catenin (vs. membranous)
  – Loss of Pten (increased pAkt, pS6)
  – Nuclear accumulation of p53