**Introduction**

Robert J. Kurman, M.D.

*The Johns Hopkins University School of Medicine*

**Pathogenesis**

- Since serous borderline tumors are rarely associated with invasive serous carcinomas it is generally believed that they are unrelated
  - But some SBTs recur as carcinomas
  - And mucinous, endometrioid, and clear cell carcinomas are often associated with their respective carcinomas
  - How can these disparate findings be reconciled?

**Pathogenesis and Clinical Implications**

- Most ovarian cancers present in an advanced stage and survival is poor
- Survival for stage I tumors is reported to be greater than 90%
- Accordingly a great effort has been made to detect ovarian cancer when it is stage I

**Pathogenesis and Clinical Implications**

- So far this effort, using CA 125 as a tumor marker and vaginal ultrasound, has been unsuccessful
- Why?
- Can something be done to improve this?
Molecular Pathology of Ovarian Carcinoma with Morphological Correlation

Kathleen R. Cho, M.D.

Molecular Genetics

- 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”)

Molecular Genetics (Endometrioid carcinomas as example)

- Does histologic type matter?
- Does tumor grade matter?
- What role can practicing pathologists expect to play in stratifying ovarian cancer patients into appropriate treatment groups?

Grading of Ovarian Carcinoma Two Approaches

Anais Malpica, M.D.
Patricia A. Shaw, M.D.

Grading

- Historically there has been no uniformly accepted grading system
- Shimizu et al have proposed a three tier system modeled after the Elston grading system for breast carcinoma
  - Applicable to all histological types of ovarian cancer
- Malpica et al have proposed a two tier system
  - Applicable only to serous carcinoma

Histotyping of Ovarian Carcinoma

Robert A. Soslow, M.D.
Histotyping

- Once borderline tumors are excluded does histotyping have any clinical relevance?
- How do we distinguish borderline endometrioid and clear cell borderline tumors from their respective carcinomas?
- How do we distinguish endometrioid from serous carcinomas when they are poorly differentiated?
- What exactly constitutes “clear cell carcinoma”?

Case Discussion
Panel and Audience

- Unknown cases posted on the ISGYP and USCAP websites
- Histotyping and grading

Pathogenesis of Ovarian Cancer Lessons from Morphology and Molecular Biology

A Proposed Model with Clinical Implications

Robert J Kurman, M.D.

Key Issues

- Epithelial carcinomas of the ovary can be divided into two categories designated Type I and Type II
- Type I carcinomas are slow growing, indolent tumors that develop from atypical proliferative (borderline) tumors
- Type II carcinomas are rapidly growing, aggressive tumors which develop de novo
- Understanding the pathogenesis of Type I and Type II carcinomas provides clues for new approaches to early detection and treatment

Histological Classification of Ovarian Tumors

FIGO Classification
Tumors of the Ovary - 1971

Cystomas
- (a) Benign cystadenomas
- (b) Cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)
- (c) Cystadencarcinomas

WHO Classification
Ovarian Tumours - 1973

Surface Epithelial-Stromal Tumors

Benign
- Cystadenoma
- Adenofibroma

Of borderline malignancy (carcinomas of LMP)
- Cystadenoma
- Adenofibroma

Malignant
- Adenocarcinoma
- Surface papillary carcinoma
- Malignant adenofibroma

Serous Borderline Tumors

The Birth of Borderline

The borderline group was carved out of the carcinoma category

and defined as a noninvasive group of tumors displaying cellular proliferation and cytologic atypia

Why was Borderline Created?

To account for
- “Intermediate” behavior between cystadenomas and frankly invasive carcinomas
- Inability to predict the outcome of an individual tumor

**Surface Epithelial-Stromal Tumors**

**Benign**
- Cystadenoma
- Adenofibroma

**Of borderline malignancy**
- Cystadenoma
- Adenofibroma

**Malignant**
- Carcinoma
- Surface papillary carcinoma
- Malignant adenofibroma

**WHO Classification**
**Ovarian Tumours - 2003**

**Histological Classification of Ovarian Tumors**

**WHO Classification**
**Ovarian Tumours - 1999**

**Dualistic Model of Ovarian Carcinogenesis**

**Type I**
- Low-grade
- Arise from precursor lesions in a stepwise fashion
  - Cystadenomas
  - Borderline Tumors
- Typically present as advanced stage

**Type II**
- High-grade
- Arise “de novo”
- Typically present as stage I
- Slow growing, indolent

Shih and Kurman
Am J Pathol 164:1511, 2004
Dualistic Model of Ovarian Carcinogenesis

Type I
- Low-grade
- Arise from precursor lesions in a stepwise fashion
  - Cystadenomas
  - Borderline Tumors
- Typically present as stage I
- Slow growing, indolent
- Generally remain low-grade

Type II
- High-grade
- Arise “de novo”
- Typically present as advanced stage
- Rapid growing, aggressive
- Can progress to high-grade

Shih and Kurman
Am J Pathol 164:1511, 2004

A “Classification” of Ovarian Carcinoma Based on Clinical, Pathologic, and Molecular Features

Type I
- Low-grade micropapillary serous carcinoma
- Mucinous carcinoma
- Endometrioid carcinoma
- Clear cell carcinoma
- Malignant Brenner tumor

Type II
- High-grade serous carcinoma
- MMMT (carcinosarcoma)

Shih and Kurman
Am J Pathol 164:1511, 2004

The Pathogenesis of Ovarian Cancer

Traditional View
- Ovarian cancer is regarded as a single disease and treated with one approach
- The reason is because most ovarian cancer is **serous** carcinoma

Serous Carcinogenesis - Current View
- Well differentiated carcinoma **progresses** to poorly differentiated serous carcinoma
- Carcinoma **begins in the ovary (stage I)**, spreads to the pelvic (stage II) and abdominal cavities (stage III) and beyond (stage IV)

Relationship of BOTs to Invasive Carcinoma
- SBTs are rarely associated with invasive carcinoma
- SBTs are a distinct entity unrelated to invasive carcinoma
- MBTs are frequently associated with invasive carcinoma
- Are some BOTs precursors and others not?
The Pathogenesis of Ovarian Cancer

- Recent advances in understanding the pathogenesis of ovarian carcinomas come from studies of serous and mucinous borderline tumors.

