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Authors

Karnezis, Anthony N

Cho, Kathleen R

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Preclinical Models of Ovarian Cancer: Pathogenesis, Problems, and Implications for Prevention

Anthony N. Karnezis and **Kathleen R. Cho**

Abstract

Preclinical models are relatively underutilized and underfunded resources for modeling the pathogenesis and prevention of ovarian cancers.[1] Several reviews have detailed the numerous published models of ovarian cancer.[2–6] In this review, we will provide an overview of experimental model systems, their strengths and limitations, and use selected models to illustrate how they can be used to address specific issues about ovarian cancer pathogenesis. We will then highlight some of the preclinical prevention studies performed to date and discuss experiments needed to address important unanswered questions about ovarian cancer prevention strategies.

BACKGROUND – ovarian cancer histotypes and origins

Ovarian carcinomas are currently subdivided into five major histotypes: high-grade serous carcinoma (HGSC), endometrioid carcinoma (EC), clear cell carcinoma (CCC), low-grade serous carcinoma (LGSC) and mucinous carcinoma (MC). The risk factors, clinicopathological, immunohistochemical and molecular features of these tumors and their precursor lesions have been the subject of several excellent reviews (Table 1).[1, 7–12] We will focus on HGSC, EC and CCC since these tumors account for the vast majority of ovarian cancer deaths, and because mouse models have been generated for these histotypes.

Unlike cancers in other organs, understanding the origins and pathogenesis of ovarian cancers has been difficult to understand partly because so-called ‘primary ovarian carcinomas’ do not resemble any of the cells types present in normal ovaries. In fact current evidence suggests that the majority of ovarian cancers may be derived from cells that are not intrinsic to the ovary. [2, 8, 9, 11, 12] This has complicated our understanding of, and ability to generate models that recapitulate human ovarian cancer pathogenesis. By extension, new approaches for preventing ovarian cancer have been difficult to test due to lack of suitable *in vivo* models.

The association of endometriosis (ectopic endometrial epithelium and stroma) with EC and CCC has been known for almost a century, and endometriosis fulfills epidemiological, histological, genetic and molecular criteria as a *bona fide* precursor of EC and CCC.[11] Several non-mutually exclusive mechanisms have been proposed to explain how endometriosis develops, including retrograde menstruation, metaplasia, vascular dissemination, or from embryonic remnants of Mullerian tissue.[13]

Anthony N. Karnezis, MD, PhD, University of British Columbia, Pathology and Laboratory Medicine, British Columbia, CANADA, PHONE: 778-839-4214, FAX: 7788394214, akarnezis@bccrc.ca.

The most clinically significant controversy surrounds the origin of HGSC. Historically, HGSCs were thought to arise from the ovarian surface epithelium (OSE), a layer of modified coelomic mesothelium/epithelium covering the ovaries.[14] The 'incessant ovulation' hypothesis[15] proposed that repeated ovulation resulted in metaplasia of the OSE to a Mullerian serous (secretory) phenotype, with subsequent accumulation of mutations that resulted in HGSC. Unlike most other cancers, only rare credible histologic precursor lesions in the ovaries had been reported.[16] The paucity of *bona fide* ovarian HGSC precursors was attributed to the fact that most of these cancers were discovered at advanced stage, and the precursors were presumably destroyed during tumor growth.

A critical paradigm shift occurred with the discovery of occult microscopic precursors of HGSC – called serous tubal intraepithelial carcinomas (STICs) – in the fallopian tube epithelium (FTE) of patients with germline *BRCA1/BRCA2* mutations undergoing risk-reducing salpingo-oophorectomy (RRSO).[17–21] Using the presence of STIC or invasive tubal carcinoma as evidence of tubal origin, possibly up to 80% of 'ovarian' HGSCs, either sporadic or in the setting of genetic predisposition, may arise in the fallopian tube.[22–26] Importantly, recent studies suggest that some STIC-like lesions likely represent mucosal metastases from other sites.[27–32] Therefore, more detailed studies are needed to determine how often STIC-like lesions in the context of HGSC represent precursors versus implants.

