Published in final edited form as:

Gynecol Oncol. 2012 December ; 127(3): 645–650. doi:10.1016/j.ygyno.2012.08.023.

New Views on the Pathogenesis of High-grade Pelvic Serous Carcinoma with Suggestions for Advancing Future Research

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Keywords

Ovarian Cancer; Pathogenesis; Seed and Soil; Fallopian Tube; Serous

Background

High-grade serous carcinomas (HSCs) of the gynecological tract typically present with advanced stage disease, which often proves fatal despite treatment[1]. Effective approaches for early detection or prevention of HSCs have not been developed [2]; however, the emergence of new insights into the pathogenesis of these tumors raise hopes that achieving this goal may be possible in the future[3–6].

Historically HSCs were widely presumed to arise from ovarian surface epithelium (OSE), which consists of a single cell layer that envelops the ovary and may line subjacent cortical invaginations and cysts[7]. Most histopathological studies have provided only weak, inconsistently replicable evidence for the existence of HSC precursors arising within OSE[8–13]; nonetheless, the view that HSCs arise from OSE has been sustained by the belief that HSCs might rapidly overgrow and destroy the precursors from which they arise. In support of this view, a recent analysis comparing OSE (not related to cysts) to cells lining ovarian inclusion cysts found that the latter demonstrate: greater proliferation; less apoptosis, DNA aneuploidy and a “quasi-neoplastic” gene expression signature[14]. Irrespective of whether some HSCs arise from ovarian inclusion cysts, it is now clear from detailed pathological study of risk-reducing salpingoophorectomy (RRSO) specimens that occult carcinomas in the fallopian tube fimbria are surprisingly common, with one prevalence estimate of 8%, based on aggregate data from several small studies(reviewed

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in[5]). However, analysis of data from Gynecologic Oncologic Group Protocol 199 (GOG-199), A Prospective Study of Risk Reducing Salpingoophorectomy and Longitudinal CA-125 Screening among Women at Increased Risk of Ovarian Cancer, found only 9 neoplastic tubal lesions in 966 RRSOs[15]. Of the 9 neoplastic lesions, 8 occurred among *BRCA* mutation carriers. Thus, the fallopian tube fimbria has emerged as an important, if not the dominant source of HSCs, at least among *BRCA* carriers, but identification of cases with a clear tubal origin remains comparatively rare secondary to tumor overgrowth, and much less is known about non-familial cases.

As current research on HSC pathogenesis focuses increasingly on the tubal fimbria, we present here a model in which the fallopian tube, OSE and ovarian stroma cooperate in the pathogenesis of HSC, with each component playing critical roles. Specifically, we provide evidence from descriptive epidemiological data to support the hypothesis that some HSCs may remain clinically occult in the ovary for months to years prior to diagnosis, offering prospects that early detection and curative intervention may be possible for these tumors. We suggest that this process is akin to the “seed and soil” hypothesis of metastasis, in which a primary cancer site represents the “seed” and the metastatic site the “soil”. In addition, we cite prior studies, including our own, showing that uterine HSCs that may be associated with tubal and/ or ovarian lesions that resemble *in-situ* carcinoma [16, 17]. However, it is unclear whether such lesions represent tubal, endometrial or multifocal primaries [18]. Thus, understanding the origins of HSC may require comprehensive, concurrent analysis of all of these structures, rather than narrowly focused attention on the tubal fimbria. However, accomplishing this goal will require improved methods of collecting and analyzing cells and tissues of the upper gynecological tract. Accordingly, we present our experience collecting OSE using a novel intra operative approach, and note other opportunities to optimize tissue processing of the ovary and tubes.

Prevalence of HSC Precursors in Fallopian Tubes

Our knowledge of the prevalence of HSC precursors is based mainly on pathological studies of RRSOs, in which precursors are undoubtedly more frequent than in the general population, because this procedure is generally restricted to women at markedly elevated risk of ovarian and fallopian tube cancer. Nonetheless, published estimates of HSC prevalence, even in this group, are almost certainly too low, for several reasons:

1. Many tubes removed as part of RRSOs are not entirely submitted for pathological processing, despite recent recommendations to do so [19, 20].
2. Even when fallopian tubes are processed “in their entirety” for histopathological study, pathologists typically examine a single 5-micron section per 2 mm-thick paraffin tissue block leaving more than 99% of tissue unexamined. Given that occult tubal intraepithelial carcinomas may measure 1-mm or less, this sampling strategy could miss small lesions. Furthermore, more extensive sectioning and histological examination of RRSOs is associated with increased detection of lesions[20–22].

3. Until recently, few pathologists had experience with the diagnosis of tubal intraepithelial carcinoma and, consequently, its diagnostic reproducibility is suboptimal, although potentially improvable with immunostains for p53 and Ki67[23].

Recognition that the prevalence of HSC precursors is higher than previously suggested implies that the lesional incidence and/or time of persistence have been underestimated. Accordingly, defining mechanisms and biomarkers related to progression of occult lesions to fully developed cancer could have important translational value for cancer prevention and early detection.

