The Role of Chromosomal Translocations in the Molecular Pathology of Sarcomas

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University of Pennsylvania School of Medicine

Overview of translocations in bone and soft tissue sarcomas

Importance of chromosomal translocations – consistency and specificity
Role in biology, diagnosis, and therapy

Translocations in alveolar rhabdomyosarcoma (RMS)
Common 2;13 translocation – generating PAX3-FKHR fusion
Single breakpoints – single fusion size
Variant 1;13 translocation – generating PAX7-FKHR fusion

Translocations in Ewing’s sarcoma
Common 11;22 translocation – generating EWS-FLI1 fusion
Breakpoint variability – generating variable sized fusions
Protein products – ETS transcription factor (FLI1) and RNA binding protein (EWS)
Variant translocations involving other ETS proteins with EWS or related FUS protein
Additional translocations in Ewing sarcoma-like tumors

EWS protein and related RNA binding proteins
Fusions involving EWS and transcription factor-encoding genes in other sarcomas
TET family of RNA binding proteins – multiple regions of homology
Additional fusions involving genes related to EWS in sarcomas
Exception – TCF12-CHN fusion in myxoid chondrosarcoma
Fusion protein function and specificity – relationship of fusion protein to target cell
Expanding story of EWS-ATF1 and related fusions – same fusion in different tumors
Clear cell sarcoma and angiomatoid fibrous histiocytoma
Other gene fusions associated with divergent tumor types

Methodologies for detection of chromosomal translocations
Considerations for use of established technologies (Southern, PCR, FISH)
Immunohistochemistry – strategy to detect fusion protein (example: DSRCT)
Available antibodies to detect fusion proteins - application to various tumors
Microarray analysis of fusion-positive sarcomas
Identification of downstream target genes and other cellular features
Example - Expression profiling of fusion positive and negative RMS
Use of microarray data to identify IHC markers for fusion-positive ARMS

Clinical utility of detection of chromosomal translocations
Differential diagnosis
Prognosis
Prognostic significance of sarcoma-associated gene fusions
Prognostic value of microarray data - example of fusion-positive ARMS
Minimal disease detection – example: Ewing’s sarcoma
Minimal disseminated disease in bone marrow
Minimal disease in peripheral blood stem cell collections

**Major Points:**

- These fusion genes are useful reagents in the differential diagnosis of bone and soft tissue sarcomas.

- The detection of these fusion products is complex because of multiple breakpoints and variant partners, and thus a negative result must be interpreted cautiously.

- The fusion of EWS or related genes to one of multiple transcription factor-encoding genes in many of these sarcomas complicates the use of EWS reagents in the differential diagnosis of these sarcomas. These fusions also raise an essential issue of the relationship of these aberrant fusion proteins to the specific tumor phenotype and target cell.

- Several examples have been found in which a fusion gene is associated with two or more completely unrelated tumor types, and thus these gene fusions are not absolutely specific for a single lineage.

- For several translocation-associated sarcomas, antibodies to the C-terminal fusion partner have been shown to be useful markers of the presence of the fusion protein.

- Microarray-based strategies to elucidate genes associated with these fusion-positive tumors and downstream targets of these fusion proteins are generating useful markers for differential diagnosis and prognosis of these tumors.

- A small number of studies have been performed to address the clinical significance of these fusion genes as minimal disease markers. In studies of Ewing’s sarcoma, potential utility has been found in the predictive value of minimal disseminated disease in bone marrow but not in the predictive value of minimal disease in peripheral blood stem cell collections.

**References:**


Barr FG: Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma, Oncogene 2001, 20:5736-5746

Davicioni E, Finckenstein FG, Shahbazian V, Buckley JD, Triche TJ, Anderson MJ: Identification of a PAX-FKHR gene expression signature that defines molecular classes and determines the prognosis of alveolar rhabdomyosarcomas, Cancer Res 2006, 66:6936-6946


Molecular Insights Into the Morphological Heterogeneity of Ovarian Carcinomas – Does Histological Type Matter?