The source of HGSCs without evidence of tubal origin (i.e. true primary ovarian or peritoneal HGSCs) is an unanswered question with important implications for our understanding of HGSC pathogenesis and prevention. Two potential ovarian sources of HGSC have been proposed - the OSE and ovarian cortical epithelial inclusion cysts (CICs).[14, 33, 34] CICs are lined by OSE-type epithelium, tubal-type epithelium or a mixture of both. Since HGSC can arise from eutopic tubal epithelium in the fallopian tube fimbria, ectopic tubal epithelium in the ovary (in the form of tubal CICs, also called ovarian endosalpingiosis) is a plausible cell of origin for true intraovarian HGSC. Similarly, endosalpingiosis involving the peritoneal surfaces may give rise to primary peritoneal HGSCs. Like endometriosis, the mechanism by which CICs form is unclear. OSE-type CICs are thought to arise by invagination and pinching off of the OSE to form cysts. Tubal-type CICs are thought to arise either by metaplasia of OSE-type CICs to a tubal phenotype or implantation of fimbrial epithelium onto the ovary with subsequent invagination and cyst formation. The formation of tubal-type CICs by implantation vs. metaplasia may influence the effectiveness of risk-reducing strategies (see below).

PRIMARY PREVENTION STRATEGIES

Oral Contraceptive Pills

The widespread use of combined oral contraceptive pills (OCPs) has provided the most effective primary prevention strategy for ovarian cancer, albeit somewhat serendipitously. Women who use OCPs are at a substantially lower risk of ovarian cancer compared to never users. OCPs are protective against HGSC, EC and CCC.[35] The protective effect is large (~40% reduction in risk with five years of OCP use and ~65% reduction with 10 years of use) and continues for at least 25 years after OCP use is stopped.[36] The mechanisms underlying the protective effect of OCPs are unclear. The long-held view that blocking

ovulation is the mechanism has been called into question by two observations. First, menopausal estrogen use is associated with an increased risk of serous and endometrioid carcinomas,[37, 38] suggesting a direct hormonal effect on carcinogenesis. Second, a full-term pregnancy is associated with a much stronger protective effect compared to a year of OCP use,[35, 39] again suggesting a mechanism other than blocking ovulation. These observations, together with the tubal origin of most HGSCs, are shifting the focus to how OCPs affect the biology of the FTE, either directly (through their action on estrogen and progesterone receptors in the tubal epithelium and stroma) or indirectly (by preventing ovulation and thereby exposure of fimbrial epithelium to potentially toxic and pro-inflammatory follicular fluid[40, 41] and blood from the ruptured follicle or retrograde menstrual fluid.[42]

Tubal Ligation

Tubal ligation is associated with a 29% decreased risk of ovarian cancer and has a stronger protective effect against endometriosis-associated cancers (approximately 50% for EC and CCC) than for HGSC (20% risk reduction).[43] Several mechanisms have been proposed to explain the protective effect of tubal ligation against ovarian cancers. One mechanism is by preventing retrograde menstruation, leading to a reduction in the prevalence of endometriosis. This hypothesis is supported by the stronger protective effect against EC and CCC, both of which are thought to arise from endometriosis.[44] The protective effect afforded by tubal ligation on HGSC could also be a result of decreased blood flow to or atrophy of the distal end of the fallopian tube or by decreased exposure of the fimbrial epithelium to blood in retrograde menstrual fluid.[42]

Risk-Reducing Bilateral Salpingo-oophorectomy (RRSO)

RRSO is recommended as a primary prevention strategy for women at high-risk of ovarian cancer, including patients with germline *BRCA1/BRCA2* mutations. RRSO is very effective, with risk reduction of approximately 80%.[45, 46] The residual risk of ovarian cancer among women who have had RRSO[47, 48] may be related to endosalpingiosis in the peritoneum.