The identification of tubal intraepithelial carcinomas in the fimbriae raises questions regarding whether HSC precursors might develop within other, more medial segments of the tube or spread secondarily to these segments. If tubal carcinomas neither arise within nor spread to medial segments of the tube, it remains important to know why these epithelial segments are resistant to malignant change, replacement or implantation. Overall, knowledge of the prevalence, extent and location of HSC precursors within the tube remains incompletely described among high-risk women, and virtually unknown among average risk women.

Studies using the SEE-FIM protocol (Sectioning and Extensively Examining the Fimbria) suggest that intraepithelial carcinoma occurs more frequently in the fimbria than the medial aspects of the tube; however, as its name SEE-FIM implies, this protocol involves exhaustive sectioning of the fimbriae longitudinally, whereas the remaining tube is only examined as cross-sections. In addition, most of the data using this approach is based on small single institution studies that include few intraepithelial carcinomas [22, 24]. Further, studies of patients who present with clinically manifest HSC have limited value for assessing whether tubal involvement is primary or secondary[25]. Most importantly, because intraepithelial carcinoma is rarely found in RRSOs obtained from women who are non-carriers of *BRCA 1* mutations, very large sample sets will be required to define the topographic distributions in this group. Defining these percentages is paramount because more than 90% of HSCs occur among women who are non-carriers, and one cannot assume that the pathogenesis of HSCs in these two groups is similar, especially because *BRCA 1* mutation carriers present with HSC a decade or more earlier than non-carriers.

Ovarian Surface Epithelium (OSE)

Historically, studies of OSE have been limited by difficulties in collecting sufficient, representative OSE samples for histologic examination and molecular analysis. OSE consists of a single cell layer, which exfoliates readily with minimal handling. Consequently, histological studies of OSE have been limited by sparse, patchy, cellular representation of the entire OSE and potentially preferential loss of rare cancer precursors during intra-operative and post-resection handling. Thus, shortcomings of prior research on the pathogenesis of HSC partly reflect a failure to adequately scrutinize the fimbria, which is nonetheless easily accomplished, and excessive attention to analysis of OSE, which is nearly impossible, without improving upon conventional histological methods. If OSE is not a source of HSC, it will be important to explore the reasons for its resistance to

transformation, because metastases to the ovary are common, suggesting that the ovary provides a fertile “soil” for the growth of many cancers.

It is clear that malignant cells (and possibly benign cells as well) are transferred from the fimbria to the ovary. However, research on the frequency and number of cells transferred from fimbria to ovary, the mechanisms that mediate adhesion of such cells to the ovary, and the micro environmental factors in the ovarian cortex that favor growth are all limited. Understanding the mechanisms by which benign cells are transferred from tube to ovary, and their fate once explanted to this new location, may inform development of new methods of ovarian cancer prevention. For example, if transfer of cells from the tubes to the ovaries represents a mechanism that leads to ovarian endosalpingiosis (*i.e.*, benign glandular inclusions that resemble fallopian tube), then studying the lesions may provide clues about factors in the ovarian microenvironment that favor cellular differentiation and senescence over malignant transformation. Given that the fimbria bears a fairly invariant orientation to the ovarian surface, mapping the gradient of ovarian endosalpingiosis against the distance from the tube may provide evidence related to cell transfer. This work could be strengthened by including stains for markers specific for müllerian-derived epithelium.

Few studies have been conducted to optimize collection of OSE. One prior investigation using a cervical cytology brush found that it is possible to collect an average of 23,000 OSE cells per ovary by brushing, which was sufficient for cytological examination, immunohistochemical studies and culture [26]. More recently, Rungruang *et al* employed a different cytology brush to collect OSE for proteomic analysis [27]. We have developed a novel OSE collection method, which we think provides abundant, representative samples that could be used to estimate the frequency of cancer precursors and early cancer within OSE (or rule out their existence), as well as to understand molecular mechanisms that are important in adhesion of cancer cells to the ovarian surface. In addition, this method may provide needed OSE controls for comparison with other samples (inclusion cysts, fimbrial epithelium and HSC).

Ovarian Surface Epithelium (OSE) Brushings

Laboratory scientists have typically prepared immortalized cultures of OSE from oophorectomy specimens for *in vitro* experimentation (reviewed in [7]). These procedures overcome limitations related to insufficient cellularity, but immortalization may alter cell biology, limiting the relevance of these studies for human pathophysiology. We have tried to develop methods to collect specimens that are adequate for histological and molecular analysis without undergoing prior culture.

Initially, we piloted several methods for collection of OSE *ex vivo*, but achieved limited success. Despite immediate, meticulous handling of the ovaries post-removal, brushing techniques often harvested few cells; when samples were cellular, blood contamination that was not easily eliminated by size-dependent filtration or lysis, posing challenges for assay performance. Accordingly, we have turned to a novel method of *in vivo* sampling.