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MAJOR HISTOLOGICAL TYPES OF OVARIAN CARCINOMAS

Serous (>50%)

Mucinous (<10%)

Endometrioid (10-20%)

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 (?)
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)

• Tissue samples: 4 normal ovaries, 99 primary ovarian carcinomas
  - 41 serous
  - 37 endometrioid
  - 13 mucinous
  - 8 clear cell
Principal Component Analysis

• Identifies a set of statistically independent projections, or components, of the expression data

• The first PC captures the greatest fraction of the overall variance in tumor gene expression compared to any other projection

• The second PC captures the greatest fraction of variance subject to being independent of the first PC

• Using any 2 PCs a pair of coordinates can be determined for each sample; tumors falling close together have more similar gene expression than tumors further apart
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)
First two principal components for 99 tumors, all probe-sets, log-transformed data
Ovarian Endometrioid Adenocarcinoma (OEA) Tumor Progression Model

Endometriosis → Atypical Endometriosis → Endometrioid Borderline Tumor → Endometrioid Adenocarcinoma

Genetic Alterations:
- Tumor suppressor genes (*PTEN, p53*)
- DNA mismatch repair genes (*MSH2, MSH6, MLH1, MLH3*)
- Oncogenes (*K-RAS, CTNNB1/β-catenin, PIK3CA*)
• 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 Overview

Nucleus

Cytoplasm

TCF Target Genes
Figure modified from review by T Reya and H Clevers (Nature Medicine, 2005)
Wnt/β-catenin/Tcf Pathway Defects
Ovarian Endometrioid Adenocarcinomas (OEAs)

- 72 primary OEAs collected (CHTN, UM, Kumamoto U.)
- Majority (60) from CHTN-GOG bank
- 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
### 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>
<th>Total</th>
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<tbody>
<tr>
<td><strong>Wnt pathway defect</strong></td>
<td>13</td>
<td>6</td>
<td>19</td>
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<tr>
<td><strong>Wnt pathway intact</strong></td>
<td>5</td>
<td>48</td>
<td>53</td>
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<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>54</td>
<td>72</td>
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\[ p = 1.2 \times 10^{-6} \] (Fisher’s exact)

<table>
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<th></th>
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<th>Stage 3 or 4</th>
<th>Total</th>
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<td>19</td>
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<tr>
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<td>53</td>
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<td><strong>Total</strong></td>
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\[ p = 1.5 \times 10^{-5} \]
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>
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<tr>
<td>OE-63T</td>
<td>CAG→TAG, exon 6, nonsense</td>
<td>None</td>
<td>None</td>
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<tr>
<td>OE-75T</td>
<td>GAT→GGT, exon 5 (Gly→Asp)</td>
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<td>None</td>
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## Mutational analysis of PIK3CA (n=72) and corresponding mutations of PTEN and CTNNB1 in OEAs

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

Correlation of *PTEN* and/or *PIK3CA* mutation with Wnt/β-cat/Tcf pathway defects in OEAs

<table>
<thead>
<tr>
<th></th>
<th>Wnt/β-cat/Tcf pathway DEFECTIVE</th>
<th>Wnt/β-cat/Tcf pathway INTACT</th>
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<tbody>
<tr>
<td><em>PTEN</em> or <em>PIK3CA</em> mutation</td>
<td>7</td>
<td>3</td>
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<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>
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</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
p53 is a Sensor of Various Stresses

Stress conditions

"Oncogenic" Stress
Excess growth signals
Activated oncogenes (e.g. RAS)

"Genotoxic stress"
UV, x, γ, rays
Carcinogens
Cytotoxic drugs

"Non-genotoxic" Stress
Oxidizing stress
Physical stress
Hypoxia?

Modulation of p53 activity

Binding to proteins

Induction of target genes
The Majority Of TP53 Mutations Are Missense Mutations
Missense Mutations are Clustered in the DNA-binding Domain

Mut. frequency
- Transactivation (1-42; 43-62) 1 %
- Proline-rich (65-97) 2.3 %
- DNA binding (102-292) 80 %
- Oligomerisation (323-356) 3.4 %
- Regulation (363-393) 0.3 %

Missense mut.
- 50.8 %
- 45.4 %
- 82.1 %
- 36.4 %
- 72.7 %
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>
<th></th>
<th>Grade 1</th>
<th>Grade 2 or 3</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Mutant p53</td>
<td>3</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>15</td>
<td>20</td>
<td>35</td>
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<td></td>
<td>18</td>
<td>54</td>
<td>72</td>
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</table>

- **p = 0.0009**

### Stage 1 or 2 vs. Stage 3 or 4

<table>
<thead>
<tr>
<th></th>
<th>Stage 1 or 2</th>
<th>Stage 3 or 4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant p53</td>
<td>13</td>
<td>24</td>
<td>37</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>31</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>28</td>
<td>72</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

<table>
<thead>
<tr>
<th></th>
<th>Wnt/β-cat and/or PI3K/Pten Pathway DEFECT</th>
<th>Wnt/β-cat and PI3K/Pten Pathways INTACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant p53</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>50</td>
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</tbody>
</table>

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

The scatter plot shows the distribution of tumors across the first two principal components. The markers represent different categories: OS, OM, OC, and OE.
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…?