Can SBTs be divided into benign and malignant subtypes?

Hierarchical Branching
- Atypical Proliferative Serous Tumor
- Micropapillary Serous Carcinoma, Noninvasive

Noninvasive Micropapillary Serous Carcinoma

- Tumors with a hierarchical pattern had significantly better outcome than those with a nonhierarchical pattern.

Noninvasive MPSC with focus of early invasion

- Noninvasive tumors with a micropapillary pattern are variants of a borderline tumor and should not be classified as a noninvasive carcinoma because …

The Proposal that Noninvasive Micropapillary Tumors were Noninvasive Carcinomas

- there is no significant difference in the outcome of typical stage I SBTs compared to stage I noninvasive MPSCs.

The Controversy

- Noninvasive tumors with a micropapillary pattern are variants of a borderline tumor and should not be classified as a noninvasive carcinoma because …
The Controversy

• True but...
  – As reported in the literature there is also no significant difference in the outcome of typical stage I SBTs compared to

* Bona fide invasive stage I carcinoma which is >90% *

The Controversy

• There is no significant difference in the outcome of typical advanced stage SBTs compared to MPSCs if stratified into implant type
• Specifically, invasive implants determine behavior
  – True but...
  – virtually all studies have shown that MPSCs are significantly more often associated with invasive implants

What are Invasive Implants?

Sharp line between implant and underlying tissue
Looks like primary SBT

Irregular infiltration into underlying tissue
Looks like well differentiated serous carcinoma

Courtesy Debra Bell

MPSC with Invasive Implant

Ovarian tumor 1979
Omentum 1979
Pelvic mass 1997

Primary tumor
Invasive implant
Recurrent carcinoma

All look the same

MPSC with Invasive Implant (1991)

Vaginal Recurrence (1993)

Micropapillary architecture
If something looks like a duck, walks like a duck, and quacks like a duck
It is a duck!

Invasive implants are low-grade serous carcinomas

Are Typical SBTs Ever Associated with Invasive Implants?

- for all practical purposes they are not
- Typical SBTs are benign tumors that have the potential to undergo malignant transformation to a low-grade micropapillary serous carcinoma (noninvasive or invasive)

- Reports of invasive implants associated with typical borderline tumors …
- almost certainly missed occult areas of carcinoma (noninvasive or invasive MPSC) in the primary SBT
- even when the tumor was adequately sampled (1 block/cm of the greatest tumor dimension)

- Patient with bilateral typical SBTs with implants in parametrium and pelvic peritoneum
- One block/cm of greatest tumor dimension

Patient with bilateral typical SBTs with implants in parametrium and pelvic peritoneum

One block/cm of greatest tumor dimension
Up to 75% of low-grade invasive MPSCs are associated with SBTs.

Over 90% of these SBTs display a micropapillary pattern.

Conclusion - SBTs with a micropapillary pattern (noninvasive MPSCs) are the immediate precursors of low-grade invasive MPSCs.

Smith Sedheve et al

Malpica et al
Amer J Surg Pathol 28:496-504,2004

Behavior of Advanced Stage LG-Invasive MPSCs Compared to SBTs that Recur as LG-Invasive MPSCs

- **Group 1** - LG-Invas MPSC (n=112) vs
- **Group 2** - SBTs recurring as LG – Invas MPSC (n=41)
- Survival **Group 1** - 82 months
- Survival **Group 2** – 192 months
- Survival **Group 2** (from relapse as MPSC to death) – 83 months

Shvartsman et al – Unpublished data
In addition to clinical and pathologic differences, Low-grade (invasive micropapillary serous) CA and High-grade (conventional serous) CA are characterized by distinctive molecular genetic changes.

**Frequency of KRAS/BRAF and p53 Mutations in Ovarian Serous Tumors**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>KRAS/BRAF</th>
<th>p53</th>
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<tbody>
<tr>
<td>SBT *</td>
<td>31/51 (61%)</td>
<td>2/25 (8%)</td>
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<tr>
<td>Low-grade Serous Ca</td>
<td>15/22 (68%)</td>
<td>1/12 (8%)</td>
</tr>
<tr>
<td>High-grade Serous Ca</td>
<td>1/140 (&lt;1%)</td>
<td>98/140 (70%) **</td>
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</table>

* Includes Atypical Proliferative Tumor and noninvasive MPSC

**Gene expression profiles in SBT, LG MPSC and HG serous carcinoma**

- Low-grade
- Normal
- High-grade

SBT and LG MPSC have similar profiles distinct from HG ser CA

**What is the Pathogenesis of Low-grade Micropapillary Serous Carcinoma?**

- Before and after images showing a border of tumor and cyst.

(Cancer Res, 64:6915, 2004)
Mutations of KRAS and BRAF Precede the Development of APSTs

Serous cystadenoma adjacent to APST
BRAF mutation
Codon 599, T1796A

APST in Serous cystadenoma
BRAF mutation
Codon 599, T1796A

(Cancer Res, 64:6915, 2004)

Pathogenesis of Low Grade Micropapillary Serous Carcinoma

APST Noninvasive LG MPSC Noninvasive LG MPSC with focus of invasion Invasive LG MPSC

What is the Origin of High-grade Serous carcinoma?

