Rationale for Developing a Specimen Bank to Study the Pathogenesis of High-Grade Serous Carcinoma: A Review of the Evidence

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Abstract

Women with clinically detected high-grade serous carcinomas (HGSC) generally present with advanced-stage disease, which portends a poor prognosis, despite extensive surgery and intensive chemotherapy. Historically, HGSCs were presumed to arise from the ovarian surface epithelium (OSE), but the inability to identify early-stage HGSCs and their putative precursors in the ovary dimmed prospects for advancing our knowledge of the pathogenesis of these tumors and translating these findings into effective prevention strategies. Over the last decade, increased BRCA1/2 mutation testing coupled with performance of risk-reducing surgeries has enabled studies that have provided strong evidence that many, but probably not all, HGSCs among BRCA1/2 mutation carriers appear to arise from the fallopian tubes, rather than from the ovaries. This shift in our understanding of the pathogenesis of HGSCs provides an important opportunity to achieve practice changing advances; however, the scarcity of clinically annotated tissues containing early lesions, particularly among women at average risk, poses challenges to progress. Accordingly, we review studies that have kindled our evolving understanding of the pathogenesis of HGSC and present the rationale for developing an epidemiologically annotated national specimen resource to support this research. Cancer Prev Res; 9(9): 713–20. ©2016 AACR.

Overview of the Problem

Ovarian carcinoma accounts for more than 22,000 incident cases and 14,000 deaths annually in the United States (1). The most common histopathologic subtype of ovarian carcinoma is high-grade serous carcinoma (HGSC), which characteristically presents with symptomatic, late-stage, high-volume disease. Even with aggressive treatment, the prognosis of advanced-stage HGSC is poor, with 5-year survival rates estimated at less than 50% (2).

Among women with deleterious BRCA1/2 mutations, risk-reducing salpingo-oophorectomy (RRSO) is effective in reducing ovarian cancer incidence and mortality (3). Unexpectedly, early pathology studies of RRSO specimens led to the identification of putative clinically occult HGSC precursors in the fimbria of the fallopian tubes, rather than in the ovarian surface epithelium (OSE), as anticipated (4). Subsequently, many studies have described putative HGSC precursors in tubes of BRCA1/2 mutation carriers (reviewed in ref. 5); however, descriptions of these lesions among noncarriers, especially in the absence of concurrent HGSC, remain rare (6, 7), and developing the specimen resource required to investigate such lesions is challenging. Herein, we review recent advances in the understanding of the pathogenesis of HGSC and provide evidence that the development of a tissue bank may facilitate translation of recent findings into improved prevention strategies.

Screening and Prevention Approaches for HGSC

To date, approaches for ovarian/tubal cancer screening and prevention in the general population (8–10) have been disappointing. Screening using CA-125 blood testing at a fixed threshold in combination with pelvic ultrasound did not reduce ovarian cancer mortality in the Prostate, Lung, Colorectal and Ovarian
Cancer Screening Trial (11) or earlier studies (summarized in ref. 12). In the United Kingdom Collaborative Trial of Ovarian Cancer Screening, serial CA-125 serum levels analyzed with the risk of ovarian cancer algorithm in combination with transvaginal ultrasound also did not demonstrate a statistically significant mortality reduction (13), despite a favorably stage shift (14). Although long-term use of oral contraceptives reduces risk of developing ovarian cancer by up to 50% (15), uptake for this indication has been limited by concerns related to increased risks of thrombotic complications, stroke, and breast cancer (16).

Despite the aforementioned challenges, the discovery that many HGSCs found among asymptomatic BRCA1/2 mutation carriers seem to arise from the fallopian tubes offers hope of achieving a breakthrough in the early detection and prevention of this disease. However, the percentage of HGSCs that originate in the fallopian tube among BRCA1/2 mutation carriers and non-carriers is unclear. Further, lack of sufficiently annotated benign gynecologic tissues, putative HGSC precursors and early-stage HGSCs from non-carriers poses an obstacle to pursuit of this work.

**Evolving Views on the Molecular Histology and Pathology of the Fallopian Tube**

Prior to implementation of RRSO as a prevention strategy among BRCA1/2 mutation carriers, pathologists rarely encountered specimens containing low-volume HGSC, and when such tumors were identified, attention was routinely focused on the ovaries (17). HGSC was presumed to develop from OSE because tumor was frequently present on the ovarian surface. OSE was presumed to represent the source of a unique progenitor of HGSC, and the risk of HGSC increases with a woman’s number of lifetime oovulations. In this model, each ovulation would subject the OSE to injury and repair that could lead to accumulation of deleterious mutations (18). Among cases of HGSC, ovarian and peritoneal involvement is often extensive, whereas tubal involvement is comparatively subtle, easily overlooked, and was seldom sought historically. Thus, the failure to identify dysplastic changes in OSE increased the risk of HGSC. Moreover, the microscopic size of almost all STIC lesions; (ii) incomplete standardization of the extent of pathology processing of gynecologic tissues is enhanced by targeted next-generation sequencing methods that may enable molecular characterization of these lesions in fixed tissues, despite their minimal size (50). These studies may also provide molecular evidence suggesting that some “STIC” lesions represent secondary deposits from endometrial carcinomas (50) and that the clonal relationships of multiple foci of STIC and carcinoma within a single woman are complex (51, 52).