Though highly effective, the optimal timing of this procedure must also be balanced against the reproductive, general health and emotional effects of surgically induced premature menopause, including increased risk of coronary heart disease, stroke, osteoporosis, and colorectal cancer, and reduced quality of life.[49, 50] Salpingectomy with delayed oophorectomy has been proposed as an alternative to RRSO[51–53] to eliminate the cancer risk from the fallopian tube at a young age while delaying surgical menopause as long as possible. At this point, the risk-benefit ratio of delaying oophorectomy and optimal timing of oophorectomy are unclear.

Risk-Reducing Salpingectomy (RRS), i.e. ‘Opportunistic’ Salpingectomy (OS)

An alternative primary prevention strategy for both high- and average-risk women is RRS/OS, defined as the removal of the fallopian tubes at the time of hysterectomy, other pelvic surgery or in place of tubal ligation. First proposed and implemented in British Columbia, OS has since been adopted in multiple centers internationally, and in the past two years the Society of Gynecologic Oncology and other professional organizations have issued recommendations to discuss the procedure as a prevention strategy with average-risk

patients undergoing relevant surgeries. RRS/OS has been shown to be safe[54] and possibly more protective than tubal ligation.[55, 56] Further detailed exploration of risk-reducing interventions in both high-risk and average-risk populations can be found in the accompanying chapters of this symposium.

PRECLINICAL MODEL SYSTEMS OF OVARIAN CARCINOMAS

The two commonly used experimental systems for *in vivo* studies of ovarian cancers are genetically engineered mouse models (GEMMs) and the spontaneous, naturally occurring laying hen model.[2–6] All mouse models are genetically induced since experimental mouse strains do not spontaneously develop ovarian carcinomas. The major GEMMs were summarized in a recent report from the Institute of Medicine.[1] Since the cell of origin for most ovarian carcinomas was long assumed to be the OSE, most GEMMs described to date have use Cre-lox technology to mutate genes involved in ovarian cancer pathogenesis in the OSE. More recently, GEMMs have been generated by mutating the secretory cells in the FTE, thereby allowing a comparison of the effects of genetics and cell of origin on the cancer phenotype. The laying hen is the only species besides humans that spontaneously develops ovarian carcinomas at a significant frequency. The laying hen model has interesting parallels to the human disease (see below), but has been underutilized, probably due to the logistical challenges associated with animal husbandry associated with chickens compared to mice, and due to the paucity of chicken-specific experimental reagents like antibodies for immunohistochemical analyses.

STRENGTHS AND LIMITATIONS OF PRECLINICAL MODELS

Several important differences exist between humans, mice and hens that influence the interpretation of results from experimental model systems and their potential relevance to human ovarian cancers (Table 2). In short, there is no spontaneous, genetically similar model of ovarian cancer that parallels the human condition.

Mouse models

The main strengths of GEMMs is that mice and humans have similar anatomy and physiology, and the genetics, cell(s) of origin and time of mutation in mice can be defined by experimental design. Unlike the laying hen model, GEMMs provide an opportunity to compare the influence of cell of origin (i.e. OSE vs. FTE) on cancer phenotype.[57] However, endosalpingiosis and endometriosis have been difficult to model in mice, presenting challenges for modeling origins of HGSC, EC, and CCC.

Endosalpingiosis-like lesions can be identified in approximately 15% of mouse ovaries that have been meticulously examined microscopically (Cho lab, unpublished data). Whether similar lesions are present in extra-ovarian sites in the mouse is unclear, as there are no published studies that have systematically addressed this question. In the mouse, implantation of detached tubal epithelium in the ovary is likely favored over other sites due to the presence of the ovarian bursa, a thin membrane surrounding the distal fallopian tube and ovary in mice but not humans. If detachment of tubal epithelium and implantation in the ovary is the mechanism by which endosalpingiosis arises, the bursa provides a physical

barrier to sites other than the ovary. Alternatively, as in humans, endosalpingiosis-like lesions in the mouse could arise from Mullerian rests or from metaplasia of the OSE, stimulated by hormones or by damage during ovulation. Studying factors associated with endosalpingiosis in the mouse could provide insights into the mechanisms by which endosalpingiosis arises in humans, with obvious implications for prevention of those HGSCs that arise outside of the fallopian tube. If ovarian endosalpingiosis is the origin of 'non-tubal' HGSCs, and it develops by metaplasia of the OSE, this may decrease the effectiveness of opportunistic salpingectomy.

