The Role of Immunohistochemistry in Surgical Pathology of the Ovary, Fallopian tube and Peritoneum

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Revisions: Ovary
Surface epithelial-stromal to Epithelial
Malignant serous to High-grade serous and Low-grade serous
Mucinous with pseudomyxoma peritonei, transitional cell carcinoma, and mixed carcinoma all removed

Revisions: Tube and Peritoneum
Reflects changes to ovary and endometrium classification (e.g. high-grade versus low-grade serous, high-grade endometrioid stromal sarcoma)

TNM and FIGO staging
Tumors of ovary, fallopian tube, and peritoneum combined
WHO 2014 Revisions: Ovary

Malignant serous to High-grade serous and Low-grade serous
Mucinous with pseudomyxoma peritonei, transitional cell carcinoma, and mixed carcinoma all removed

Ovarian cancer histotypes

Serous – high grade
Serous – low grade
Clear cell
Endometrioid
Mucinous

Ovarian carcinoma subtypes differ from each other with respect to:
1. Genetic risk factors
2. Precursor lesions
3. Patterns of spread
4. Molecular events during oncogenesis
5. Response to chemotherapy
6. Outcome

Revisions: Ovary

Malignant serous to High-grade serous and Low-grade serous
Mucinous with pseudomyxoma peritonei, transitional cell carcinoma, and mixed carcinoma all removed
High-grade Serous Carcinoma

- Chromosomal instability/aneuploidy (100%) 
- p53 mutations (>90%), BRCA loss (30-45%)

High-grade Serous Carcinoma

- few other recurrent mutations 
- numerous SCNA 
- Homologous recombination

MOST HIGH-GRAD SEROUS CARCINOMAS OF OVARY ORIGINATE IN THE FALLOPIAN TUBE!


Fimbria submitted in toto
Cases with STIC and/or FT mucosal invasive
HGSC: fallopian tube primary
Cases with all or fimbriated end of FT incorporated into a large adnexal mass: fallopian tube primary
If ovarian mass and FT mucosa is uninvolved: ovarian primary
If no or minimal ovarian surface involvement and FT mucosa is uninvolved: primary peritoneal
Genetic alterations in low grade serous carcinomas and serous borderline tumors

Usually diploid (82.5% of advanced stage SBT - Yue et al. Int J Gynecol Pathol 2003)
Fewer genetic abnormalities than serous ca by CGH (Staebler et al. Hum Pathol 2002)

High Grade vs Low Grade
Serous

p53
(p16)
High Grade Serous vs Endometrioid

WT1
(p53)

Revisions: Ovary

Surface epithelial-stromal to Epithelial
Malignant serous to High-grade serous and Low-grade serous
Mucinous with pseudomyxoma peritonei, transitional cell carcinoma, and mixed carcinoma all removed
Mesenchymal and Mixed epithelial and mesenchymal added

Mucinous tumors with pseudomyxoma peritonei

Almost all metastases from GI tract

CDX-2 and CK20 strongly positive, CK7 negative
Mucinous vs Endometrioid

ER
(CK20)

Mucinous vs High Grade Serous

ER
WT1

Transitional cell carcinoma

Unrelated to Brenner tumor/rare malignant Brenner tumor
p53, ER and WT1 staining profile same as HGSC
Associated with BRCA1/2 mutations
Now considered a variant of HGSC
Revisions: Ovary in 2007

Surface epithelial-stromal to Epithelial
Malignant serous to High-grade serous and Low-grade serous
Mucinous with pseudomyxoma peritonei, transitional cell carcinoma, and mixed carcinoma all removed
Mesenchymal and Mixed epithelial and mesenchymal added

“Careful study of ovarian tumors often reveals two or even three or more cell types.”

Sternberg’s Textbook of Pathology (2007)

Implications of mixed carcinomas being common
ovarian carcinoma subtypes are closely related
differences between subtypes are unlikely to be relevant
Ovarian carcinoma subtypes are not reproducibly diagnosable by pathologists in 2007.

What are the problems with histopathological assessment of cell type?

