The Henry Moon Lecture

Pelvic Serous Carcinogenesis: The Subtle, the Obvious, and the Unknown

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Ovarian cancer strikes nearly twenty-five thousand women yearly and kills over three in five, primarily because it is discovered after it has spread to the pelvic surfaces. While many ovarian cancers begin in the ovary, recent evidence has shown that a significant percentage of these tumors - particularly serous carcinomas - arise in the distal fallopian tube. Risk reducing surgery, designed to remove the tubes and ovaries from asymptomatic women who have inherited risk factors such as mutations in the BRCA1 and BRCA2 genes, will detect an early cancer in from 5-10%. In 80% of these, the early cancer will be discovered in the distal tube, not the ovary. From 35-45 percent of women with established (symptomatic) serous ovarian or peritoneal cancer, an early cancer will be detected in the distal fallopian tube. Rarely, the examination of the distal fallopian tubes of women who undergo routine hysterectomy for benign diseases will reveal an early tubal cancer. There is evidence, albeit based on small numbers of cases, that early interception of pelvic serous cancer when it is confined to the fallopian tube will often be followed by cure or a long disease-free interval. Many current ovarian cancer detection and prevention strategies do not account for the possibility that many of these cancers arise in the distal fallopian tube.

In order to maximize the chances that an early tubal cancer will be detected, we have proposed a variation on the conventional protocol for tubal examination that provides for more extensive examination of the fimbria. This protocol, which entails Sectioning and Extensively Examining the Fimbriated end (SEE-FIM), exposes approximately 60% more surface area of the fimbria for examination. We perform this protocol in the analysis of all risk-reducing surgeries in women with and without documented BRCA1 or BRCA2 mutations. We also submit the fimbriated end as a routine in all salpingectomies, irrespective of the indications for surgery. Our work in this area has uncovered three findings of interest that will be discussed. First, we have shown that a significant minority of pelvic serous cancers - between 35 and 45 per cent - have a plausible origin in the distal fallopian tube in the form of a tubal intraepithelial carcinoma, and that a higher percentage of early carcinomas detected in BRCA+ women arise in the fimbria. Second, we have uncovered a putative precursor - the p53 signature - that we feel is an early step in this process in which benign mucosa is subject to inactivation of p53. Third, we recently expanded the precursor concept to include other early epithelial perturbations, termed secretory cell outgrowths or SCOUTs, which contain loss of PAX2 expression. Loss of both PAX2 and p53 function characterize these early steps and are bringing early serous carcinogenesis in the tube into sharper focus.

Lee, Miron et al, J of Pathology 2007
Rabban et al, AJSP 2009
Xian, Miron et al, J of Pathology 2010
Roh et al, Mod Pathol 2010 (in press)
Chen et al, J of pathology 2010 (in press)
Rabban et al, AJSP 2010
Henry Moon Lecture

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Outline
• Background
• Pelvic serous carcinoma and the fallopian tube
• The carcinogenic sequence
  – Tubal intraepithelial carcinoma (TIC)
  – p53 signatures
  – Li Fraumeni
  – Secretory cell outgrowths (SCOUTs)
• Ongoing studies

The Problem
Ovarian Cancer:
The leading cause of gynecological cancer death
• 22,500 new cases in the US every year
• 7 in 10 cannot be completely removed at surgery (advanced stage)
• 15,000 die of their disease
• No screening test

Inclusion-related tumors
• Are associated with endometriotic cysts
• Comprise a wide range of tumors that indicate a gradual progression (precursor to cancer) with mutations in:
  – K-ras
  – pTEN
  – Beta catenin

Cortical Inclusion Cysts

Inclusion-Related Tumors

Adenofibroma
Borderline Serous
Endometrioid ACA
Ovarian Carcinomas

- **Pathogenesis**
  - Some tumors appear to arise from inclusion cysts, such as endometrioid carcinomas (from endometriosis), mucinous cystadenocarcinomas, lower grade serous neoplasms.
  - The origin of the most lethal carcinomas (serous) has been controversial.