The Usual Type of Serous Carcinoma

Is high-grade and rarely associated with SBTs

Grade 3 nuclei

High-grade Serous Carcinoma Pathogenesis

- The precursors of high-grade serous carcinomas are not well characterized
- It has been proposed that they develop “de novo”

From the surface epithelium or inclusion cysts

Bell DA, Scully RE
Cancer 73:1859-64, 1994

Development of Conventional (high-grade) serous carcinoma

Mutation of p53 is a very early event in the development of high-grade serous carcinoma

Courtesy Jeff Boyd
Dual Pathways of Serous Carcinogenesis
Early Events

Low-grade pathway

High-grade pathway

Low-grade serous carcinoma

High-grade serous carcinoma

KRAS or BRAF Mutation

p53 Mutation

Grade is a Defining Feature of Invasive Serous Carcinomas

- Serous carcinoma can be divided into two distinct types
  - Low-grade micropapillary serous carcinoma
  - High-grade serous carcinoma
- Not an issue of progression, these are distinct and separate tumor types

High-grade Serous Carcinoma (HG Ser CA) Developing from an Atypical Proliferative Serous Tumor (APST)

Case A

Case B

Identical KRAS mutation in APST and HG Ser CA
No p53 mutation
Progression in the Type I Pathway
Serous Tumors

Cystadenoma
APST
Noninvasive MPSC
HG Ser CA
Invasive MPSC
Very rare event

Mucinous Borderline Tumors (MBTs)

- Survival of stage I MBTs is 100%
- Survival of advanced stage MBTs reported in the literature is 50% but
- Over 80% of advanced stage MBTs reported are associated with pseudomyxoma peritonei (PMP)
- It is now known that PMP results from a ruptured mucinous appendiceal adenoma
- Ovarian involvement is secondary

Advanced Stage (MBTs)?

- Several studies have now shown that what appear to be ovarian MBTs are in fact metastatic carcinomas
- typically from the upper GI tract (biliary tree and pancreas) or cervix

Ovarian Tumor

Advanced Stage (MBTs)?

- Once MBTs associated with PMP (>80%) are eliminated from consideration and
- Metastatic carcinomas involving the ovaries that masquerade as MBTs are excluded

Cervical Tumor

Ovarian Tumor

HPV – 16 in situ hybridization
Advanced Stage (MBTs)?

There are none!

Molecular genetic studies using KRAS mutations as a marker

- Approximately 50% of mucinous carcinomas, MBTs, and mucinous cystadenomas have KRAS mutations of codon 12 and 13
- Using laser capture microdissection, the identical KRAS mutation is present in all three of the tumor components in the same case (mucinous cystadenoma, MBT, and mucinous carcinoma)


Relationship of MBTs to Mucinous Carcinoma

- Clinical, morphologic, and molecular data suggest there is a progression

Mucinous Carcinoma

- Mean size of both primary mucinous carcinomas and MBTs is - 18 cm
- Majority of primary mucinous carcinomas are well differentiated and unilateral at presentation
- Primary mucinous carcinomas are often focal developing within MBTs and mucinous cystadenomas
- Survival for stage I is >90%

Borderline Endometrioid and Clear Cell Tumors

- Not a single well documented case associated with malignant behavior reported since the category was introduced by FIGO in 1971 and incorporated into the WHO classification in 1973
- Frequently associated with their respective carcinomas

The Relationship of Endometrioid Adenocarcinoma To Endometrioid Borderline Tumors

Genetic Alterations: PTEN, K-Ras, β-catenin, Microsatellite Instability

Courtesy Kathy Cho
**Endometrioid Carcinoma**
- Frequently presents as a well differentiated stage Ia tumor associated with an atypical proliferative endometrioid tumor/adenofibroma
- Survival for these tumors is close to 100%
- Advanced stage tumors are relatively uncommon when classified using strict criteria

**Clear Cell Carcinoma**
- Poorly understood because it has not been well characterized
- In literature and based on personal experience “clear cell carcinoma” includes
  - Solid endometrioid carcinomas with extensive secretory change
  - Poorly differentiated carcinomas containing cells with clear cytoplasm
  - Classic clear cell carcinoma (tubulocystic, papillary and solid patterns)

**Summary of Type I Tumors**
- Low-grade
- Develop in a slow, stepwise fashion from
  - Cystadenomas
  - Atypical proliferative (borderline) tumors
- Similar to Type I endometrial carcinomas - complex hyperplasia and complex atypical hyperplasia precursors
- Typically present as stage I
- Are generally Indolent and remain low-grade
- Some progress

**Summary of Type II Tumors**
- High-grade adenocarcinoma
  - Includes high-grade serous carcinoma, MMMT, adenocarcinoma NOS, and undifferentiated carcinoma
  - Preliminary molecular data suggests they are similar
- Arise “de novo”
- Present in advanced stage
- Highly aggressive

**Dualistic Model of Ovarian Carcinogenesis**

**Screening for Ovarian Cancer**
- Understanding pathogenesis clarifies misconceptions in the current approaches to early detection
- Ovarian cancer is not a single disease
- Appreciation of diverse molecular pathways of carcinogenesis will permit more customized approaches to detection
Goal of Early Detection

- **Detect stage I ovarian carcinoma**
- Survival of stage I disease is > 90%
- 75-80% of ovarian carcinoma presents as advanced stage disease implying 20-25% are stage I
- Most patients will be successfully treated by surgery
- There will be limited need for cytotoxic chemotherapy

Detection of Stage I Ovarian Cancer

- The vast majority of “ovarian cancers” are serous carcinomas but …
- how many are stage I?

### Carcinomas Stage Distribution (n=220)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Stage I</th>
<th>Stage II-IV</th>
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</thead>
<tbody>
<tr>
<td>Serous</td>
<td>4%</td>
<td>96%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>83%</td>
<td>17%</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>53%</td>
<td>47%</td>
</tr>
<tr>
<td>Clear cell</td>
<td>36%</td>
<td>64%</td>
</tr>
<tr>
<td>Brenner</td>
<td>100%</td>
<td>0%</td>
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<tr>
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Bilaterality and Size

- Serous carcinomas tend to be small (in the ovary) and bilateral at presentation
- The bulk of the tumor is often outside of the ovary

High-grade Serous Carcinoma
Detection of Stage I Ovarian Serous Carcinoma

- Since ovarian carcinomas are small, even when there is extraovarian spread
- They will not be detected by pelvic exam or transvaginal ultrasound
- Is it possible to detect stage I serous carcinoma?