In addition, the development of genetically engineered mouse models that recapitulate the origin of HGSC from the fallopian tube epithelium may suggest new prevention strategies (57, 58).
represent the best available approach to study the biology of these lesions.

**Detection and Characterization of HGSC and Putative Precursors**

The Sectioning and Extensively Examining the Fimbria pathology protocol ("SEE-Fim") was developed to enable detailed comprehensive microscopic study of the fallopian tube in RRSO specimens (Fig. 1; ref. 49). Dissemination of data regarding detection of STIC at RRSO, and guidelines that emphasize microscopic examination of the tube when cancer is present, have undoubtedly led to increased use of SEE-Fim (61). However, pathology processing of surgical specimens removed from women with wild-type \textit{BRCA1/2} for benign indications is likely more variable, particularly if the tubes and the ovaries appear unremarkable on microscopic examination of the "representative sections" initially submitted for histologic processing.

Among 523 sequential surgical pathology specimens removed for benign indications that were processed according to a modified SEE-Fim approach for research, 4 STICs and 11 additional examples of epithelial atypia were identified (47). A recent study found STICs in 3 (0.17\%) of 1,747 specimens from women 50 years of age and older who neither harbored a concurrent pelvic or uterine HGSC, nor were known \textit{BRCA1/2} mutation carriers (E. Meserve and C. Crum, unpublished). Experience suggests that if these specimens had been processed routinely, many STIC lesions may have been missed. In contrast, among 966 high-risk women with or without deleterious \textit{BRCA1} mutations who elected immediate risk-reducing surgery in Gynecologic Oncology Group Protocol-0199, STICs were identified in four and invasive fallopian tube cancers in five women (5). Among women who are not \textit{BRCA1/2} carriers, STIC is infrequent; however, the absolute number of STICs in this group may be substantial given that these women account for 85\% to 90\% of HGSCs in the population. Further, germline mutations in genes other than \textit{BRCA1/2} may increase risk of HGSC and these women may also harbor STIC or other cancer precursors (62).

The "molecular histology" of the fallopian tube, broadly conceptualized as the morphology, molecular biology, and function of benign tubal tissues in relation to risk exposures has not been extensively studied; however, similarities have been found between the transcriptome of benign tubal epithelium of \textit{BRCA1/2} carriers and HGSC (63, 64), prompting a hypothesis that mutation carriers may respond abnormally to post-ovulatory inflammation (65). In addition, stretches of p53 immunopositive cells have been identified in approximately 24\% of carriers of \textit{BRCA1/2} mutations and 33\% of women undergoing benign surgery (ref. 27; Fig. 2). These "p53 signatures," which may appear cytologically normal or show only mild cytologic atypia, are not highly proliferative, but frequently demonstrate \textit{TP53} mutations and stain positively for \textit{γH2Ax}, a histone that is phosphorylated by ATM kinase at sites of double-strand DNA breaks. Compared with STIC and HGSC, p53 signatures are much more common, especially with intensive scrutiny (59), suggesting that many would not progress to neoplasia if left intact, although a minority of such lesions may represent early steps in carcinogenesis. Areas of secretory cell outgrowths (SCOUTs) composed of stretches of non-ciliated cells expressing wild-type p53 have also been recognized in otherwise histopathologically unremarkable fallopian

**Figure 1.**

Macroscopic appearance of fallopian tube demonstrating SEE-Fim protocol (A–C). Approach to longitudinal sectioning of fimbria (B) and preparing cross-sections of tubes (C). Hematoxylin and eosin-stained section of fimbria (D). This figure was published in Diagnostic Gynecology and Obstetrics Pathology, Christopher Crum, Marissa Nucci and Kenneth Lee, Chapter 21, The Fallopian Tube and Broad Ligaments, p. 701, copyright Elsevier.
Fallopian Tube Pathology in Clinical Practice and Translational Research

The interobserver reproducibility of the diagnosis of STIC based on morphology is suboptimal. Although use of immunohistochemical stains may improve agreement (28, 31, 67–69), expert consensus is the only available measure of diagnostic accuracy. Establishing reproducible and accurate diagnoses of STIC is a prerequisite for developing clinical studies to improve management. Accurate diagnosis of STIC will likely pose an increasing clinical problem, as BRCA1/2 mutation testing, performance of RRSO, and meticulous examination of surgically removed fallopian tube increases. Moreover, only 6% to 10% of STICs encountered in RRSOs of women with BRCA1/2 mutations have an outcome of metastatic HGSC, raising important questions about the risk of progression of this putative early form of HGSC (70, 71).