Mice do not spontaneously develop endometriosis, but several experimental models of mouse endometriosis have been developed.[58, 59] In contrast to the human menstrual cycle, the mouse endometrium is resorbed rather than shed in the estrus cycle. This suggests that the lack of spontaneous endometriosis in mice may be due to the lack of retrograde menstruation and not from the absence of hormone-induced metaplasia or from differentiation of ectopic Mullerian remnants, since there is not an obvious reason for the latter two processes to differ in mice and humans. For genetic models of endometriosis, there are practical limitations to the genes that can be studied, since the genes involved in the generation of experimental endometriosis (e.g. *KRAS*) are not used in most existing EC or CCC GEMMs.

Laying hen model

The strength of the hen model is that it is a spontaneous model of ovarian cancer with important parallels to the human disease. At 2–3 years of age, when a hen has undergone a comparable number of ovulations as a woman entering menopause, the incidence of ovarian cancer is up to 4%, similar to the lifetime risk of ovarian cancer in women (0.35%–8.8%).[60] The incidence increases to 30–60% after 4–6 years.[61, 62] The hens develop ovarian tumors with peritoneal spread and ascites, similar to women with advanced-stage disease. Four histotypes of ovarian cancer have been described: HGSC, EC, CCC and MC.[63]

The most obvious differences concern avian vs. mammalian anatomy and physiology. The adult hen has a single ovary, fallopian tube (oviduct) and uterus (shell organ). The oviduct lacks finger-like fimbria and instead starts with a funnel-shaped infundibulum that catches the yolk from the ovary. The oviductal magnum, which roughly corresponds to the ampulla of the human fallopian tube, produces and deposits the egg white, and the uterus is involved in synthesis of the shell. The lack of endosalpingiosis, endometriosis (or even a cycling endometrium), and oviductal fimbria suggest the OSE is the cell of origin of hen ovarian cancers, possibly in CICs.[64]

Importantly, the four histotypes of ovarian cancer described in hens have not undergone the detailed molecular characterization and immunohistochemical analysis of histotype-specific proteins to rigorously validate them as parallels of their human ovarian cancer counterparts. Therefore, a detailed cross-species analysis is overdue to compare and contrast the histologic, immunophenotypic and molecular features of human, hen and mouse models of ovarian cancers. It is plausible that hen ovarian cancers have greater similarities with a subset of human ovarian cancers that may originate in the OSE and the GEMMs that model them.

SPECIFIC MODELS AND PREVENTION STUDIES

An ideal GEMM is one that recapitulates the cell of origin, genetics, histopathology, immunophenotype and clinical behavior of the human disease. In addition, it is useful to compare models mutating the same genes in two different cells of origin to determine which model more fully recapitulates the human situation.

Inactivation of *Brca1/2, Trp53,* and *Pten* in Pax8-expressing tubal secretory cells induces fimbrial STICs that spread to the ovary and peritoneum as HGSC.[65] We have generated similar results by inactivating various combinations of *Brca1, Trp53*, *Rb1* and *Nf1* in Ovgp1-expressing tubal epithelial cells.[66] Importantly, salpingectomy completely prevented the development of STIC and HGSC.[65] In contrast, oophorectomy did not prevent STIC formation but reduced peritoneal metastases. This suggests that the ovary creates a permissive environment for advanced disease, either through endocrine effects or through its ability to locally support the growth of clones capable of metastasis. The latter is supported by recent clonality studies of human HGSC.[67]

Chemoprevention studies in the hen model – hormones, aspirin, diet

Chemoprevention studies with progestins in the hen model have provided important support for the 'incessant ovulation' hypothesis. In one study, medroxyprogesterone acetate (MPA, Depo-Provera) resulted in a 15% reduction in ovarian cancer compared to controls.[68] In a subsequent trial, oral contraceptives containing MPA alone or in combination with estradiol resulted in a 91% or 81% risk reduction of ovarian cancer, respectively;[69] estradiol alone did not increase the incidence of tumors compared to controls. The doses of MPA in these studies inhibited ovulation. Furthermore, a 'restricted ovulatory' chicken that ovulates less frequently than control hens showed an 89% reduction in the incidence of ovarian cancers.[70] Together, these studies support the data from women indicating that inhibiting ovulation, or factors associated with ovulation, significantly reduces the risk of ovarian cancer.