Is Ovarian Serous Carcinoma Ever Stage I?

- Thoroughly staged, stage I serous carcinoma sent for consultation because of microscopic lesion in the omentum thought to represent mesothelial hyperplasia
Is Ovarian Serous Carcinoma Ever Stage I?

- *Hardly ever!*

Other Problems

- Not all "ovarian" serous carcinomas start in the ovary
  - Serous carcinomas identical to those in the ovary can develop after bilateral salpingo oophorectomy
  - These are primary peritoneal serous carcinomas which at diagnosis are *stage II or III*

Other Problems

- Not all "ovarian" serous carcinomas start in the ovary
  - Small serous carcinomas detected in the fimbria of women with BRCA mutations undergoing prophylactic oophorectomy
  - Not previously recognized because fallopian tubes are not routinely serially sectioned?
  - These tumors are *stage II “ovarian” carcinomas at diagnosis*

Problems in the Detection of Stage I Ovarian Serous Carcinoma

Summary

- Serous carcinomas that start in the ovary spread rapidly
  - Lead time is brief and nearly always present in *advanced stage*
    - Cervix model does not apply
- Some serous carcinomas start in the peritoneum or fallopian tube
  - These are *advanced stage* at diagnosis
Detection of Stage I Ovarian Serous Carcinoma (Type II)

**Bottom Line**

- It will be virtually impossible to detect stage I serous carcinoma using presently available technology

Implications for Treatment of High-grade Serous Carcinoma (Type II Tumor)

- The most important predictor of outcome (more important than stage) is whether the tumor has been adequately debulked (optimal cytoreduction)
- Over time this has shifted from <2cm to <1.5 cm to <1cm

Implications for Treatment of Serous Carcinoma (Type II Tumor)

**A Paradigm Shift**

- Successful treatment will depend on detection of minimal (microscopic to 1 cm) ovarian serous carcinoma (stage is irrelevant)
- Using a panel of sensitive and specific molecular markers that precede development of morphologically recognizable precursors
- Treatment will be instituted based on marker detection only

Classification of Ovarian Tumors

- Why do SBTs have an intermediate behavior?
- Because they are a heterogeneous group mainly benign (atypical proliferative) and …
- a small number of noninvasive low-grade MPSCs which can implant (so-called invasive implants but in fact are metastatic low-grade carcinomas)

Evolution in our Understanding of Ovarian Carcinogenesis

- Creation of the borderline category was an important achievement that focused our attention on a subset of tumors that had previously been buried in the category of carcinoma
- Over the last 30 years significant advances have elucidated our understanding of these tumors

Evolution in our Understanding of Ovarian Carcinogenesis

- The borderline category and the concept that there is a group of tumors whose behavior is unpredictable has now outlived its usefulness and needs to be replaced
Classification of Ovarian Tumors

- Cystadenoma/adenofibroma
- Atypical proliferative tumor
- Noninvasive (intraepithelial) carcinoma
- Invasive carcinoma (Type I)
  - Low-grade micropapillary serous
  - Mucinous
  - Endometrioid
  - Clear cell
  - Malignant Brenner tumor
- High-grade carcinoma (Type II)

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

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:

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

Ovarian Endometrioid Adenocarcinoma (OEA) Tumor Progression Model

- Endometriosis
- Atypical Endometriosis
- Endometriosis with Epithelial Hyperplasia
- Endometrioid Adenocarcinoma

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

- Ovarian carcinomas arise through a multistep 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

What are we learning about ovarian cancer?

- OvCA Samples
- High-throughput analysis of RNA and DNA

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)
Wnt Signaling: Importance and overview...

- 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/β-catenin/Tcf Pathway Defects
Ovarian Endometrioid Adenocarcinomas (OEs)

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

Results
- Missense mutations found in 18 OEs (25%)
- OEs with CTNNB1 mutations show nuclear accumulation of β-cat by immunohistochemical staining

Other Wnt/β-cat/Tcf Pathway Defects in
Ovarian Endometrioid Adenocarcinomas: APC

- CTNNB1 or APC mutations present in 26% of OEs

WNT PATHWAY DEFECTS:
CORRELATION WITH LOW TUMOR GRADE
AND STAGE

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<tr>
<th></th>
<th>Grade 1</th>
<th>Grade 2 or 3</th>
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<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>
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<td></td>
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p = 1.2 X 10^-4
(Fisher’s exact)

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<td>four</td>
<td>44</td>
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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 5-9)</th>
<th>CTNNB1 mutation (exons 3)</th>
<th>K-RAS mutation (exons 12 and 13)</th>
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<tr>
<td>OE-13T</td>
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Mutational analysis of PIK3CA (n=72) and corresponding mutations of PTEN and CTNNB1 in OEAs

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<tr>
<th>Tumor ID</th>
<th>PIK3CA mutation</th>
<th>PTEN mutation (exons 5-9)</th>
<th>CTNNB1 mutation (exons 3)</th>
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</thead>
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<tr>
<td>OE-13T</td>
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<tr>
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<td>OE-38T</td>
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</table>

Mutations in the Wnt/β-catenin and PI3K/Pten/Akt Pathways Frequently Co-Occur in OEAs

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

<table>
<thead>
<tr>
<th>PTEN or PIK3CA mutation</th>
<th>Wnt/β-catenin pathway defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN or PIK3CA mutation</td>
<td>DEFECTIVE</td>
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<tr>
<td>Wild type PTEN and PIK3CA</td>
<td>12</td>
</tr>
<tr>
<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

IARC TP53 DATABASE

The Majority Of TP53 Mutations Are Missense Mutations

Missense Mutations are Clustered in the DNA-binding Domain
**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>
</tr>
</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>
</tr>
<tr>
<td></td>
<td>18</td>
<td>54</td>
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<tr>
<td></td>
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<td>72</td>
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\[ p = .0009 \]

