High-grade Serous Carcinoma: Latent precursors, surrogate precursors, premalignant atypias and intra-epithelial carcinomas

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Introduction

In recent studies of the origin(s) of pelvic serous carcinoma, two concepts have emerged; the fallopian tube as a major source for these tumors and a carcinogenic sequence in the distal fallopian tube. The first has immediate implications for both the early detection and classification of this disease. The second impacts on the pathogenesis of tubal malignancies and has important implications for the histologic diagnosis. In particular, the discovery of benign appearing secretory cell outgrowths (SCOUTs) in the fallopian tube that contain functional gene perturbations shared with cancer requires a reassessment of the precursor concept. This exercise requires the separation of innocuous clonal expansions or outgrowths of no clinical significance from those with the potential, albeit low, to metastasize.

Background

Based on the World Health Organization (WHO) definition and criteria set forth by other authors the following must be met to establish the diagnosis of primary tubal carcinoma: (1) the main tumor is in the fallopian tube and arises from the endosalpinx, (2) histologic features reflect a tubal differentiation pattern, (3) if the tubal wall is involved, the transition between malignant and benign tubal epithelium should be detectable, and (4) the fallopian tube contains more tumor than any other sites (i.e. ovary, endometrium or peritoneum). 1 2 3 4

Tumors meeting all the above criteria are uncommon, making primary tubal carcinoma an extremely rare entity, which explains in part the fact that this disease is reported at a rate of less than one in 30 ovarian carcinomas. Much of this disparity is rooted in criteria 1 and 4 above, i.e. the requirement that the main portion of the tumor be centered in the tube and comprise the bulk of the tumor mass. Moreover, the term “ovarian cancer” has had an unshakeable grip on both laymen and the medical profession for the past 50 years, both eluding efforts to seriously reassess the origins of pelvic serous cancer and continuously fostered by programs that stressed serologic detection in the face of a disease that invariably presented at high stage, even in closely followed high risk populations.

A number of publications have suggested a strong relationship between the distal fallopian tube and pelvic serous cancer, both sporadic and associated with BRCA1 and BRCA2 mutations. 5 6 7 8 This discussion puts forth the most recent evidence in support of a tubal origin for pelvic serous cancer but focuses specifically on the newer entities that comprise its precursor spectrum, their relationship, direct or indirect, to cancer, and their relevance to diagnostic practice.

Precursors and their surrogates in the fallopian tube

Although the cervix is the prototype for a viral induced carcinogenic sequence – one which is repeated in other anogenital sites and the oropharynx – the pathway to malignancy in other epithelial surfaces in other organs is one of accumulated genetic changes. This scenario is seen in the bladder, esophagus Barrett’s metaplasia), prostate, pancreas and colon. 9 10 11 12 13 Each organ has a continuum of microscopic epithelial changes that reflect the gradual, age-related accumulation of genetic or epigenetic disturbances in gene function. Until recently, such a spectrum was not appreciated in the fallopian tube because the field of ovarian cancer concentrated primarily on an ovarian source. However, of late, the concept of precursors in the fallopian tube has emerged, initiated by the reports of p53 positive foci within the oviductal mucosa and subsequently,
the direct link between these benign or “latent” precursors and early malignancy. Finally, the discovery of so-called “precursor correlates” – mucosal events remote to the most common site of tumor initiation but emblematic of the same functional gene perturbations – introduces the notion that the carcinogenic sequence can be separated in both space and time. The underpinning of these site specific and more globally distributed changes in the oviduct is the “secretory cell outgrowth” or SCOUT.