Developing Models of Ovarian Cancer in Mice

Developing Models of Ovarian Cancer in Mice

\[ Pten^{loxP/loxP} \text{ transgenic mice provided by T. Mak (Ontario Cancer Institute, University of Toronto)} \]

\[ Apc^{loxP/loxP} \text{ transgenic mice provided by T. Noda (The Cancer Institute, Tokyo, Japan)} \]

\[ \{ Apc^{loxP/loxP}, Pten^{loxP/loxP} \} \quad \text{Cohorts of mice injected with AdenoCre (5 X 10^7 pfu) in the ovarian bursa; mice monitored for tumor growth} \]
OEA-like Tumors Arise in the Setting of APC and PTEN Inactivation in the Ovarian Surface Epithelium

A

B

C

D

E

% Survival

Weeks after AdCre injection

Weeks after AdCre injection

Days

0 5 10 15 20

0 10 20 30 40 50 60 70 80 90 100

0 2.5 5 7.5 10 12.5 15 17.5

0 4.142 8.72 14.785

0.092
APC-/PTEN- OEA-like Murine Tumors: Inhibition by Rapamycin

p=.00069, one-sided T-test on log-transform of tumor volume
Bioluminescence Imaging Strategy for Mouse Model of Ovarian Cancer

- Obtain ROSA26 (lox-stop-lox luciferase) mice (Kaelin and colleagues)
- Generate APC$^{loxP/loxp/PTEN^{loxP/loxp}/ROSA26^{L-S-L-Luc/+}}$ mice
- Inject ovarian bursa with AdCre, monitor tumor progression and response to drugs in vivo
Bioluminescence Imaging

1. $\text{Apc}^{\text{loxP/loxP}};\text{Pten}^{\text{loxP/loxP}};\text{R}26^{\text{L-S-L-Luc/+}}$

2. $\text{R}26^{\text{L-S-L-Luc}}$

6 wks

7 wks

8 wks

9 wks
What can pathologists do to help…?

• Current morphological classification provides useful information - classification schemes continuing to evolve

• 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 $\beta$-catenin (vs. membranous)
  – Loss of Pten (increased pAkt, pS6)
  – Nuclear accumulation of p53
The molecular genetics of endometrial cancer

Lora Hedrick Ellenson, M.D.
Department of Pathology and Laboratory Medicine
Weill Medical College of Cornell University
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

NI epithelium

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>
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<tbody>
<tr>
<td><strong>PTEN</strong></td>
<td>62%</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MI</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>
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</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 → CAH → Endometrioid 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

Atrophy → EIC → Serous Ca
Fundamental Questions

• Are mutations in *PTEN* sufficient for the development of CAH or UEC?

• What is the relationship of *PTEN* mutations and MI in the development of CAH and UEC?
Endometrial Tumorigenesis

Estrogen

\[ \text{NI epithelium} \rightarrow \text{SH} \rightarrow \text{CH} \rightarrow \text{CAH} \rightarrow \text{Endometrioid Ca} \]

Atrophy

\[ \text{EIC} \rightarrow \text{Serous Ca} \]

\[ PTEN \]

\[ \text{MI} \]

\[ k-ras \]

\[ p53 \]
Endometrial Tumorigenesis

NI epithelium

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
**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>
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<tbody>
<tr>
<td>16</td>
<td>7</td>
<td>71.4%</td>
<td>0</td>
<td>1.14±1.07</td>
<td>NA</td>
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<tr>
<td>24</td>
<td>9</td>
<td>88.9%</td>
<td>0</td>
<td>9.78±5.91</td>
<td>30</td>
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<tr>
<td>32</td>
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<td>100%</td>
<td>0</td>
<td>18.56±8.57</td>
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<tr>
<td>40</td>
<td>8</td>
<td>100%</td>
<td>2 (25%)</td>
<td>28.75±15.34</td>
<td>60</td>
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Immunohistochemical Analysis of Endometrial Lesions
Histologic analysis of *Pten*+/−/*M lh1*−/− 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>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>7</td>
<td>5 (71.4)</td>
<td>0</td>
<td>1.14±1.07</td>
<td>0.09±0.10</td>
<td>NA</td>
</tr>
</tbody>
</table>
MI and Pten LOH

(a) and (b) show different patterns in samples 1, 2, and 3.