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<th>Stage 3 or 4</th>
</tr>
</thead>
<tbody>
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<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Wild type p53</td>
<td>31</td>
<td>4</td>
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<tr>
<td></td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>72</td>
</tr>
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</table>

\[ p = 3 \times 10^{-6} \]

**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>

\[ p = 1.5 \times 10^{-6} \]

**First two principal components for 99 tumors, all probe-sets, log-transformed data**

**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 OEs are characterized by frequent Wnt/β-catenin and PI3K/Pten pathway defects, infrequent p53 mutations, favorable outcome
  - High grade OEs are characterized by frequent p53 mutations, infrequent Wnt/β-catenin and PI3K/Pten pathway defects, poorer outcome

- High grade OEs 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

Grading of Ovarian Cancer

- Histologic grade has been found to be an important prognostic factor in cases of ovarian carcinoma
- However, there is no system that is universally used for grading these neoplasms
- At present, it seems that ovarian cancer represents a heterogeneous group of diseases
- Different histologic parameters may need to be used for grading these neoplasms
- Nuclear grade is the biological behavior of selected tumors

Bibliography


8. Silva EG and Gershenson DM. Standardized histologic grading of epithelial ovarian cancer: Elusive after all.
Grading of Ovarian Cancer

• Introduction
  – Traditionally ovarian cancer has been considered one disease
  – Diverse grading systems have been used to grade these neoplasms regardless of the tumor histotype

• The FIGO grading system
  – Based on architectural features, grade of the tumor depends on the ratio of glandular or papillary structures to solid tumor
    • Grade 1, < 5% solid
    • Grade 2, 5% to 50% solid
    • Grade 3, >50% solid

• The WHO system
  – Based on the pathologist’s impression of both architectural and cytologic features
  – Categories not defined according to a quantitative method

• Ovarian carcinoma appears to be a heterogeneous group of tumors, rather than a single disease
  • Therefore, a universal grading system for this heterogeneous group most likely would not accommodate the inherent differences among these diverse tumors
Grading of Ovarian Cancer

- Considering this heterogeneity, it would be more appropriate to use different parameters in order to grade the cases within each histologic type.

Serous Carcinoma

- The recently proposed two-tier grading system (M.D. Anderson grading system) has emerged as a good method to segregate serous carcinomas that have different molecular, pathogenetic, histologic, immunohistochemical, and clinical features.

Serous Carcinoma

- The two-tier grading system (M.D. Anderson grading system) is based primarily on the assessment of nuclear atypia with the mitotic rate used as a secondary feature.
- Two grades:
  - Low
  - High

Low Grade Serous Carcinoma

- Definition
  - A serous carcinoma characterized by the presence of uniform cells with mild to moderate nuclear atypia, and usually a low mitotic index (≤ 12 mitoses per 10 HPFs)
High Grade Serous Carcinoma

- Definition
  - A serous carcinoma characterized by the presence of pleomorphic cells with marked nuclear atypia (≥3:1 variation in size and shape), and a high mitotic index (>12 mitoses per 10 HPFs)
Low vs. High Grade Serous Carcinoma
Differences in Pathogenesis: Morphologic Evidence

- Association with a serous neoplasm of low malignant potential
  - 60% of the low grade serous carcinomas
  - 2% of the high grade serous carcinomas

Malpica A et al, 2004

Low vs. High Grade Serous Carcinoma
Differences in Pathogenesis: Molecular Evidence

- Ovarian Tumorigenesis Model
  - Type I, tumors that arise in a stepwise manner from borderline (low malignant potential) tumors
    - Low grade serous carcinoma, prototypic type I tumor
  - BRAF and KRAS mutations

Singer G et al, 2002

Low vs. High Grade Serous Carcinoma
Differences in Pathogenesis: Molecular Evidence

- Ovarian Tumorigenesis Model
  - Type II, de novo development (as yet no recognizable precursor lesion identified)
    - High grade serous carcinoma, prototypic type II tumor
    - p53 mutation

Singer G et al, 2002

Low vs. High Grade Serous Carcinoma: Immunohistochemical Differences

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Low Grade Serous Ca</th>
<th>High Grade Serous Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>18%</td>
<td>64%</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>5%</td>
<td>26%</td>
</tr>
<tr>
<td>MIB1, mean index</td>
<td>23.0%</td>
<td>55.4%</td>
</tr>
<tr>
<td>Her-2 neu</td>
<td>4.5%</td>
<td>36%</td>
</tr>
<tr>
<td>C-Kit</td>
<td>4.5%</td>
<td>30%</td>
</tr>
</tbody>
</table>

O’Neill CJ et al, 2005
Low vs. High Grade Serous Carcinoma: Differences in Biologic Behavior

- Low grade serous carcinoma appears to be less responsive to conventional chemotherapy than high grade serous carcinoma.
- Patients with low grade serous carcinoma have a longer overall survival than patients with high grade serous carcinoma.


Low vs. High Grade Serous Carcinoma: Frequently Asked Questions

- What is the incidence of low grade serous carcinoma?
  - Uncommon tumor
  - Less than 10% of ovarian serous carcinomas (MDACC unpublished data)
  - 1/102 cases of advanced stage ovarian cancer (Gilks CB, 2004)
- Is the term low grade serous carcinoma used in a standard fashion?
  - No
  - Some gynecological pathologists use this term to designate the micropapillary/cribriform variant of a serous tumor of low malignant potential.

Malpica A et al, in press 2007

Low vs. High Grade Serous Carcinoma: Frequently Asked Questions

- What is the reproducibility of the two-tier grading system for ovarian serous carcinoma?
  - Overall kappa among 9 different observers = 0.909
  - The inter-observer kappas ranged from 0.717 to 1.000 in the first round of the review and from 0.701 to 1.000 in the second review.