Secretory cell clones with increased p53 immunostaining (p53 signatures): It is widely accepted that a precursor lesion often precedes an invasive carcinoma. This can be seen in the cervix where a cervical intraepithelial neoplasia can progress to an invasive carcinoma. However, such precursor lesion was never previously identified in serous carcinogenesis. In the fallopian tube, STICs are presumed to be an early and non-invasive carcinoma, but potentially lethal phase of malignancy that will eventually spread if not detected; hence, STICs cannot be considered as precursor lesions. While the normal fallopian tubal epithelium consists of a mixture of ciliated and secretory cells, STIC and invasive serous carcinoma are comprised of only secretory cells. Piek et al. noted that prophylactically removed fallopian tubes from BRCA positive patients contained dysplastic changes that had an increase in p53 expression and are secretory cell type. Since p53 mutation is considered integral to the development of both STICs and invasive carcinomas, Lee et al. analyzed a series of BRCA positive women and controls for p53 positivity. They required the presence of at least 12 consecutive secretory p53 positive nuclei, given that these cells sometimes intermixed with normal ciliated cells. Discrete segments of secretory cells with strong nuclear p53 immunostaining in benign-appearing tubal mucosa was identified and were designated as “p53 signature” (Figure 2A). These precursor lesions of the fallopian tube epithelium share many features with high-grade serous carcinomas, including the cell type involved, evidence of DNA damage, and p53 mutations. In addition, the p53 signatures were found most commonly in the fimbria, a site similar to STICs, supporting a tubal precursor lesion. A series of studies have identified numerous links between p53 signatures and pelvic serous cancer, establishing this entity as either a direct precursor or a reflection of the early events leading to pelvic serous carcinoma.

Surrogate precursors (Secretory cell outgrowths (SCOUTs))
The p53 signature is characterized by a linear expansion of a homogeneous population of tubal secretory cell outgrowth (SCOUT), presumably as a result of interruption in the process of normal differentiation. This process is most conspicuous in the setting of p53 mutations and DNA damage, as demonstrated by intense p53 nuclear staining and H2AX staining. However, because carcinogenesis is classically multi-genic, we surmised that the oviductal mucosa could also give rise to benign clonal expansions (SCOUTs) that reflected disturbances in genes other than p53. Recently, down-regulation of Pax2, a member of the pair box (PAX) gene family expressed in müllerian duct derivatives, has been documented in both early endometrial carcinogenesis, including normal endometrial glandular epithelium, and pelvic serous carcinomas, implicating this gene in the carcinogenesis of gynecologic tract malignancy. We have recently demonstrated that Pax2 expression is consistently down-regulated in SCOUTs. Hence, similar to the endometrium, SCOUTs appear to signify a latent serous precancer commonly harboring loss of Pax2. The subset of p53-positive SCOUTs (e.g. “p53 signatures”) seen in continuation with STICs and associated invasive serous carcinomas show concomitant loss of both Pax2 and p53 function, further linking the fallopian tube precursor to pelvic serous carcinogenesis and supporting the accepted multi-hit model for this malignancy. It is still not entirely clear whether Pax2 and p53 function in the same pathway leading to serous carcinogenesis. Several pieces of evidence suggest that Pax2 and p53 might function independently. First, p53 signatures (inactivation of p53 function) occur more frequently in the fimbria. In contrast, SCOUTs are more widely distributed in the fallopian tube. Thus, exposure to genotoxic injury, while sufficient in some loci to produce p53 signatures, is not necessarily required to produce dysregulation of Pax2. The fact that SCOUTs are so widely distributed is at odds with their direct role in serous carcinogenesis. Thus the term surrogate precursor is more appropriate, in that SCOUTs signal the presence of gene perturbations that are associated with serous cancer.

Premalignant tubal epithelial atypia
Secretory cell outgrowths, including most with p53 mutations, are not accompanied by either atypia or more than a mild increase in proliferative index and are very common in the fallopian tube. Over one half of controls will exhibit an uninterrupted sequence of secretory cells with strong p53 immunostaining. PAX2-null outgrowths with variable ciliated differentiation are seen in a high percentage of fallopian tubes, albeit more commonly in women in the older age groups or with co-existing pelvic serous cancer. In contrast, expansile or proliferative p53 positive epithelia, variously termed proliferative p53 signatures or tubal intraepithelial lesions in transition, are uncommon and bear some resemblance to tubal intraepithelial carcinomas. In contrast, they exhibit preserved epithelial polarity, a lower proliferative index, frequent admixtures of ciliated cells and in our experience, less consistent staining for markers associated with ovarian cancer such as p16. Because they are much larger than most p53 signatures, often spanning hundreds of nuclei, they probably merit consideration as intraepithelial neoplasms; however, a more generic term such as premalignant epithelial atypia clearly distinguishes them from both STICs and latent precursors such as p53 signatures.