(c) displays markers M through 5, with indications of MUT and WT.
LOH of Pten and additional loci on chromosome 19
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
OGP Immunohistochemistry on human tissue

CAH

H&E

OGP

UEC
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
Endometrial Tumorigenesis

Estrogen

SH  CH  1  CAH  2  Endometrioid Ca

NI epithelium

3  4

Atrophy  EIC  Serous Ca

1. Complex hyperplasia vs Complex atypical hyperplasia
   Profiling  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


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 between PTEN and estrogen pathway
Alterations in hormone status

CD1 Pten het No rx 50 weeks

CD1 wt ovx/estrogen 24 weeks

CD1 Pten het ovx 26 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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Suzanne Baker- St. Jude
Ken Korach- NIEHS
Molecular Characterization of Neoplasms of the Pancreas

March 2, 2008

Ralph H. Hruban, M.D.
Professor of Pathology and Oncology
The Sol Goldman Pancreatic Cancer Research Center
Disclosure

• Dr. Hruban has the potential to receive milestone payments and royalties from Anza Therapeutics as a result of the mesothelin invention
“If you've seen one Redwood, you've seen them all”

Ronald Reagan
(3/12/1966)
As paraphrased by Jerry Brown
“If you’ve seen one pancreatic cancer, you’ve seen them all”
Examples

1. Medullary carcinoma and microsatellite instability
2. Undifferentiated carcinomas and E-cadherin loss
3. Beta-catenin gene mutations in solid-pseudopapillary neoplasms
4. KRAS2 gene mutations in undifferentiated carcinomas with osteoclast-like giant cells
5. Chromosome 11p loss in pancreatoblastoma
6. PIK3CA and STK11 gene mutations in Intraductal Papillary Mucinous Neoplasms
Medullary Carcinoma

Poorly differentiated, Syncytial growth pattern, Pushing boarders
Microsatellite Instability (MSI)

1. MSI status has prognostic value—median survival for MSI cases of 62 months, versus 10 months (hazard ratio = 5.6; P = 0.007)

2. MSI status may have therapeutic implications—Fluorouracil (5FU)-based adjuvant chemotherapy benefits patients with stage II or stage III colon cancer with microsatellite-stable tumors but not those with tumors exhibiting high-frequency microsatellite instability

Nakata et al., Clin Cancer Res. 2002; 8: 2536-40.

Microsatellite Stable

Microsatellite Unstable

Microsatellite Instability (MSI)

3. Has implications for other family members
   • The medullary phenotype is highly associated with a family history of cancer in first-degree relatives (P < 0.001).


Medullary Carcinoma

Microsatellite Instability

Good Prognosis, Not 5-FU, Family Hx

Medullary
Undifferentiated Carcinoma

- A malignant epithelial neoplasm with a significant component showing no glandular structures or other features to indicate a definite direction of differentiation
- Mean survival of 5.2 months after diagnosis
Undifferentiated Carcinoma
Cytokeratin
E-cadherin Expression

<table>
<thead>
<tr>
<th>Proportion of cancers with E-cadherin loss</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noncohesive carcinoma</strong></td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
</tr>
<tr>
<td>- Anaplastic</td>
</tr>
<tr>
<td>- UCOCGC</td>
</tr>
<tr>
<td>Signet ring</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td><strong>Cohesive carcinoma</strong></td>
</tr>
<tr>
<td>Ductal adenocarcinoma</td>
</tr>
<tr>
<td>- Moderate</td>
</tr>
<tr>
<td>- Poor</td>
</tr>
<tr>
<td>Colloid carcinoma</td>
</tr>
<tr>
<td>- Moderate</td>
</tr>
<tr>
<td>- Poor</td>
</tr>
<tr>
<td>Overall</td>
</tr>
</tbody>
</table>

p<0.001

Winter and Iacobuzio, Clinical Cancer Research
E-cadherin
Survival in Relation to E-cadherin Status in Resection Specimens

Historical reference, n=1252

Winter and Iacobuzio, Clinical Cancer Research
Undifferentiated Carcinomas

- Loss of E-cadherin
- Non-cohesive
- Poor Prognosis
Undifferentiated Carcinoma with Osteoclast-like Giant Cells