Malpica A et al, in press 2007

Endometrioid Carcinoma

- Grading system similar to the FIGO grading system used for endometrial endometrioid adenocarcinoma.

Grade 1: less than 5% solid areas
Endometrioid Carcinoma

Grade 2: 5 to 50% solid areas

Endometrioid Carcinoma

Grade 3: more than 50% solid areas

Endometrioid Carcinoma

- Survival rate of patients with grade 1 or 2 tumors is higher than those with grade 3 tumors

Kline RC et al, 1990

Clear Cell Carcinoma

- By definition a high grade carcinoma
- The WHO recommendation is not to grade

Clear Cell Carcinoma

- A high incidence of stage I disease
- Poor response to platinum-based therapy
- Controversial results regarding the survival of patients with this type of tumor when compared to patients with serous carcinoma
Transitional Cell Carcinoma

• The WHO recommendation is to grade according to the criteria used for transitional cell carcinoma of the urinary tract
• In reality, most (if not all) cases are high grade

Transitional Cell Carcinoma

• WHO criteria
  – Low grade
    • Uniformly enlarged nuclei, with mild alteration of the polarity and mild differences in shape, contour, and chromatin distribution
    • Infrequent mitoses
  – High grade
    • Marked variation in nuclear polarity, size, shape, and chromatin distribution
    • Frequent mitoses

Transitional Cell Carcinoma

• Ovarian carcinomas with more than 50% of a TCC component appear to have a better response to chemotherapy
  – Especially if the TCC component is also predominant in the metastases

Mucinous Carcinoma

• Non-invasive carcinoma (Intraepithelial)
  • Marked atypia of the epithelium
• Invasive carcinoma
  – Expansile or confluent type
    • Confluent glandular pattern uninterrupted by normal ovarian stroma occupying an area measuring more than 5 mm in diameter (The Johns Hopkins’ group criterion) or more than 10 mm² (WHO criterion)
  – Infiltrative type
    • Small glands, nests of cells or individual cells infiltrating the stroma in an area measuring more than 5 mm in diameter (The Johns Hopkins’ group criterion) or more than 10 mm² (WHO criterion)
Mucinous Carcinoma

- Prognosis
  - Intraepithelial mucinous carcinoma
    - Risk of recurrence for stage I cases: 5.8%
  - Invasive carcinoma
    - 5-year survival of 91% for stage I cases; advanced stage cases all died of disease (Riopel MA et al, 1999)
    - Infiltrative stromal invasion appears to be more aggressive than expansile invasion (Lee KR and Scully RE, 2000 and Rodriguez IM and Prat J, 2002)

Undifferentiated Carcinoma

- By definition a high grade carcinoma
- The 5-year survival of patients with undifferentiated carcinoma is worse than that of patients with serous carcinoma or transitional cell carcinoma

Grading of Ovarian Cancer

Summary
- Serous Carcinoma
  - Categories:
    - High grade
    - Low grade
  - Criteria: Degree of cytologic atypia (primary feature) and mitotic index (secondary feature)
- Endometrioid Carcinoma
  - Categories: Grades 1, 2, and 3
  - Criterion: amount of solid component
Grading of Ovarian Cancer

Summary

- **Mucinous Carcinoma**
  - Categories:
    - Non-invasive (intraepithelial)
    - Invasive
      - Expansile or confluent vs. infiltrative
  - Criteria:
    - Marked cytologic atypia (non-invasive carcinoma)

- **Transitional cell carcinoma**
  - Categories:
    - Almost always high grade
  - Criteria: WHO criteria for urothelial carcinoma
  - High grade carcinomas (by definition)
    - Clear cell carcinoma
    - Undifferentiated carcinoma

International Society of Gynecological Pathologists
San Diego 2007

Dr. Patricia Shaw
University Health Network
University of Toronto
Toronto, Canada

Key Issues

- Review of the three-tiered grading system as developed by Dr. Silverberg and colleagues
- Does this system fulfill the criteria desired in a grading system?
  - Clinical relevance
  - Applicability to all major histological types
  - Inter-observer reproducibility
An ideal grading system should be:

- Applicable to all histological types EOC
- Clinically relevant
- Reproducible - Intra- and inter-observer
- Easy to apply in clinical setting
- Widely adopted

Dr. Silverberg and colleagues developed a 3-tiered system:

- Shimizu et al. Gynecologic Oncology 1998

**Architectural Grade**

<table>
<thead>
<tr>
<th>Glandular</th>
<th>Papillary</th>
<th>Solid</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
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</table>

**Nuclear Grade**

<table>
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**Mitotic Count Score**

<table>
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</tr>
<tr>
<td>2</td>
<td>10-24</td>
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<td>3</td>
<td>&gt; 24</td>
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</table>
Are these features clinically relevant? And to all major histological subtypes?

<table>
<thead>
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<tr>
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<td>NS</td>
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<tr>
<td>TCC</td>
<td>--</td>
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<tr>
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<td>0.0039</td>
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</table>

Shimizu Gynecol Oncol 1998

<table>
<thead>
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Shimizu Gynecol Oncol 1998

<table>
<thead>
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<th>Type</th>
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Shimizu Gynecol Oncol 1998

<table>
<thead>
<tr>
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<tr>
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<table>
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</tr>
<tr>
<td>8-9</td>
<td>3</td>
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</tbody>
</table>

Shimizu Cancer 1998

Is this grading system clinically relevant? And for all major histological subtypes?
Silverberg Grade and Survival

<table>
<thead>
<tr>
<th>Type</th>
<th>Stage I-II p-value</th>
<th>Stage III-IV p-value</th>
</tr>
</thead>
<tbody>
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<tr>
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<tr>
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<td>0.0000</td>
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</tbody>
</table>

Shimizu Gynecol Oncol 1998

Prognostic Factors: Stage I-II Ovarian Cancer (Multivariate)

Significant:
- Silverberg grade (p=0.0001)
- Performance status (p=0.0004)

Not significant:
- FIGO grade
- Age
- Histologic sub-type

Prognostic Factors: Stage III-IV Ovarian Cancer (Multivariate)

Significant:
- Silverberg grade
- Response to chemotherapy
- Post-surgical residual disease
- Performance status
- Histological subtypes - Mucinous/TCC

Not Significant:
- FIGO grade
- Age

Is this grading system reproducible?