Practically speaking, there are three separable forms of p53 positive (or less commonly p53 null) oviductal epithelium with sufficient atypia to warrant concern. The first is premalignant atypia, in essence an expansile or proliferative p53 signature. The size criteria are not fixed, but they typically encompass at least an entire plical surface or its equivalent. They have not acquired the attributes of malignancy but clearly have acquired a growth advantage. When discovered in prophylactic specimens such atypias will usually be discovered and if so, should be classified as epithelial atypias that do not fulfill the criteria for malignancy.

Tubal intraepithelial carcinoma
Tubal intraepithelial carcinomas are typically serous (STICs) and differ from premalignant atypia by a high nuclear-cytoplasmic ratio and most importantly, conspicuous loss of polarity with variable stratification. The latter is often associated with small clusters of un-polarized cells on the surface, or small intraepithelial fractures splitting off groups of cells. Immunostains for a Mib1 will typically reveal an index of greater than 70% in at least a portion of the lesion although the most important parameters are histologic. I emphasize the latter because I have seen STICs with very low MIB1 indices associated with metastatic serous carcinomas. We have been studying markers linked to cell polarity that might aid in separating premalignant atypias from STICs, one being p-ERM. This marker has a distinctive luminal distribution in benign and premalignant epithelia. Similar staining can be found in STICs but the latter typically display a combination of loss of luminal p-ERM staining, irregular staining pattern reflecting the loss of normal cellular orientation, including staining of individual cell membranes. This progression from one pattern to the other is not abrupt, but appears to reflect the underlying changes in biology (This is shown in the poster by Ning et al).

STICs can be divided into "early" and "late" varieties. Early ones, by definition, are typically encountered in risk reducing salpingo-oophorectomy specimens and may not always display as striking atypia as that seen in intraepithelial carcinomas accompanying stromal invasion or metastases. These can be viewed as localized tubal intraepithelial carcinomas. The implication is that they carry some but not an absolute risk of recurrence. The STICs associated with invasion and/or metastasis can be viewed as metastasizing tubal intraepithelial carcinomas. The clinical implications of the above classification should be clear.

Do peritoneal carcinomas come from the oviduct?
So-called primary peritoneal serous cancers are so defined because there is disseminated disease without a clear source. We know that they bear a close resemblance to presumed fimbrial/ovarian carcinomas and that in approximately one-half, a STIC can be implicated in their pathogenesis. We also know that approximately 6% of BRCA+ women who undergo RRSO will develop a pelvic serous cancer at a later time point. We know that some of these cases are likely the consequence of a disseminated STIC, either by prospective follow-up or retrospective discovery of STIC in the RRSO specimen. What is not clear is the pathogenesis of pelvic cancers that develop despite documented normal fallopian tubes. This is difficult to establish given the fact that some STICS are sufficiently small to be overlooked. Thus, until large prospective studies are done with exhaustive examination of the oviducts, the exact percentage in which the tubes can be excluded will not be known. If occult disease in the fallopian tubes gave rise to these peritoneal tumors, there are two potential scenarios. In the first, an occult STIC would shed cells to the peritoneum that survived but did not grow.
immediately. The acquisition of additional mutations would result in a malignancy. In the second normal tubal cells would become ensconced in the peritoneum and undergo malignant transformation. This is difficult to believe given the fact that the peritoneal surface is remote to the fimbrial-ovarian region, where most tumors develop and would not be exposed to the same carcinogenic stimuli. Two additional possibilities exist. The first is endometriosis, which unquestionably gives rise to high-grade müllerian carcinomas on occasion. The last is a source in the ovary such as the ovarian surface epithelium or another cell type that has yet to be discovered.