Our OSE collection method employs the Tao Brush™, which consists of a long, sheathed brush with soft bristles that can be retracted within a casing, thereby protecting samples as

the instrument is withdrawn. The length of the brush is ideal for sampling the majority of the surface of the ovary. We employed this approach to sample ovaries from more than 70 patients at Magee Women's Hospital under direct visualization during laparoscopic, abdominal and vaginal surgery. This procedure can be performed prior to oophorectomy with limited risk of bleeding, enabling a fresh cell collection that is suitable for microscopic study and/ or extraction of RNA for gene expression analysis, without *in vitro* culture or manipulation (Figure 1). Cells are collected at the beginning of surgery prior to ligation of the ovarian vascular pedicle, and directly handling of the ovaries by the surgeon is scrupulously avoided. Brushes are used to prepare microscopic slides and cell suspensions. Assessment of 5 samples collected with this protocol demonstrated that single touch preps made from brushings contained about 3,000 – 5,000 OSE cells per slide with few erythrocytes, and that cell suspensions prepared from the brushes yielded an average of 10 ug (7.8 – 15.0 ug) of high quality RNA per sample.

Molecular epidemiological studies of OSE could facilitate the investigation of several research aims. Comparing molecular profiles of OSE obtained from women with strong ovarian cancer risk factors with those at low-risk may provide clues regarding the role of OSE in the pathogenesis of HSC. This approach mirrors a prior analysis that showed a lack of association between ovarian cancer risk factors and detection of ovarian cysts by transvaginal ultrasound (TVU)[28]. This study found that ovarian cancer risk factors were unrelated to detection of ovarian inclusion cysts, which foreshadowed clinical trial results showing the ineffectiveness of TVU screening[2]. By analogy, if OSE is involved in ovarian carcinogenesis, the molecular profile of OSE should vary among subjects with different ovarian cancer risk factors. This rationale would apply to a scenario in which ovarian cancers arise from OSE, but might also apply to more complex models in which cells shed from the tube attach to OSE, implant, and then undergo malignant change or progression to an aggressive phenotype within the ovary prior to wider dissemination (see below).

Challenges to Evaluating Fallopian Tube Epithelium and Ovarian Stroma

The interpretation of subtle cytological changes in tubal epithelium is challenging; poor preservation and suboptimal fixation can render this task difficult at best, unreliable at worst. A recent inter-observer study found that agreement among expert gynecological pathologists in the diagnosis of fallopian tubal *in-situ* carcinoma was poor, although results were improved in a repeat review of the same cases when aided by immunohistochemical stains for p53 and Ki67 [23]. These challenges are compounded by the surgical use of surgical electrocautery during RRSO, which induces artifacts in cellular architecture and nuclear detail. Furthermore, pathologists typically fix tubal tissue for histological processing by placing them *unopened* in formalin, which results in delayed fixation of the mucosa until formalin diffuses through the wall of the tube. Efforts to process tubes according to modified procedures warrant exploration. For example, gentle insufflation of tubes with formalin or opening tubes longitudinally and pinning the tissue flat to achieve rapid fixation of the mucosa under slight uniform tension may provide more reproducible, optimized fixation.

Unlike OSE, tubal fimbrial epithelium is securely attached to its basement membrane, and therefore, readily amenable to collection and analysis. However, molecular analysis of the

tubal mucosae throughout its length is required to evaluate whether and how frequently HSC precursors develop outside the fimbria or spread medially. Studies using SEE-FIM begin to address this issue, but larger datasets that include greater numbers of intraepithelial carcinomas and earlier putative precursors would be informative. This question is important both to understand the pathogenesis of HSC and to determine whether lesions occur within the most medial part of the tube, which is embedded in the uterine wall. Current surgical practice leaves this short intramural segment of the fallopian tube *in situ*, if concurrent hysterectomy is not performed[29].

To our knowledge, no one has yet documented a malignancy arising in this seldom-examined portion of the fallopian tube, but technical challenges posed by this location are likely to make such an observation exceedingly difficult to accomplish. Nonetheless, if the latter is sometimes involved by early HSC, hysterectomy may be needed for more complete prophylaxis among high-risk women. If HSC can extend medially early in its development, it may be amenable to endoscopic or cytobrush sampling through the uterus. Furthermore, endometrial intraepithelial carcinoma (EIC), the presumptive precursor of uterine serous carcinoma, may present with tubal or ovarian lesions that either mimic *in-situ* carcinoma of these organs or represent a multifocal process of carcinogenesis [16, 17]. Tang et al reported that tubal in-situ carcinoma was found in 19% of ovarian, 14% of endometrial and 29% of primary peritoneal serous carcinomas[30] and Jarboe et al identified tubal in-situ carcinoma in 5 of 22 cases of endometrial serous carcinoma, which were minimally or non-invasive[18]. It is notable that EIC has been identified in RRSOs (Sherman ME, personal observation) even though such lesions are typically uncommon at young ages.