Anecdotal observations suggest that cells from STIC lesions may exfoliate from the fallopian tube mucosa and implant on the ovary or peritoneum without invading through the basement membrane of the tube (79). Staging procedures may demonstrate invasive HGSC in cases initially diagnosed as STIC (80). Interest in the topic of prophylactic salpingectomy with deferred oophorectomy will likely magnify unaddressed concerns regarding whether detection of STIC or STIC-like lesions necessitates immediate oophorectomy, and possibly, formal cancer staging. In fact, clinical observations (81) and studies of animal models (53) suggest that ovarian involvement may potentiate the malignant behavior of early HGSC. Further, the value of offering BRCA1/2 genetic testing to women with incidental STIC is unknown. It is also unclear whether high-risk women who undergo salpingectomy will return for delayed oophorectomy, and if so, when that should be performed to maximize cancer risk reduction, while minimizing negative effects of estrogen deprivation, including osteoporosis and cardiovascular disease.

National Gynecological Specimen Bank: Considerations

The overarching goal of creating a national gynecological specimen bank would be to provide epidemiologically annotated samples to the research community to pursue high-quality research related to the pathogenesis of early-stage HGSC. Although investigators have collected RRSO samples, and a campaign promoting ‘opportunistic salpingectomy’ with benign tube epithelium, but whether this is a variant of normal or a subtle alteration associated with greater cancer risk is also uncertain (66).
Historically, pathologists have examined grossly unremarkable fallopian tubes sparingly, mainly for documentation purposes; however, clinical practices are likely changing. Thus, by leveraging the shift toward routinely examining tubes more thoroughly, it may be practical to efficiently identify the rare cases of STIC among non-carriers of *BRCA1*/2 mutations, without vastly modifying routine pathology protocols for research. Specifically, electronic searches of surgical pathology reports may be sufficient to identify a useful number of women with STIC, even if such cases are rare. Further, more extensive sampling of the ovary and endometrium may reveal unsuspected non-tubal HGSC precursors, such as endometrioid intraepithelial carcinoma, the probable precursor of uterine serous carcinoma (82).

*BRCA1*/2 carriers are diagnosed with HGSC at earlier ages, respond better to treatment, and in a recent meta-analysis, had improved survival compared with non-carriers at a median of 6.3 years (83). Further, studies suggest HGSC comprises multiple histopathologic patterns, which may be differentially associated with loss of *BRCA1*/2 function, STIC, age at onset or prognosis (42, 43). Similarly, HGSC may include multiple molecular subtypes with different clinical behaviors (84). Accordingly, the hypothesis that most HGSCs among non-carriers develop from STICs represents an untested hypothesis, which could be evaluated using tissue bank resources. Defining whether tubal lesions are associated with HGSC among women who are not carriers of *BRCA1*/2 mutations would be useful, either confirming a common approach to HGSC prevention, irrespective of mutation status, or redirecting attention to other approaches.

The proposed bank would collect pathology specimens from three contexts: (i) selected procedures performed for benign indications, such as hysterectomy or surgical sterilization; (ii) RRSO or risk-reducing salpingectomy; and (iii) HGSC, especially stages I, II, or IIIA (Fig. 3). An important aspect of the resource would be the collection of specimens from non-carriers that were removed for benign indications, but which revealed occult STIC or minimal HGSC on microscopic review. In addition, the bank would collect tissues from all RRSOs, HGSC cases, especially those defined as stage I or II or stage IIIA1i (disease volume ≤ 10 mm), and a judiciously selected sample of matching normal tissues from benign surgeries, including fallopian tubes. Each sample would be annotated with minimal medical history as required to estimate risk of developing HGSC within a reasonable logistical framework (85). Centers contributing specimens to the bank would agree to process pathology material according to a standard protocol (Fig. 1). Given that SEE-Fim processing is recommended for cases with STIC or HGSC (61) and that many pathologists are probably examining the tubal fimbria routinely, finding pathology laboratories that are currently processing samples that can identify HGSC precursors and early HGSC may be possible, without altering existing practices. This would enable a post hoc selection of a small percentage of specimens from a large pool by re-contacting patients after surgery for consent as needed and further collection of data and specimens. A survey of pathology services

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**Figure 3.**

Centers participating in the proposed bank would perform SEE-Fim on all fallopian tubes for microscopic examination. The bank would include the following specimens: RRSO, risk-reducing salpingectomy, any specimen with a diagnosis of STIC, or HGSC (multiple annotated samples of primary and metastatic deposits), SEE-Fim processing, and extensive endometrial sampling to assess the presence of early uterine serous carcinoma. Benign specimens would be selected randomly to create a set of tissues for comparison with those showing putative or diagnostic lesions. Clinical and epidemiologic annotation and source of germline DNA (e.g., unused blood drawn clinically) would be collected as permitted. Residual liquid-based cytology samples would also be banked.