(Yes)
- Shimizu et al
- Sato et al

Validation of Silverberg System by others:

- Mayr and Diebold 2000
- Sato et al 2002
- Shaw et al 2002
- Gilks et al 2006

Histological Features of Hereditary Ovarian Carcinoma

<table>
<thead>
<tr>
<th>BRCA1/2 Control</th>
<th>p-value</th>
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</thead>
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<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>3</td>
<td>50%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Silverberg Grade</th>
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</thead>
<tbody>
<tr>
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<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>25%</td>
</tr>
<tr>
<td>3</td>
<td>75%</td>
</tr>
</tbody>
</table>

Shaw et al. Int J Gynecol Pathol 2002
Histotyping ovarian cancers
Robert A. Soslow, MD

Key Issues
• Review morphology, immunophenotype, genotype and differential diagnosis*
• Emphasize endometrioid and clear cell tumors
• Suggest refinements to diagnostic criteria

Rationale for “histotyping”
• Distinct disease entities
  – BRCA1 and 2
  – Tumor progression from:
    • Endometriosis
    • Borderline tumor
• Diagnostic criteria for carcinoma
• Carcinoma grading
• Therapeutic relevance

Introduction
• WHO classification
  – Serous
  – Mucinous
  – Endometrioid
  – Clear cell
  – Transitional
  – Squamous
  – Mixed epithelial
  – Undifferentiated

WHO critique
• WHO approach
  – Morphologically based, but not entirely objective
• Problems
  – Mucinous carcinoma
  – Poorly differentiated endometrioid carcinoma
  – Transitional cell carcinoma
  – Mixed epithelial carcinoma

Serous carcinoma: morphology

- **Overview**
- **Problems**
  - Architecture
    - Glandular
    - Cribriform
    - Microcystic
    - Trabecular
  - Cytology
    - Clear cells
    - Signet ring cells
    - Spindle cells

Serous carcinoma: immunophenotype

- All serous
  - WT1 (>70%)
- Low-grade serous
  - ER/PR
- High-grade serous
  - P53 (overexpression, >70%)
  - P16
  - Variable ER/PR
  - Variable loss of BRCA1

Serous carcinoma: genotype

- Low grade serous
  - BRAF mutation
  - K-ras mutation
- High grade serous
  - P53 mutation
  - BRCA1 or 2 abnormalities (sporadic)
  - BRCA1 or 2 mutation (familial)

Serous carcinoma: pathogenesis

Inclusion cyst
BRCA-1 mutation
“Dysplasia”
TP53 mutation
BRCA-1 abnl
K-ras
High grade carcinoma
Surface Epithelium
Borderline tumor (BT)
Micropapillary BT
Low grade carcinoma


High grade serous carcinoma: pathogenesis

Low grade serous carcinoma: pathogenesis

Low grade serous carcinoma: differential diagnosis

- Glandular, cribriform, miccystic, trabecular:
  - Mucinous, endometrioid, clear cell, transitional
- Clear cells, signet ring cells:
  - Clear cell, endometrioid, mucinous

Ovarian carcinoma classification: serous

- Broad range of histologic features
  - Slit-like spaces, irregular luminal contours
- Frequent WT1
- Low-grade: serous borderline tumor, BRAF/K-ras, ER/PR
- High-grade: tubal intraepithelial carcinoma, p53, p16, loss of BRCA1, BRCA1 or 2 family
- Other entities are excluded

Serous tumors: prevalence

- 80-85% of ovarian carcinomas
- 95% of stage III-IV ovarian carcinoma
- Low stage serous carcinomas are rare
  - ~25% of stage I/II carcinomas are serous
  - ~25% of stage I/II serous carcinomas are stage I

(Intestinal) Mucinous tumors: morphology

• Overview
• Problems
  – Primary versus metastasis
  – Paucity of intracytoplasmic mucin/prevalence of extracellular mucin
  – Borderline tumor versus carcinoma

Mucinous tumors: immunophenotype

• Immunophenotype
  – CK7>20 (GI ddx)
  – Negative racemase and β-catenin (GI ddx)
  – Negative p16 (Endocervical ddx)
  – Negative ER (Endometrioid ddx)
  – Retained SMAD4/DPC4 (Pancreatic ddx)
  – Negative mesothelin and fascin (Pancreatic ddx)

Mucinous carcinoma: pathogenesis

Mucinous cystadenoma

\[ \xrightarrow{\text{K-ras}} \]

Intestinal mucinous BT

\[ \xrightarrow{\text{}} \]

Intestinal mucinous carcinoma

Mucinous tumors: borderline versus carcinoma

• Expansile invasion
  – Large cribriform glands
  – Extensive gland fusion
  – Complex papillary architecture
• Destructive invasion

Mucinous tumors: differential diagnosis

• Exclude metastasis
• Scant intracytoplasmic mucin
  – Endometrioid
• Extracellular mucin
  – Pseudomyxoma peritonei
  – Low grade serous carcinoma (cribriform)


Mucinous tumors: features favoring metastasis

- Bilateral disease
- Surface involvement
- Destructive stromal invasion
- Nodular growth pattern
- Single cells/signet ring cells
- Vascular invasion


Ovarian carcinoma classification: intestinal mucinous

- Intracytoplasmic mucin, expansile invasion
- Intestinal mucinous borderline tumor
- CK7>20, retained SMAD4
- Negative racemase, β-catenin, ER, p16, mesothelin, fascin
- K-ras
- Other entities are excluded: exclude metastasis

Mucinous tumors: prevalence

- Only <3% of all ovarian carcinomas
- >2/3 are stage I
- ~15% of all stage I tumors