Finally, the ovarian stromais likely to play an important role in the pathogenesis of HSC, but methods for defining the relevant tissue compartments of the ovarian cortex, sampling these areas in a targeted fashion and preparing them for molecular analysis have not yet been developed. Molecular analysis of stromais generally difficult because of its sparse cellularity. Furthermore, novel methods to assess enzyme activity and concentrations of hormones and growth factors may be needed to complement more frequently performed analyses, such as gene expression or proteomics. The increased HSC risk associated with menopausal hormone replacement [31] and the protective effect of oral contraceptives [32] suggest that hormones are important in the etiology of HSC. Tissue studies may resolve whether use of exogenous menopausal hormones acts to promote the growth of precursors or contributes to cancer initiation. Similarly, it is unclear whether oral contraceptives are protective because they inhibit ovulation or because they directly affect the tubal epithelium.

The Sojourn Time for Progression of HSC Precursors to Clinical Cancer: Data Supporting a Two-Phase Model

Few models have been developed to estimate the sojourn time of HSC from cancer initiation to symptomatic clinical detection. Using published data related to the prevalence and size of occult and clinically-evident HSCs, Brown proposed that HSC may remain clinically occult for more than 5 years, on average, prior to detection[33]. However, such analyses are limited by the quality and scarcity of data regarding the characteristics of occult HSC and cannot readily account for then atural history of HSC precursors, which is probably variable and

potentially influenced by prior medical history. Our knowledge of the characteristics of occult HSCs is mainly derived from findings in RRSO surgical material obtained as a risk-reduction strategy in women with *BRCA1/2* mutations, and many such patients have received systemic treatment for breast cancer or have taken oral contraceptives or tamoxifen as chemoprevention for HSC or breast cancer respectively [15]. Although many patients undergo RRSO for elevated risk, the vast majority of occult tubal carcinomas are found among *BRCA1* mutation carriers [15]. In GOG 199, a multi-center non-randomized trial comparing RRSO to with a novel ovarian cancer screening strategy (the Risk of Ovarian Cancer Algorithm), 51% of 928 women enrolled in the RRSO arm had a prior history of breast cancer, 32% had prior systemic chemotherapy, 26% had taken tamoxifen and 77% had taken oral contraceptives [34]. These exposures could alter the prevalence of HSC precursor and/ or their natural history.

Ecological data suggest the possibility that at least some HSCs remain confined to the ovary for years. Specifically, the average age of women diagnosed with tubal *in-situ* carcinoma is younger than that for women with invasive carcinoma, and women with stage 1 HSC are younger than those with stage 3 or 4 cancers (Table 1). Based on a literature review that included descriptions of 38 microscopic cancers, Yates *et al* found that 23 were confined to the tube and 8 to the ovary, whereas 6 involved both sites and one was consistent with primary peritoneal carcinoma. Similarly, in GOG-199, when minimal total carcinoma burden was found, microscopic carcinoma was generally found in the tube [15]. These data support the hypothesis that many HSCs originate in the tubes and involve the ovaries secondarily, but remain occult for a period prior to dissemination [35]. The results also leave open the possibility that HSCs might arise from the tubes or the ovaries in different women.

Many menstrual and reproductive factors linked to “ovarian cancer” occur early in life, although HSCs are diagnosed among older women. However, it is unclear whether epidemiological risk factors influence the initiation of HSC precursors, their progression or both. In addition, authors have postulated that the entity “primary peritoneal serous carcinoma (PPSC)” is a misnomer. The identification of occult tubal *in situ* carcinoma in patients with PPSC and theoretical arguments suggest that PPSCs arise from endosalpingiosis or cells shed from occult malignant or pre-malignant tubal lesions [36, 37]. PPSCs are sometimes diagnosed following salpingoophorectomy. If PPSCs arise from cells of tubal origin, then the interval between surgery and diagnosis would provide information about the speed with which HSC develops. Although data are limited, PPSCs reportedly have been diagnosed more than 25 years following gynecological surgery, and in two of the larger series, the mean intervals were 5.3 years [38] and 8 years [39]. These data suggest that it may take several years for HSCs to arise from its precursors.

Ovarian Surface Epithelium (OSE) and Subjacent Stroma: Fertile Soil for the Pathogenesis of High- grade Serous Carcinoma (HSC)?

Historically, pelvic HSCs have been classified as ovarian cancers because the bulk of disease in most women is located in the ovaries and peritoneum. As recently highlighted [35], even when early HSCs are detected, the majority of the tumor volume consists of multiple nodules involving the ovarian surface and subjacent stroma, a pattern more

consistent with metastatic spread to the ovary than with primary origin. Given that few high-stage HSCs occur without ovarian involvement, and that primary peritoneal carcinomas are rare, one could hypothesize that exposure to the ovarian microenvironment contributes to the aggressiveness of these cancers. In fact, the identification of multiple nodules of carcinoma on the ovarian surface in association with tubal intraepithelial carcinoma suggests that transfer of malignant cells from the tube to the ovary may occur repeatedly and that HSCs that present with dominant masses may nonetheless derive from the confluence of multiple discrete foci, possibly endowed with divergent biology. Thus, HSC may represent a multiclonal cancer characterized by molecular heterogeneity from the outset. Translocation of cells from the fimbria to the ovary could occur frequently by direct contact, but physical proximity alone may not explain why such cells adhere to the ovary and grow once deposited.