**Women > 25 years of age**

- Gynecologic surgery for benign indications with removal of one or both fallopian tube(s)
- Risk-reducing salpingo-oophorectomy or salpingectomy, irrespective of final pathologic diagnoses
- Surgery for HGSC, stages I, II, or IIIA

**Procedures**

- Consent to research using de-identified tissues
- Residual blood collected on day of surgery
- Medical abstract: one page, including medications
- Reports: CA-125, radiologic imaging

**Pathology processing benign surgery**

- Routine processing
- Fimbria submitted in total
- RRSO, STIC, or HGSC
  - Complete SEE-Fim protocol
  - Submit the majority of the endometrium

**Pathology central review**

- Benign: pathology, including fimbria
- Ki67, p53 stains
- Package formalin-fixed and possibly residual wet tissues for temporary storage and later triage

Submit all material to bank

Submit stratified random sample of material to bank
laboratories to assess usual tissue sampling procedures for specimens by clinical indication as would be needed to develop a pilot project is ongoing.

The bank could be pilot tested in pathology laboratories that perform SEE-Fim on all tubes and meticulously sample ovaries and endometrium. Benign surgical pathology specimens removed from non-carriers could be handled using a two-stage approach. Specifically, the fimbria of fallopian tubes from procedures with a benign diagnosis would be processed in their entirety for clinical diagnosis and later centrally reviewed for research. On a rolling basis, a stratified random sample of benign specimens without STIC or HGSC would be chosen with oversampling of those at greatest risk (85). These samples could be used in comparative molecular analyses.

Goals of Research Using Banked Gynecologic Tissue Samples

Potentially, data from medical charts could be supplemented by questionnaires. Data and materials from the proposed bank could be used to address a wide range of potential questions related to the pathogenesis of HGSC, including (but not limited) to those defined below.

- Does the molecular histology of the fallopian tube, particularly the epithelium of the fimbria and/or its microenvironment, vary by critical factors including BRCA1/2 mutation status, age, menopausal status, family history of breast or ovarian cancer, medications, parity or other factors?
  - Are factors associated with risk of developing HGSC associated with the ‘omic’ profile of the benign appearing tubal epithelium?
  - How do molecular profiles of the fimbria and non-fimbria tubal epithelia compare, and what are the similarities and differences?
  - Does the frequency of detecting p53 protein overexpression by immunohistochemistry vary by risk of HGSC among carriers and among non-carriers?
- Does the frequency, extent or molecular profile of microdissected ‘p53 signatures’ vary by risk factors among non-carriers or carriers of deleterious BRCA1/2 mutations? Are certain specific p53 mutations in ‘p53 signatures’ related to HGSC, while other mutations are not?
  - Are ovarian cancer risk factors associated with important characteristics of the microenvironment, including number and immunophenotype of mononuclear cells, microvessel density, collagen, or matrix factors or biophysical characteristics?
  - Are ovarian cancer risk factors associated with markers of cell stress, DNA damage, DNA repair, proliferation, apoptosis, inflammation, and telomere length in benign appearing tubal epithelium?
- How do molecular profiles of STIC, normal appearing epithelium adjacent to STIC and small foci of HGSC deposits compare within and between patients? What evidence is there for clonal relationships between classes of lesions and metastatic deposits and what specific molecular abnormalities are likely drivers of early events in the pathogenesis of these lesions?
- How do molecular profiles of benign appearing fallopian tube epithelium among women with small cancers that are not associated with STIC compare with those that are associated with STIC?
- How heterogeneous is the molecular profile of HGSC and does it vary by age and ovarian cancer risk factors? Do molecular signatures vary by proposed histological subtypes of HGSC?
  - Is there evidence of intratumoral molecular heterogeneity at the earliest stages of HGSC?
  - Given that ovarian involvement may be linked to accelerated dissemination of malignant cells, are there differences in gene expression between tubal and ovarian foci of HGSC?

Conclusions

The development of a national gynecologic tissue bank to study early-stage HGSC and its precursors holds promise for enabling researchers to identify improved methods for early cancer detection and prevention because an important challenge to conducting this research is the scarcity of carefully annotated tissue specimens representing different hypothesized stages in the development of HGSC. However, assembling this resource would require a complex multi-institutional effort, substantial investment, and equitable access based on objective merit of proposed studies. Accordingly, assessment of feasibility and pilot testing to define a cost-effective approach are important prerequisites for considering this project.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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