Irreproducible!
Overall moderate reproducibility (kappa = 0.55) Lund et al. APMIS 1991
Problems with reproducibility most marked with mixed, unclassified, undifferentiated, and serous vs. endometrioid – Cramer et al. Arch Pathol Lab Med 1987

Implications of lack of reproducibility
patients given different diagnoses in different centers
results of studies on natural history provide conflicting results (e.g. clear cell carcinoma in NA/Europe vs Asia)
impossible to move forward with good studies on molecular pathology or clinical trials of new treatments
in 2007

subtype does not matter, independent of tumor stage and grade

“with few exceptions, the clinical presentation, treatment, and results of treatment are similar for all (cell) types of tumor”

CDM Fletcher ed. In Diagnostic Histopathology of Tumors (2007)

Implications of “no clinical significance associated with ovarian ca subtype diagnosis”

why bother?

natural history cannot be studied

subtype specific clinical trials/treatment is impossible

patients with non-high-grade serous carcinomas ignored

in 2015

What has changed?
It is now possible for pathologists to routinely subclassify ovarian carcinomas into a small number of reproducible, clinically relevant groups.

### Ovarian cancer histotypes

- Serous – high grade
- Serous – low grade
- Clear cell
- Endometrioid
- Mucinous

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Mixed carcinomas of the ovary are rare, with the exception of the endometriosis-associated subtypes

Review of 871 cases of ovarian carcinoma with classification by current criteria:

1. 1.7% mixed
2. Mixed ECa + CCC/ECa + LGSC accounted for most cases
3. 41% of “mixed” carcinomas showed uniform immunophenotype throughout
Clear Cell Carcinoma

- most diploid or tetraploid
- Lynch Syndrome
- ARID1A, PIK3A
- Met amp
- HNF1B
- hypoxic growth, angiogenesis and glucose metabolism

High Grade Serous vs Clear Cell

WT1
HNF-1beta/NapsinA
ER
Clear Cell vs Endometrioid

ER
HNF-1beta/NapsinA

Mismatch repair gene expression

MLH1
MSH2
PMS2
MSH6
Endometrioid Carcinoma

- Lynch Syndrome
- PTEN, MSI, β-catenin, ARID1A

Endometrioid vs Low Grade Serous

WT1

Immunomarkers used for Ovarian Carcinoma Histotype Diagnosis

WT1
ER
NapsinA
p53
CK20
NapsinA

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

p53
### Limitations of our menu of Abs
- Initially validated for FFPE samples only
- Expansion of validation/controls to include bone marrow biopsies
- Careful attention to pre-analytical variables (neutral buffered formalin, adequate fixation)
- Cytology specimens?

### Liquid-based cytology specimens
- Popular
- Good diagnostic yield (at least in era of “benign vs malignant” distinction)
- Increasing need for subtype diagnosis for management
- What to use as controls for immunostaining?
Background on handling of cytology specimens at Vancouver General Hospital

Peritoneal and pleural fluids specimens: fixed in CytoLyt -> thin prep cytology and cell block (in formalin)

Pragmatic Considerations for Fluid Specimens

Some recurrent differential diagnoses (e.g. adenocarcinoma vs mesothelioma) so a limited panel of Abs used

Often an accompanying or subsequent surgical pathology specimens, so direct comparisons are possible

Material and Methods

Histologically confirmed cases of serous carcinoma with positive ascitic fluid/peritoneal washings.

Cell block preparation:
1. Received fresh, centrifuged 5 min 1200G, supernatant removed.
2. ThinPrep CytoLyt ® Solution (Hologic Inc), centrifuged 5 min 1200G, supernatant removed
3. HistoGel (Thermo Scientific) 4°C for 5 min.
4. 10% Formalin for minimum of 24 hrs.

Material and Methods

Surgical blocks: Fixed in 10% formalin within 3 hours of removal for minimum of 24 hours.

IHC performed with automated Ventana systems protocol including heat antigen retrieval.

ER (Labvision, SP1, 1:50).
WT-1 (DAKO, 6F-H2, 1:100).
Results

26 cases: 23 high, 3 low-grade serous carcinoma. Ave age at surgery 68 (44-92) years old.