Increased numbers of lifetime ovulations is an established risk factor for ovarian cancer. The mechanisms that underlie this association have been variously ascribed to repeated ovulation-related bouts of OSE injury and repair, local release of pro-carcinogenic inflammatory molecules and elevated cumulative exposure to sex-steroid and/ or peptide hormones[40–42]. It is also notable that, compared with benign OSE, ovarian cancers show increased expression of adhesion molecules, including e-cadherin and integrins. The influence of gonadotrophin releasing hormones, follicle stimulating hormone, luteinizing hormone, activin, inhibin and sex-steroid hormones on OSE, immortalized OSE and ovarian cancer are complex and have recently been reviewed [43]. Although readers are referred to the literature for details [40–42], three ideas emerge regarding the pathogenesis of HSC: 1) hormones may mediate effects of some ovarian cancer risk factors; 2) hormones alter the biology of OSE and 3) alteration of OSE biology could affect the ability of malignant cells of the fimbria to adhere and grow within the ovary, form inclusion cysts or undergo malignant change.

Historically, models of carcinogenesis focused largely, if not exclusively on understanding mechanisms by which epithelial cells or their progenitors bypass physiological constraints on cell growth and expand autonomously to form tumors. More contemporary views might widen concerns to include how natural selection operates on epithelial-stromal interactions to favor tumor formation. In this vein, accumulating evidence suggests that there are numerous potential interactions between epithelial ovarian cancer cells and stroma, as summarized recently by Schauer *et al*[40]. Notably, ovarian cancer cells such as SKOV3 have been shown to induce phenotypic shifts in normal fibroblasts, which diminish their anti-carcinogenic properties, resulting in the formation of cancer associated fibroblasts (CAFs) that secrete growth factors, chemokines and matrix re-modeling enzymes. The origin of CAFs in ovarian cancer remains obscure, but proposed candidates include fibroblasts, either active or senescent, bone marrow-derived cells, or stem cells. Given that the ovarian cortex is rich in stromal cells, and that some ovarian carcinomas are associated with non-invasive tubal lesions, we hypothesize that the ovary might play a critical role in the development of HSC by providing the precursors of CAFs. This hypothesis could be evaluated by comparing stromal gene expression signatures, hormone levels and enzyme activity in the ovarian cortex of women with and without HSC. Furthermore, it would be

useful to know how established risk factors affect the molecular biology of ovarian stroma among healthy women.

Accumulating evidence suggests that tumor-specific metastatic patterns are non-random. According to a leading paradigm, first proposed by Paget as the “seed and soil” hypothesis in 1889 [44], cancers spread to sites at which the microenvironment favors their growth, now recognized to reflect a “soil” conducive to cell adhesion, angiogenesis, immune evasion, stimulation of cell division and survival, and minimization of apoptosis. The specificity and applicability of the seed and soil hypothesis to HSC is supported by studies of patients with HSC who received peritoneal – venous shunts for palliation of intractable ascites. At post-mortem examination, women with shunts have predominantly peritoneal disease, despite the introduction of tumor cells directly into the blood [45]. By analogy, deposition of normal, HSC precursor cells or HSC onto the ovary may be common, but many of these cells may die, undergo senescence or differentiate into innocuous structures, such as endosalpingiosis. Mechanistic studies examining interactions between ovarian cancer cells and pleural derived mesothelium implicated complex mechanisms involving fibronectin, adhesion molecules and generation of contractile force in the dispersal of the mesothelial layer and attachment of tumor cells to subjacent stroma [46]. Co-culture studies also suggest that adipocytes may influence migration and invasiveness of ovarian cancer cells, with possible implications for interactions between HSC and omental fat [47].

Apart from the biological consequences of aging and use of menopausal hormones, few factors related to HSC are known to act in later life. However, after menopause, the ovarian stroma continues to produce androgens and possibly other factors that could act locally in the microenvironment to promote growth of HSC, without producing measurable analyte changes in blood. Thus, prevention and early detection strategies could emerge from studies of the ovarian stroma (“the soil”), the tube (“the seed”) or OSE (its point of implantation).

Conclusion

Progress towards understanding the pathogenesis of HSC has been slow. Until recently, research has focused on the biology of OSE, once presumed to represent the epithelial source of HSC and its precursors. A new paradigm in which HSC arises from the tube rather than the ovary sheds light on the pathogenesis of this disease, and thus on prospects for developing effective methods for early detection and prevention. The development of models using cultured fallopian tube cells may provide increased understanding of ovarian carcinogenesis and clues about strategies for prevention or markers for early detection [48–50]. However, in translational research, an agnostic approach in which the tube in its entirety, OSE, the ovarian stroma and the endometrium are comprehensively analyzed in parallel may insure our systematic progress towards more complete understanding. To achieve this aim, better means of studying this entire anatomic/physiologic unit should be developed and tested for suitability in the types of analyses that will be required to unravel the early pathogenesis of HSC. This work could begin with fundamental investigation of normal structure and molecular function in benign tissues to define the “molecular histology” of these organs, thus providing a firm basis for analysis of the molecular pathology of HSC precursors and HSC.