Seromucinous tumors

- Definition and synonyms
- Similarities with low grade serous tumors
  - Architecture
  - Clinical profile
- Differences with intestinal mucinous tumors
  - Morphology
  - Clinical profile
- Similarities with endometrioid tumors
  - Endometriosis

Endometrioid tumors: morphology

- Overview
- Problems
  - Architecture:
    - Cribriform
    - Trabecular
    - Papillary
  - Cytology:
    - Intracytoplasmic mucin
    - Clear cells
    - High nuclear grade
  - Borderline tumor versus carcinoma

Endometrioid tumors: immunophenotype

- ER/PR
- β-catenin
- Not WT1
- P53 in “high grade” examples
Endometrioid tumors: genotype

- CTNNB-1 (β-catenin)
- PTEN
- Microsatellite instability (MSI)

Endometrioid carcinoma: pathogenesis

Endometriosis (clonal)

Complex atypical hyperplasia/Endometrioid BT

Familial

EMS
PTEN
CTNNB1

Sporadic

Low grade carcinoma

Endometrioid carcinoma: invasion patterns

- 13 grade 1 and 2 endometrioid carcinomas (8 IA, 5 IC), median F/U 81 mos:
  - 9 expansile (9/9 NED)
  - 4 expansile and destructive* (3/4 NED)

- Low grade, stage I endometrioid carcinoma with expansive invasion: VERY limited malignant potential

Chen S, et al. Mod Pathol 18:903-11, 2005

Endometrioid tumors: borderline versus carcinoma

- Expansile invasion
  - Large cribriform glands
  - Extensive gland fusion
  - Complex papillary architecture
- Destructive invasion


Endometrioid tumors: invasion patterns
Endometrioid tumors: differential diagnosis
- Cribriform, papillary, trabecular:
  - Serous, transitional
- Intracytoplasmic mucin, clear cells, high nuclear grade:
  - Metastasis, mucinous, clear cell, serous

Endometrioid tumors: prevalence
- 10% of ovarian carcinomas
- Most common stage I carcinoma (~50%)
- Most endometrioid carcinomas are Stage I or II (>2/3)

Ovarian carcinoma classification: endometrioid
- Endometrial-like, metaplasias, secretory change, expansile invasion
- Endometriosis, endometrioid borderline tumor, endometrioid uterine carcinoma
- ER/PR, β-catenin; not WT1
- CTNNB-1 (β-catenin), PTEN, MSI-H
- Other entities are excluded

Clear cell tumors: morphology
- Overview
- Problems
  - Architecture:
    - Papillary
    - Solid
  - Cytology:
    - Clear cytoplasm
    - Oxyphilic variant
  - Borderline tumor versus carcinoma

Clear cell tumors: diagnostic reproducibility, immunophenotype, lessons learned
- Clear cell carcinomas have a limited and distinctive architectural repertoire and immunophenotype
- Mixed epithelial tumors containing clear cells (MET-C) are not reproducibly diagnosed
- MET-Cs are seldom clear cell carcinomas—most are serous carcinomas

Interobserver agreement

<table>
<thead>
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<th>Diagnosis</th>
<th>Kappa</th>
<th>Degree of agreement beyond chance</th>
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<tbody>
<tr>
<td>CCC</td>
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<td>Almost perfect</td>
</tr>
<tr>
<td>SC</td>
<td>0.59</td>
<td>Moderate</td>
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<tr>
<td>MET-C</td>
<td>0.32</td>
<td>Fair</td>
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<tr>
<td>Overall</td>
<td>0.62</td>
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Han G, et al. USCAP 2007, abstract
Clear cell immunophenotype

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<th>WT1</th>
<th>ER</th>
<th>BRCA1</th>
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</table>

Han G, et al. USCAP 2007, abstract

Clear cell tumors: immunophenotype

- Immunophenotype
  - Paucity of ER/PR
  - Lack of WT1
  - Variable p53 expression
  - Low proliferation rate

Clear cell tumors: genotype

- Mutations:
  - PTEN
  - TGF beta R2
  - K-RAS
- MSI-H

Clear cell carcinoma: pathogenesis

Endometriosis

Atypical endometriosis or Endometrioid carcinoma

(Clear cell borderline tumor)

Clear cell carcinoma

Clear cell tumors: borderline versus carcinoma

- Adenofibromatous
  - Destructive invasion greater than microinvasion: carcinoma
    - What counts as invasion?
    - Pure borderline tumors are almost never encountered
- Papillary
  - Essentially always considered carcinoma
    - Importance of cytologic characteristics
    - Nuclear grade?
      - Ddx with papillary endometrioid carcinoma and serous borderline tumor

Clear cell tumors: differential diagnosis
- Papillary, tubulocystic, solid
  - Serous, seromucinous, endometrioid
- Hobnail cells, clear cells
  - Serous, seromucinous, endometrioid

Ovarian carcinoma classification: clear cell
- Papillary, tubulocystic, solid, hobnail, frequently clear cytoplasm
- Endometriosis, clear cell borderline tumor
- Low ER/PR, WT1, p53, mib-1
- MSI-H, PTEN
- Lack of features that define other entities
  - Metaplasias, secretory changes
  - Multilayering, serrated luminal profiles

Clear cell tumors: prevalence
- 5% of ovarian carcinomas
- Disproportionately represented in stages I and II
- 25% of stage I and II carcinomas are clear cell
- Most clear cell carcinomas are low stage at presentation (>2/3)


Transitional cell carcinoma
- Definition
- Similarities with high grade serous tumors
  - Morphology
  - Immunophenotype
  - ?Clinical profile
- Differences with urothelial carcinoma

Malignant surface epithelial tumors: late 20th century, West

Malignant surface epithelial tumors: early 21st century, West

Case Discussion
Panel and Audience

- Unknown cases posted on the ISGYP and USCAP websites
- Histotyping and grading