Tumour cells not identified in re-cut from one surgical specimen + insufficient number of tumour cells in one section re-cut from cell block.

Statistical analysis performed on remaining 25 (WT-1) and 24 (ER) cases.

Results: Zonal pattern of WT-1 (A) and ER (C) staining at well fixed/ peripheral portions of the specimen versus WT-1 (B) and ER (D) in poorly fixed/central portions of specimen.

For our study: assessment of expression performed on well fixed portions only.

Results: high-grade serous carcinoma

A A B B C C D D E E

Surgical specimens: H+E (A), WT-1 (B) and ER (D).

Cytological preparations: WT-1 (C) and ER (E).

Results: low-grade serous carcinoma

A A B B C C D D E E

Surgical specimens: H+E (A), WT-1 (B) and ER (D).

Cytological preparations: WT-1 (C) and ER (E).
Results

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<th>WT-1 expression</th>
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<td>Cyto -ve</td>
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WT-1: +ve in 96% (24/25) of surgical specimens, 84% (21/25) of matched cytological preparations. Difference not statistically significant (p=0.35).

ER: +ve in 96% (23/24) of surgical specimens and 66%, (16/24 ) of matched cytological preparations. Statistically significant difference (p=0.02).

Prior chemotherapy did not alter expression of ER and WT-1, as previously described.


Conclusion

Useful information can be obtained based on alcohol-fixed liquid-based cytology specimen.

Results must be interpreted with caution, as false negative results can occur.

W.H.O. Histological Classification of SCST of the Ovary
FOXL2 402C⇒G (C134W) mutation

- Missense point mutation: 402C⇒G (C134W) in FOXL2 (Single base substitution).

- Very characteristic for aGCT

Introduction

Histomorphology

FOXL2 mutation

Immunostaining with FOXL2

Correlation of FOXL2 mutation with immunostaining

FOXL2 immunostaining versus other immunomarkers

Synopsis

Material

- 498 ovarian tumors (from all the categories) were examined using whole sections and tissue microarrays (TMA).

- Expression of FOXL2 was evaluated by immunohistochemistry using avidin-labelled monoclonal anti-FOXL2 antibody (Imgenex®, 1:25 dilution).
Results

There was positive nuclear staining for FOXL2 in 97/498 (19.74%) cases.

The FOXL2 immunopositivity showed remarkable predilection for sex cord-stromal tumors:

- 41 of 42 (97.6%) aGCT,
- 9 of 9 (100%) juvenile GCT,
- 9 of 9 (100%) fibromas,
- 3 of 3 (100%) fibrosarcomas,
- 3 of 4 (75%) thecomas,
- 20 of 40 (50%) Sertoli-Leydig cell tumors,
- and 3 of 4 (75%) sex-cord tumors, unclassified, showed FOXL2 positivity.

In contrast, all epithelial tumors (whether primary ovarian surface epithelial or metastatic tumors) showed complete negativity for FOXL2.

Likewise, tumors in other categories such as germ cell tumors and mesenchymal tumors not of ovarian stromal lineage did not show immunoreactivity for FOXL2.
All different types of aGCT show immunopositivity with FOXL2

Sertoli cell tumors/Sertoli-Leydig cell tumors: 20/40

10 retiform Sertoli cell tumors: 1/10

Sex cord-stromal tumor, unclassified: 3/4

SCTAT: 2/2
All tumors in the group of fibroma/cellular fibroma/fibrosarcoma show immunoreactivity with FOXL2 (12/12)

Steroid cell tumor: 1/1

Leydig cell tumors: 0/6
• All carcinomas did not show immunoreactivity with FOXL2.

Mesenchymal tumors not of ovarian stromal lineage did not show immunoreactivity for FOXL2.
Germ cell tumors of the ovary did not show immunoreactivity to FOXL2.

To distinguish between SCSTs and non-SCSTs, such as:

* aGCT and other mimickers:
  - Luteinized aGCT vs pregnancy luteoma.
  - aGCT vs carcinoid tumor.
  - aGCT vs small cell carcinoma, hyperca type.
  - aGCT vs poorly differentiated carcinoma.

* SLCT and other mimickers:
  - SLCT vs endometrioid carcinoma.