References

1. Choi M, Fuller CD, Thomas CR Jr, Wang SJ. Conditional survival in ovarian cancer: results from the SEER dataset 1988–2001. *Gynecol Oncol.* 2008; 109:203–9. [PubMed: 18329082]
2. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, Reding DJ, Greenlee RT, Yokochi LA, Kessel B, Crawford ED, Church TR, Andriole GL, Weissfeld JL, Fouad MN, Chia D, O'Brien B, Ragard LR, Clapp JD, Rathmell JM, Riley TL, Hartge P, Pinsky PF, Zhu CS, Izmirlian G, Kramer BS, Miller AB, Xu JL, Prorok PC, Gohagan JK, Berg CD. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. *JAMA.* 2011; 305:2295–303. [PubMed: 21642681]
3. Kurman RJ, Shih Ie M. Molecular pathogenesis and extra ovarian origin of epithelial ovarian cancer--shifting the paradigm. *Hum Pathol.* 2011; 42:918–31. [PubMed: 21683865]
4. Dubeau L. The cell of origin of ovarian epithelial tumours. *Lancet Oncol.* 2008; 9:1191–7. [PubMed: 19038766]
5. Crum CP, McKeon FD, Xian W. The oviduct and ovarian cancer: causality, clinical implications, and “targeted prevention”. *Clin Obstet Gynecol.* 2012; 55:24–35. [PubMed: 22343226]
6. Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. *J Pathol.* 2001; 195:451–6. [PubMed: 11745677]
7. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev.* 2001; 22:255–88. [PubMed: 11294827]
8. Sherman ME, Lee JS, Burks RT, Struewing JP, Kurman RJ, Hartge P. Histopathologic features of ovaries at increased risk for carcinoma. A case-control analysis. *Int J Gynecol Pathol.* 1999; 18:151–7. [PubMed: 10202673]
9. Werness BA, Afify AM, Bielat KL, Eltabbakh GH, Piver MS, Paterson JM. Altered surface and cyst epithelium of ovaries removed prophylactically from women with a family history of ovarian cancer. *Hum Pathol.* 1999; 30:151–7. [PubMed: 10029442]
10. Resta L, Russo S, Colucci GA, Prat J. Morphologic precursors of ovarian epithelial tumors. *Obstet Gynecol.* 1993; 82:181–6. [PubMed: 8336861]
11. Gusberg SB, Deligdisch L. Ovarian dysplasia. A study of identical twins. *Cancer.* 1984; 54:1–4. [PubMed: 6539159]
12. Plaxe SC, Deligdisch L, Dottino PR, Cohen CJ. Ovarian intraepithelial neoplasia demonstrated in patients with stage I ovarian carcinoma. *Gynecol Oncol.* 1990; 38:367–72. [PubMed: 2227550]
13. Mittal KR, Zeleniuch-Jacquotte A, Cooper JL, Demopoulos RI. Contralateral ovary in unilateral ovarian carcinoma: a search for preneoplastic lesions. *Int J Gynecol Pathol.* 1993; 12:59–63. [PubMed: 8418079]
14. Pothuri B, Leitao MM, Levine DA, Viale A, Olshen AB, Arroyo C, Bogomolny F, Olvera N, Lin O, Soslow RA, Robson ME, Offit K, Barakat RR, Boyd J. Genetic analysis of the early natural history of epithelial ovarian carcinoma. *PLoS One.* 2010; 5:e10358. [PubMed: 20436685]
15. Mai PL, Piedmonte M, Ioffe B, Ronnett BM, Le LV, Mutch DG, Cohn DE, Thigpen JT, Tewari KS, Disilvestro P, Kauff ND, Lu KH, Greene MH. Pathological findings at risk-reducing salpingoophorectomy among women at increased ovarian cancer risk: Results from GOG-199 [Abstract]. *J Clin Oncol.* 2012; (Suppl):1519. [PubMed: 22430272]
16. Ambros RA, Sherman ME, Zahn CM, Bitterman P, Kurman RJ. Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation. *Hum Pathol.* 1995; 26:1260–7. [PubMed: 7590702]
17. Sherman ME, Bitterman P, Rosenshein NB, Delgado G, Kurman RJ. Uterine serous carcinoma. A morphologically diverse neoplasm with unifying clinicopathologic features. *Am J Surg Pathol.* 1992; 16:600–10. [PubMed: 1599038]
18. Jarboe EA, Miron A, Carlson JW, Hirsch MS, Kindelberger D, Mutter GL, Crum CP, Nucci MR. Coexisting intraepithelial serous carcinomas of the endometrium and fallopian tube: frequency and potential significance. *Int J Gynecol Pathol.* 2009; 28:308–15. [PubMed: 19483636]