Aspirin use in women is associated with a reduced risk of ovarian cancer (odds ratio 0.91).[71] In hens, dietary aspirin reduced the incidence of advanced stage cancers (62.1% aspirin, 85.2% controls) but not overall ovarian cancer incidence.[72] The tumors were not subtyped, so it is unclear whether the results reflected a general or histotype-specific inhibitory effect.

Diets rich in omega-3 fatty acids are associated with reduced inflammation and cancer in humans.[73–75] Flaxseed is a rich source of one form of this nutrient, alpha-linolenic acid. Similar to the results obtained with aspirin, dietary flaxseed in the hen decreased the incidence of late stage ovarian cancers and prolonged survival.[76] The effect on cancer correlated with decreased expression of cyclooxygenases 1 and 2 and lower levels of the pro-inflammatory eicosanoid prostaglandin E2 in normal hen ovaries.[77, 78] Together, these data suggest that the suppression of inflammation, either pharmacologically or through diet, can inhibit aspects of ovarian carcinogenesis.

Inhibiting STIC progression to HGSC in the mouse

The GEMMs of HGSC described by the Drapkin[65] and Cho[66] laboratories recapitulate the genetics (*Trp53* and *Brca1* or *Brca2* mutation), tubal cell of origin and histopathological

features seen in many human HGSCs (fimbrial STIC and subsequent tubo-ovarian HGSC). Therefore, these are excellent model systems to study the effects of additional mutations (*Pten, Rb1)* and chemoprevention strategies (described above) on the kinetics of progression from normal tubal epithelium to STIC to HGSC.

In addition, these models can be used to study the biological mechanisms by which various chemoprevention strategies exert their effects. The origin of most human HGSC in the fimbria, together with evidence mentioned above, suggests that the ability of progestin-containing OCPs to inhibit ovulation *per se* may not be the primary proximate cause of the decrease in HGSC. Instead, their protective effect against HGSC may occur via direct effects on the fallopian tube fimbria (as tubal epithelial and stromal cells express progesterone receptors) and indirectly by inhibiting the exposure of fimbrial epithelium to the inflammatory and possibly genotoxic effects of follicular fluid, thereby linking the 'incessant ovulation' hypothesis to the biology of the fallopian tube. The fact that oophorectomy does not prevent the development of STICs in the *Trp53; Brca1; Pten* mouse model[65] suggests the role of follicular fluid exposure in HGSC initiation is minor in this model. Therefore, it is important to study whether progestins regulate aspects of fallopian tube biology that may explain their chemopreventive effects (e.g. by inhibiting cell proliferation[79, 80] or altering the immune cell repertoire[81]) and to determine whether pregnancy has similar effects.

The appropriate management of incidentally identified STICs in salpingectomy specimens from patients without a known genetic risk of ovarian cancer is an important unanswered question. Current evidence indicates the risk of subsequent ovarian HGSC is low (at least within the published follow-up periods), and it is unclear how to predict who will recur/progress.[82–86] Using GEMMs, it is possible to test the effects of various genetic combinations (e.g. *Trp53; Brca1; Pten* vs. *Trp53; Brca1; Rb1*) on the response of mouse STICs to treatment (e.g. chemotherapy, PARP inhibitors, other novel therapies, etc.) and their likelihood of progression to HGSC and use the data to develop genetically-based predictive and prognostic algorithms that can hopefully be applied to patients with incidental STICs.