* Sclerosing stromal tumor vs signet-ring carcinoma.
Cannot distinguish between tumors in SCST category, such as
- Thecoma and aGCT (the distinction is important because of the difference in tumor behavior).
- SLCT and aGCT (but not significant- the two tumors of the same degree of malignancy).

Testing for the specific mutation of aGCT should help in the distinction.

• Molecular confirmation of FOXL2 (402C→G) mutation will distinguish aGCT from other tumors that co-express FOXL2 and histomorphologically mimic aGCT.

- FOXL2 immunostaining was correlated with the immunostaining with other markers.
- "Traditional markers" of SCSTs:
  - Alpha-Inhibin
  - Calretinin
  - CD99
  - Melan A
### FOXL2 versus other Markers: GCT (adult and juvenile)

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### FOXL2 versus other Markers (SCT/SLCT, n=19)

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### FOXL2 versus other Markers: Fibrothecoma group (n=10)

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### Cellular fibroma

- **Inhibin**
- **CD99**
- **Calretinin**
- **Melan A**

### Thecoma

- **Inhibin**
- **CD99**
- **Calretinin**
- **Melan A**

### FOXL2 versus other Markers: Leydig cell tumor

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Conclusions

- FOXL2 is superior to inhibin and calretinin in most of the SCST subtypes. This specifically applies to GCT and fibrothecoma groups.

- In those SCST subtypes in which FOXL2 can be negative, inhibin and calretinin can be added. This specifically applies to groups of Sertoli cell tumors and Steroid cell tumors (including Leydig cell tumors and stromal luteomas).

### Inhibin/Calretinin vs. FOXL2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>FOXL2</th>
<th>Inhibin</th>
<th>CD99</th>
<th>Calretinin</th>
<th>Melan A</th>
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<tr>
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<td>2</td>
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<tr>
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</table>

**Inhibin/Calretinin**

- Inhibin: Cytoplasmic/ Calretinin: Nuclear and cytoplasmic
- Usually diffuse, if positive.
- If positive, can be patchy or focal
- Can be positive in certain tumors in non-SCST categories
- Highly specific for SCST
- Higher rate of positivity in Steroid cell group and some Sertoli cell tumors (in which FOXL2 is usually –ve).

**FOXL2**

- Nuclear only
- Higher rate of positivity in GCT and fibrothecoma groups
- Highly specific for SCST
- Generally diffuse, if positive
A Proposed Diagnostic Scheme for Sex cord-stromal Tumors (SCST)

- Histomorphology suggestive of a SCST
- FOXL2 Immunomarker: Can be combined with other markers (e.g., inhibin and calretinin)
- Mutational Genetic Analysis for FOXL2 (402C→G)

Consider other differential Dx within SCST category
Case Presentation

31 year old with unilateral ovarian mass, smooth surface, no evidence of extra-ovarian spread
Seen in consultation: original Dx of adult granulosa cell tumor

Figure 4: Comparison of an age-matched control population with a population based cohort of Molecularly Defined AGCTs with and without recurrent disease.
SMALL CELL CARCINOMA - HYPERCALCEMIC TYPE

Rare
Young women
Hypercalcemia in 50-70% of cases
Typically stage I at presentation, but aggressive
Specimens

26 patient samples, ages 5-39 yrs
- 7 germline DNA
- 14 tumour DNA
- 23 paraffin blocks

2 cell lines: BIN67*, SCCOHT-1

Whole genome and/or exome sequencing
SMARCA4/BRG1 immunohistochemistry

*Gamwell, Vanderhyden, Orphanet J Rare Diseases 2013.
**SMARCA4 Mutations**

- 11/14 tumours (79%)
- 2/7 germlines (29%)
- 2/2 cell lines

**SWI/SNF ATP-Dependent Chromatin Remodeling Complex**

- BRG1 LOSS
  - 20/23 tumours (87%)
  - 2/2 cell lines
  - 0/50 GCT, DG, YST
  - 2 CCC/1000 (0.2%) ovarian tumours
  - Diagnostic marker

Figure 1, Karnezis et al.
Acknowledgements

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- The OvCaRe Team!