19. Rabban JT, Barnes M, Chen LM, Powell CB, Crawford B, Zaloudek CJ. Ovarian pathology in risk-reducing salpingo-oophorectomies from women with BRCA mutations, emphasizing the differential diagnosis of occult primary and metastatic carcinoma. *Am J Surg Pathol*. 2009; 33:1125–36. [PubMed: 19440148]
20. Powell CB, Kenley E, Chen LM, Crawford B, McLennan J, Zaloudek C, Komaromy M, Beattie M, Ziegler J. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. *J Clin Oncol*. 2005; 23:127–32. [PubMed: 15625367]
21. Rabban JT, Krasik E, Chen LM, Powell CB, Crawford B, Zaloudek CJ. Multistep level sections to detect occult fallopian tube carcinoma in risk-reducing salpingo-oophorectomies from women with BRCA mutations: implications for defining an optimal specimen dissection protocol. *Am J Surg Pathol*. 2009; 33:1878–85. [PubMed: 19898224]
22. Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, Garber JE, Cramer DW, Crum CP. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol*. 2006; 30:230–6. [PubMed: 16434898]
23. Visvanathan K, Vang R, Shaw P, Gross A, Soslow R, Parkash V, Shih Ie M, Kurman RJ. Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study. *Am J Surg Pathol*. 2011; 35:1766–75. [PubMed: 21989347]
24. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol*. 2007; 211:26–35. [PubMed: 17117391]
25. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, Callahan MJ, Garner EO, Gordon RW, Birch C, Berkowitz RS, Muto MG, Crum CP. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol*. 2007; 31:161–9. [PubMed: 17255760]
26. Nicosia SV, Wilbanks GD, Saunders B, Mayer J, Cardosi RJ, Kruk PA, Cheng J, Bai W, Coppola D, Fiorica J. Cytology of human ovarian surface epithelial brushings. *Cancer*. 2004; 102:1–10. [PubMed: 14968412]
27. Rungruang B, Hood BL, Sun M, Hoskins E, Conrads TP, Zorn KK. Novel surgical approaches for sampling the ovarian surface epithelium and proximal fluid proteome. *J Proteome Res*. 2010; 9:6071–6. [PubMed: 20873867]
28. Hartge P, Hayes R, Reding D, Sherman ME, Prorok P, Schiffman M, Buys S. Complex ovarian cysts in postmenopausal women are not associated with ovarian cancer risk factors: preliminary data from the prostate, lung, colon, and ovarian cancer screening trial. *Am J Obstet Gynecol*. 2000; 183:1232–7. [PubMed: 11084571]
29. Cass I, Walts A, Karlan BY. Does risk-reducing bilateral salpingo-oophorectomy leave behind residual tube? *Gynecol Oncol*. 2010; 117:27–31. [PubMed: 20110118]
30. Tang S, Onuma K, Deb P, Wang E, Lytwyn A, Sur M, Daya D. Frequency of serous tubal intraepithelial carcinoma in various gynecologic malignancies: a study of 300 consecutive cases. *Int J Gynecol Pathol*. 2012; 31:103–10. [PubMed: 22317864]
31. Pearce CL, Chung K, Pike MC, Wu AH. Increased ovarian cancer risk associated with menopausal estrogen therapy is reduced by adding a progestin. *Cancer*. 2009; 115:531–9. [PubMed: 19127543]
32. Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. *Lancet*. 2008; 371:303–14. [PubMed: 18294997]
33. Brown PO, Palmer C. The preclinical natural history of serous ovarian cancer: defining the target for early detection. *PLoS Med*. 2009; 6:e1000114. [PubMed: 19636370]
34. Greene MH, Piedmonte M, Alberts D, Gail M, Hensley M, Miner Z, Mai PL, Loud J, Rodriguez G, Basil J, Boggess J, Schwartz PE, Kelley JL, Wakeley KE, Minasian L, Skates S. A prospective study of risk-reducing salpingo-oophorectomy and longitudinal CA-125 screening among women at increased genetic risk of ovarian cancer: design and baseline characteristics: a Gynecologic Oncology Group study. *Cancer Epidemiol Biomarkers Prev*. 2008; 17:594–604. [PubMed: 18349277]