11 Epstein JI. Precursor lesions to prostatic adenocarcinoma. Virchows Arch. 2009;454:1-16


Monte NM, Webster KA, Neuberg D, Dressler GR, Mutter GL. Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. Cancer Res. 2010;70:6225-32

STICs, SCOUTs, p53 Signatures and the road to Pelvic Serous Cancer

Christopher P. Crum, MD

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Outline

• Latent precursors to pelvic serous cancer
  – Pathogenesis
• Serous tubal intraepithelial neoplasia
  – Progression sequence
  – Clinical management
• “Surrogate precursors”
  – A risk model?
Outline

• Precursors to pelvic serous cancer
  – Pathogenesis

• Serous tubal intraepithelial neoplasia
  – Progression sequence
  – Clinical management

• “Surrogate precursors”
  – A risk model?
Where do Pelvic Serous Cancers Come From?

• Possibilities
  – Ovarian surface mesothelial to mullerian epithelial transitions leading to cortical cysts
  – Transported endometrial or salpingeal epithelium
  – \textit{In situ} transformation of salpingeal epithelium with subsequent spread
  – De novo transformation of mullerian (or endometriotic) epithelium in the pelvis
  – Something we haven’t discovered yet.
Early serous carcinoma in the Fimbria
A substantial percentage of high-grade serous carcinomas can be traced to the oviduct.

A Precursor to Pelvic Serous Carcinoma

• The p53 Signature
  – Morphologically benign process involving secretory cells
  – Self limited, with variable atypia
  – Accumulation of p53 with p53 mutation
  – Predominates in the distal fallopian tube
  – Exhibits evidence of DNA damage response (H2AX)
p53 Signatures (latent precursors)

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 2007
p53 Signature

STIC

Lee 2007
The Distal Oviduct in 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

Xian 2010
p53 Signatures predominate in the distal tube and frequency has no clear link to BRCA status

Evidence of Transition from p53 Signature to Malignancy

Lee, Miron 2007, Carlson 2008

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Serous Carcinoma

STIC

P53 signature
Questions

• Do I need to look for p53 signatures?
  – No

• Does the presence of p53 signatures alone confer an increased risk of malignancy?
  – Uncertain given their high prevalence

• Should I immunostain benign appearing tubes for p53 signatures every time I see a mildly atypical epithelium?
  – No
Outline

• Latent precursors to pelvic serous cancer
  – Pathogenesis

• Serous tubal intraepithelial neoplasia
  – Progression sequence
  – Clinical management

• “Surrogate precursors”
  – A risk model?
Practical Issues

• Thorough examination of the oviducts of women with malignancy or at risk for malignancy.
• Discriminating early malignancies from early points in the neoplastic spectrum
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
Functional Steps in Serous Cancer Developments

• P53 signature – non expansile
• Non-diagnostic tubal atypia.
  – Expansile or proliferative p53 signatures
  – Tubal intraepithelial lesions in transition
  – Tubal intraepithelial lesions
• High grade tubal intraepithelial neoplasia
  – Localized STICs (RRSO)
  – Metastasizing STICs (High stage cancers)
STIC

- Proliferating neoplastic secretory cells
- Presumably metastasizes via exfoliation
  - Disorganized or stratified growth with loss of normal cell orientations (polarity), more stratified
  - Diffuse or completely absent p53 staining
  - Mib1 index is high (>40% in at least some foci) in most cases but can be deceptively low
  - Strong Cyclin E, EZH2, p16 staining, etc. in many but not all

TIC – loss of polarity

TIC – intra-epithelial fractures
Some molding but not TIC

Nuclear enlargement but not TIC
Non-diagnostic tubal atypia

- Expansile/proliferative p53 signature
- Presumably has no risk of metastasizing
  - Cohesive cellular growth
  - Preserved orientation, more pseudostratified, presence of ciliated cells
  - Diffuse or completely absent p53 staining
  - Mib1 index is variable
  - Weak or variable Cyclin E, EZH2, p16 staining