- Malignant epithelial neoplasm composed of large benign appearing multinucleated giant cells admixed with atypical neoplastic mononuclear cells
- Highly aggressive neoplasms with a mean survival of <12 months
Macrophage
**KRAS2 Gene Mutations in the Components of an UCOCGC**

Osteoclast-like Giant Cell Tumors are undifferentiated carcinomas that arise directly from intraductal epithelial precursors – “Undifferentiated Carcinomas with Osteoclast-like Giant Cells”
Middle-aged Male with FAMMM Syndrome caused by a \textit{p16-Leiden} deletion, Jaundice and Weight Loss
Biopsy of an ampullary lesion was initially interpreted as reactive, but KRAS2 gene sequencing revealed a codon 12 mutation. A resection was performed revealing an UCOCGC of the pancreas

J-B Koorstra and GJA Offerhaus
Immunolabeling for p16 showed loss of expression in the mononuclear cells and retained expression in the giant cells.
Undifferentiated Carcinoma with Osteoclast-Like Giant Cells

KRAS2 gene mutation

Epithelial with Reactive Giant Cells

Poor Prognosis
Solid-Pseudopapillary Neoplasm
Solid-Pseudopapillary Neoplasms

- Clinically, the vast majority occur in young women (20’s), with a female to male ratio of 10-20:1
- Grossly well demarcated masses. On cross section, they are cystic and solid with areas of hemorrhage and necrosis
Solid-Pseudopapillary Neoplasms

• >90% have β-catenin mutations
• KRAS2 wild-type
• 15% TP53 mutations
• 0% DPC4, p16

Abraham et al., American Journal of Pathology. 2002;160:1361-1369
Solid-pseudopapillary or Pancreatic Endocrine Neoplasm?
Solid-Pseudopapillary Neoplasm

- Has therapeutic implications - Surgical resection, even the surgical resection of metastases is the treatment of choice
Solid-Pseudopapillary Neoplasm

Loss of β-catenin \[\rightarrow\] Non-cohesive \[\rightarrow\] Great Prognosis
Pancreatoblastoma
Pancreatoblastoma

- Malignant neoplasms showing multiple lines of differentiation including acinar differentiation and squamoid nests
- Endocrine and ductal differentiation may also be seen
- Occur primarily in children (1-15 years) - Previously called infantile pancreatic carcinoma
Abraham et al., *American Journal of Pathology*. 2001;159:1619-1627
Genetic Alteration in Pancreatoblastomas

- Associated with Beckwith-Wiedemann Syndrome
- 86% LOH on 11p*

Similar to other infantile embryonal tumors such as hepatoblastomas
- Hepatoblastoma
- Nephroblastoma
- Pleuropulmonary blastoma

Am J Pathol 159:1619
Pancreatoblastoma

LOH on 11p

Squamoid nests and acinar cells

Unified with other Primitive Neoplasms
Intraductal Papillary Mucinous Neoplasms
Unique Genetic Changes

- **PIK3CA**: Four mis-sense PIK3CA gene mutations in 36 IPMNs (11%)
- **STK11/LKB1**: Sequence analysis of a pancreatic cancer from a patient with PJS revealed loss of the wild-type allele of the STK11/LKB1 gene
- **STK11/LKB1**: Inactivation of STK11/LKB1, by homozygous deletions or somatic sequence mutations coupled with loss of heterozygosity, was also demonstrated in 4-6% of 127 sporadic pancreatic and biliary adenocarcinomas.


Peutz-Jeghers Syndrome - 132 fold increased risk of pancreatic cancer
Screening implications
ENDOSCOPIC ULTRASONOGRAPHY (EUS)

- High frequency US + endoscopy
- Screened 109 patients with PJS or a strong family history of pancreatic cancer

Canto et al, Clin Gastroenterol Hepatol. 2004 Jul;2(7):606-21
Canto et al, Clin Gastroenterol Hepatol. 2006; 4:766-81
Peutz-Jeghers Syndrome

47 y.o. W/F with 1.5 cm lesion

CT

EUS
IPMN with Carcinoma-In-Situ

Canto et al, Clin Gastroenterol Hepatol. 2004 Jul;2(7):606-21
Canto et al, Clin Gastroenterol Hepatol. 2006; 4:766-81
Almost half of the reduction in breast cancer mortality over the last 25 years has come from mammography

MOLECULAR ↔ MORPHOLOGY

PROGNOSIS

TREATMENT
Selected References