35. Yates MS, Meyer LA, Deavers MT, Daniels MS, Keeler ER, Mok SC, Gershenson DM, Lu KH. Microscopic and early-stage ovarian cancers in BRCA1/2 mutation carriers: building a model for early BRCA-associated tumorigenesis. *Cancer Prev Res (Phila)*. 2011; 4:463–70. [PubMed: 21278312]
36. Seidman JD, Zhao P, Yemelyanova A. “Primary peritoneal” high-grade serous carcinoma is very likely metastatic from serous tubal intraepithelial carcinoma: assessing the new paradigm of ovarian and pelvic serous carcinogenesis and its implications for screening for ovarian cancer. *Gynecol Oncol*. 2011; 120:470–3. [PubMed: 21159368]
37. Carlson JW, Miron A, Jarboe EA, Parast MM, Hirsch MS, Lee Y, Muto MG, Kindelberger D, Crum CP. Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. *J Clin Oncol*. 2008; 26:4160–5. [PubMed: 18757330]
38. Finch A, Beiner M, Lubinski J, Lynch HT, Moller P, Rosen B, Murphy J, Ghadirian P, Friedman E, Foulkes WD, Kim-Sing C, Wagner T, Tung N, Couch F, Stoppa-Lyonnet D, Ainsworth P, Daly M, Pasini B, Gershoni-Baruch R, Eng C, Olopade OI, McLennan J, Karlan B, Weitzel J, Sun P, Narod SA. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation. *JAMA*. 2006; 296:185–92. [PubMed: 16835424]
39. Rutter JL, Wacholder S, Chetrit A, Lubin F, Menczer J, Ebbers S, Tucker MA, Struewing JP, Hartge P. Gynecologic surgeries and risk of ovarian cancer in women with BRCA1 and BRCA2 Ashkenazi founder mutations: an Israeli population-based case-control study. *J Natl Cancer Inst*. 2003; 95:1072–8. [PubMed: 12865453]
40. Schauer IG, Sood AK, Mok S, Liu J. Cancer-associated fibroblasts and their putative role in potentiating the initiation and development of epithelial ovarian cancer. *Neoplasia*. 2011; 13:393–405. [PubMed: 21532880]
41. Shan W, Yang G, Liu J. The inflammatory network: bridging senescent stroma and epithelial tumorigenesis. *Front Biosci*. 2009; 14:4044–57.
42. Saad AF, Hu W, Sood AK. Microenvironment and pathogenesis of epithelial ovarian cancer. *Horm Cancer*. 2010; 1:277–90. [PubMed: 21761359]
43. Leung PC, Choi JH. Endocrine signaling in ovarian surface epithelium and cancer. *Hum Reprod Update*. 2007; 13:143–62. [PubMed: 17071638]
44. Paget S. The distribution of secondary growths in cancer of the breast 1889. *Cancer Metastasis Rev*. 1989; 8:98–101. [PubMed: 2673568]
45. Tarin D, Price JE, Kettlewell MG, Souter RG, Vass AC, Crossley B. Mechanisms of human tumor metastasis studied in patients with peritoneovenous shunts. *Cancer Res*. 1984; 44:3584–92. [PubMed: 6744281]
46. Iwanicki MP, Davidowitz RA, Ng MR, Besser A, Muranen T, Merritt M, Danuser G, Ince T, Brugge JS. Ovarian cancer spheroids use myosin-generated force to clear the mesothelium. *Cancer Discov*. 2011; 1:144–157. [PubMed: 22303516]
47. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, Romero IL, Carey MS, Mills GB, Hotamisligil GS, Yamada SD, Peter ME, Gwin K, Lengyel E. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med*. 2011; 17:1498–503. [PubMed: 22037646]
48. Jazaeri AA, Bryant JL, Park H, Li H, Dahiya N, Stoler MH, Ferriss JS, Dutta A. Molecular requirements for transformation of fallopian tube epithelial cells into serous carcinoma. *Neoplasia*. 2011; 13:899–911. [PubMed: 22028616]
49. Karst AM, Levanon K, Drapkin R. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. *Proc Natl Acad Sci U S A*. 2011; 108:7547–52. [PubMed: 21502498]
50. Levanon K, Ng V, Piao HY, Zhang Y, Chang MC, Roh MH, Kindelberger DW, Hirsch MS, Crum CP, Marto JA, Drapkin R. Primary ex vivo cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. *Oncogene*. 2010; 29:1103–13. [PubMed: 19935705]

Figure 1.

Ovarian surface epithelial cells were collected intra-operatively with a Tao brush. Brushings were touched to a microscopic slide and then prepared as a cell suspension. RNA was extracted from 5 randomly selected samples, demonstrating molecular preservation. A. Touch prep shows an abundant, pure population of ovarian surface epithelial cells (hematoxylin and eosin, 6.25 X; inset 22.5 X).

Table 1

Age at diagnosis by stage of grade 3/4 serous ovarian carcinoma

Calendar period	Age at diagnosis (mean +/- 95% C.I.)			P-value
	Localized	Regional	Distant	
1995–2000	61.7 (60.9–62.5)	62.9 (62.4–63.3)	63.5 (63.3–63.7)	<0.0001
200–2003	61.4 (60.3–62.5)	62.8 (62.3–63.4)	63.3 (63.1–63.6)	0.0005
2004–2008	61.7 (60.8–62.5)	62.7 (62.2–63.2)	63.3 (63.2–63.5)	<0.0001

Data from the North American Association of Central Cancer Registries. Serous carcinoma defined as ICD-O-3 codes: serous (8441, 8442, 8460, 8461, 8462, 9014). Restricted to women age 30 years and older and truncated at 85 years. C.I. = Confidence interval. P-value by analysis of variance.