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BRCA as a Model for Early Ovarian Cancer

Up to 50% of women with BRCA mutations (BRCA+) will develop epithelial ovarian cancer without intervention

Opportunity to evaluate the tubes and ovaries in women who undergo risk-reducing salpingo-oophorectomy detect tumors early in their natural history


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SEE-FIM Protocol

- Sectioning and extensively examining the fimbriated end
- Based on the hypothesis that the fimbriated end is unique and susceptible to tubal neoplasia

Medeiros et al 2006
Serous tubal intraepithelial carcinoma (STIC)

- Proliferating neoplastic secretory cells
- 80+% of early carcinomas in BRCA+ women
- Metastasizes via exfoliation
  - Fimbrial location
  - Stratified growth with loss of polarity
  - Diffuse or completely absent p53 staining
  - High (>70% in at least some foci) MIB-1 index
  - Diffuse Cyclin E, p16 staining

“Early” STICs (BRCA+) may have a better prognosis

<table>
<thead>
<tr>
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<th>Cyto</th>
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<th>FU</th>
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<td>Neg</td>
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“Late STICs” are seen with advanced disease

- From 30-50% of women with ovarian or primary peritoneal serous carcinoma have a STIC
- Presumably the STIC was the source
- STICs share identical p53 mutations with the ovarian and peritoneal carcinomas

Kindelberger 2007, Carlson 2008

Ovarian serous carcinoma associated with tubal intraepithelial carcinoma (TIC)

Two pathways for the development of ovarian serous carcinoma

Endosalpingiosis
Mullerian metaplasia
Endometrial transport

Exfoliated tumor cells from TIC or invasive carcinoma

Mullerian inclusions
Precancerous condition
Carcinoma

Ovary or Peritoneum
How many pelvic serous carcinomas come from the fallopian tube?

- Based on RRSO in BRCA+ asymptomatic women, 80+% arise in the distal fallopian tube.
- Based on symptomatic women (all groups), from 30-50% may be linked to the distal tube.
- Is there a separate population that is not identified by RRSO alone?

Pelvic Carcinoma in BRCA+ Women

Symptomatic vs Asymptomatic


P53 Signatures

A Pecursor to Pelvic Serous Carcinoma

- Should be common
- Should be in the same location as the cancer
- Should arise from the same cell type
- Should fulfill some but not all of the biologic attributes of malignancy
- Should share some of the same risk factors as malignancy
- Should be seen in continuity with malignancy

p53 Signatures (latent precursors)

Early Serous Cancer

Benign Mucosa

Immuno-localization of p53 protein (associated with mutation) can be found in both early serous carcinomas and benign tubal mucosa (p53 signatures)

“p53 Signature”

Intense p53 nuclear accumulation in non-neoplastic tubal mucosa

Lee et al 2006
**p53 Signatures dominate in the distal tube and frequency has little or no relationship to BRCA status**

![Graph showing p53 Signatures frequency](image)


**Evidence of Transition from p53 Signature to Malignancy**

- Juxtaposition of signatures and Carcinoma
- Proliferative p53 signatures
  - P53 positive
  - Generally polarized with pseudo-stratified epithelium
  - Ciliated and secretory cells
  - Moderate MIB1 immunoreactivity
  - Do not fulfill the criteria for STIC

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**Proliferative p53 signatures**

![Images showing proliferative p53 signatures](image)
The Tubal “p53 Signature” is a Precursor to Pelvic Serous Carcinoma

- In this model, the proposed precursor (p53 signature) shares the following with serous carcinoma:
  - Involves secretory cells (BCL-2+, HMFG2+)
  - Predominates in the distal fallopian tube (fimbria)
  - Exhibits evidence of DNA damage (γ-H2AX)
  - Frequent p53 mutations by LCM and direct sequencing
  - Evidence of transition lesions
  - Seen in continuity with STIC.
  - Similar epidemiologic profile as ovarian cancer