Non-diagnostic atypia – preservation of polarity and cilia
Cell Orientation (polarity)

- P-ERM (Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558))
  - Cell adhesion, membrane ruffling, and microvilli formation
  - Altered distribution pattern in TICs

Ning et al abstract 1217
P-ERM in p53 Signature
P-ERM in STIC

Normal Mucosa

STIC
“Early” STICs are the first manifestation of malignancy in most asymptomatic BRCA+ women and when isolated confer a favorable outcome

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“Late” STIC with Mets)
But is there some other pathway to pelvic serous cancer?

• Possibilities
  – Ovarian surface mesothelial to müllerian epithelial transitions leading to cortical cysts
  – Transported endometrial or salpingeal epithelium with subsequent transformation
  – De novo transformation of mullerian (or endometriotic) epithelium in the pelvis
  – Something we haven’t discovered yet.
Early pelvic cancer in asymptomatic BRCA+ women

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<td>Hirst (2009)</td>
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<td>4(9)</td>
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<td><strong>TOTAL</strong></td>
<td>490</td>
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* Updated to 2010, 17/20 associated with TIC (85%)
STIC in symptomatic women with pelvic cancer

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<td>Seidman (2011)</td>
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Pelvic Carcinoma in BRCA+ Women

(S)ymptomatic vs (A)symptomatic

Outline

• Latent precursors to pelvic serous cancer
  – Pathogenesis
• Serous tubal intraepithelial neoplasia
  – Progression sequence
  – Clinical management
• “Surrogate precursors”
  – A risk model?
What else is going on in the 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 immunostaining.
Secretory cell outgrowths (SCOUTs)

- p53 Signatures
- p53 mutations
- H2AX++, HMGA2+, PAX2(-)
- Immediate precursor to STIC
- Fimbrial
- Common in all populations
  
  Chen 2010, Mehra 2011

- Generic SCOUT
- Normal p53 genotype
- H2AX (-), BCL2++, PAX2 (-)
- Not an immediate precursor to STIC
- No regional predilection
- Higher frequency with serous cancer
  
  Chen 2010, Mehra 2011

www.obgynpath.org
PAX2-null SCOUT

SCOUT Differentiation

Secret                  Secretory/Ciliated      Papillary

A  B  C

D  E  F
Papillary SCOUTs

Some secretory cell outgrowths closely resemble low grade serous neoplasms

Laury et al 2011
Location of SCOUTs in the Oviduct

Quick 2011, in press
PAX2-Null SCOUTs increase in frequency in oviducts associated with serous tumors

Quick 2011

www.obgynpath.org
Quantifying SCOUTs as a function of epithelial perimeter

Image courtesy of A Laury and M Quick
PAX2-Null SCOUTs (average frequency per section)

Bijron et al abstract 1093
Objective confirmation of the elevated frequency of PAX2-null SCOUTs in oviducts of women with serous neoplasia (Quick et al, Laury et al, Chen et al)
SCOUT
- PAX2 null
- ALDH +/-
- RCN1+
- LEF1+
- P53wt
- Histologically benign

p53 Signature
- PAX2 null
- ALDH -
- RCN1+
- LEF1+
- P53mut
- Histologically benign

STIC
- PAX2 null
- ALDH -
- RCN1+
- LEF1+
- P53mut
- Histologically malignant
Expanding the glossary for serous carcinogenesis in the oviduct

- **Pelvic serous cancer** – a widely distributed serous carcinoma
- **Latent precursor** – p53 signature; common and typically self limited.
- **Surrogate precursor** – PAX2-null SCOUT; not be directly linked to, but shares functional gene perturbations with, malignancy
- **Premalignant epithelial atypia** – an expanded or proliferative p53 signature with atypia; must be distinguished from STIC
- **STIC** – a non-invasive malignancy of the fallopian tube with metastatic potential.