Li Fraumeni Syndrome

- Association with an inherited mutation in one p53 allele.
- Prone to breast, other cancers, but not ovarian.
- Status of the fallopian tube uncertain.
- Associated with abundant p53 signatures in the distal fallopian tube

Early Serous Carcinogenesis

- The BRCA Model
  - Repetitive genotoxic stress to the distal tube causes p53 signatures which probably exist in every fallopian tube
  - Only one additional “hit” is required to inactivate BRCA
- The Li Fraumeni Model
  - Patient is born with the first “hit”, therefore less genotoxic stress is required to produce the second hit (p53 signature)
  - Odds of BRCA inactivation comparable to the general population.
  - Underscores the vulnerability of the distal tube.

Expanding the precursor definition

- We know that:
  - P53 signatures occur and result from a loss of p53 function
  - The distal tube is highly susceptible to this phenomenon
  - Genotoxic injury is cumulative and targets the secretory cell
- We suspect that:
  - The process should be multi-genic
  - Other genetic events are involved
Secretory Cell Outgrowths in the Fallopian tube (SCOUTs)

- Non-ciliated (secretory?) cells are essentially immature cells, and some mature (ciliate) under normal circumstances.
- The arrangement of the two populations is variable.
- Some secretory cell outgrowths (SCOUTs) can be recognized by p53 immuno-staining.

Clonal Mixed Pattern (endometrium)

SCOUTs and Malignancy

SCOUTs are significantly associated with serous carcinoma, both in absolute frequency and in frequency per tubal cross-section.
SCOUTs

- Possible explanations
  - Inconsequential variation in hormonal response – doubt.
  - Clonal expansion of a genetically altered cell that is either:
    - a stem cell that is not programmed to undergoing maturation (ciliation)
    - a secretory cell that has some capacity for ciliation
    - both scenarios

### Coordinated loss of PTEN and Pax2 Expression during and prior to Endometrial Neoplasia

![H&E, Pten, Pax2](images)

G. L. Mutter (www.endometrium.org)

### Pax2 (A)/p53 (N) SCOUTs are p53(wt)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Source</th>
<th>Tumor status</th>
<th>E2-E9</th>
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Chen 2010
Pax2 (A)/p53 (A) SCOUTs are p53(mut)

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>Exons tested</th>
<th>WT/Mut</th>
<th>Nucleotide Change</th>
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<td>missense</td>
<td>Contiguous with SCOUT (Figure 4)</td>
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P53(A)/Pax2(A) SCOUT (p53 signature)

P53 and Pax2 adjacent to serous carcinoma

Differential distribution of p53(A) and p53(N) SCOUTs

<table>
<thead>
<tr>
<th>Study</th>
<th>SCOUT</th>
<th>Fimbria (F)</th>
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<td>37</td>
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P53(N) SCOUTs do not have a predilection for the distal fallopian tube

PAX2, p53 and Serous Carcinogenesis

Chen 2010
Tung 2009

P53(A)/Pax2(A) SCOUT adjacent to serous carcinoma

Chen 2010
Rabban 2010

Mutter 2010
High-Grade Pelvic Cancers

HG Serous
Mixed
HG Serous/Emoid

HE p53 p16 Pax2 Roh et al

Heterogeneous population

Loss of PAX2

SCOUT (homogeneous non-ciliated population)
Wide distribution

Loss of Pax2 Loss of p53

p53 Signature (homogeneous Non-ciliated population with loss of p53 function)
Fimbrial Location

Loss of BRCA pathway

Intraperithelial carcinoma
Fimbrial location

Current Studies

• Other genes associated with precursor development
• Stem cell correlates to the tubal secretory epithelium
• Epidemiology of SCOUTs

Working Hypothesis

• SCOUTs might arise from cells with and without (stem?) the propensity to undergo ciliated maturation
• A second pathway (PAX2) with a broad geographic distribution is commonly perturbed and coordinates with p53 in a unique site (distal tube).
• Interruption of a single pathway involved in serous carcinogenesis could significantly influence cancer risk.

Pelvic Ovarian Cancer Interception Project

www.pointproject.org