Progression of endometriosis to endometrioid or clear cell carcinoma

Ovarian EC and CCC together account for approximately one fourth of ovarian carcinomas.[1, 87] Though relatively common among ovarian carcinomas, they are rare compared to endometriosis, which affects up to 5–10% of the female population.[88, 89] Though endometriosis is a known precursor of EC and CCC, what constitutes 'high-risk' endometriosis, i.e. endometriosis at risk of progression to EC or CCC, is currently unknown. Though endometriosis can occur throughout the abdomen and pelvis and even outside of the peritoneal cavity, EC and CCC almost exclusively develop in the ovary. Therefore, even though endometriosis at other sites can also harbor oncogenic mutations,[90] the ovarian microenvironment seems unique in its ability to support the progression of endometriosis to cancer.

Several GEMMs of EC[57, 91–95] and one model of CCC[96] have been reported. A recent study compared the effect of mutation of *Apc* and *Pten* in the OSE vs. oviductal epithelium.[57] The

histology, global gene expression profile and clinical behavior of the oviduct-derived mouse ECs more closely matched the human disease. This study stresses the importance of cell of origin in determining the cancer phenotype.

While all these models are useful to study the pathogenesis of EC and CCC, several issues limit their potential utility for studying specific aspects and strategies of prevention. For example, the models recapitulate many aspects of the human cancers, but none of the cancers in these models appear to arise from endometriosis. In addition, the lack of a menstrual cycle or spontaneous endometriosis in the mouse raise questions about the relevance of testing tubal ligation as a prevention strategy in current GEMMs, since a major way tubal ligation is thought to work is by preventing retrograde menstruation. Progestins in OCPs inhibit cancer development in the hen,[68, 69] and it will be informative to test their ability to do so in existing GEMMs of EC and CCC. However, since OCPs are thought to exert their chemopreventive effect by decreasing proliferation in endometriosis, the lack of this precursor in existing GEMMs may limit our ability to study the relevant biology seen in women. Therefore, the adaptation of existing endometriosis models to the study of cancer pathogenesis and prevention should be a priority.

CONCLUSIONS

Mouse and hen models of ovarian cancer provide excellent platforms to test important cancer pathogenesis hypotheses and prevention strategies. The extra-ovarian origin of many ovarian carcinomas has generated significant challenges for the generation of accurate preclinical models and yet offered tremendous opportunities for cancer prevention in women – e.g., opportunistic salpingectomy. A more thorough understanding of the multiple different cells and tissues of origin of the various ovarian cancer histotypes will hopefully lead to higher fidelity preclinical models and therefore better platforms to test and refine cancer prevention strategies.

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Table 1

Ovarian carcinoma origins, precursors and prevention strategies.

Cancer Histotype	Possible Cells and Tissues of Origin	Proximal Precursor Lesion or Tumor	Prevention Strategies
HGSC	Fallopian tube secretory epithelial cell or progenitor cell in fallopian tube fimbria or ovarian endosalpingiosis, Ovarian surface epithelium	Serous tubal intraepithelial carcinoma (STIC)	RRSO, opportunistic salpingectomy, oral contraceptives, tubal ligation
EC, CCC	Epithelial cells in endometriosis or in adenofibroma	Atypical endometriosis, Endometrioid or clear cell borderline tumor	Tubal ligation, opportunistic salpingectomy

Legend: CCC, clear cell carcinoma; EC, endometrioid carcinoma; HGSC, high-grade serous carcinoma; RRSO, risk-reducing salpingo-oophorectomy

Table 2

Comparison between experimental ovarian cancer model systems and humans.

Variables		Human	Mouse	Hen
Anatomic	Fallopian tube fimbria	Yes	Yes	No
	Ovarian bursa	No	Yes	No
	Uterus	Yes	Yes	Yes[a]
Histologic	Endometriosis	Yes	No[b]	No
	Endosalpingiosis	Yes	Yes	No
Physiologic	Menstrual cycle	Yes	No	No
	Estrus cycle	No	Yes	No
Clinical	Spontaneous cancers	Yes	No	Yes
Molecular	Confirmed histotypes	Yes	Yes	No[c]
Experimental	Genetically tractable	N/A	Yes	No

Legend:

[a]The hen uterus, also called the shell organ, does not have a cycling endometrium like the mammalian uterus but instead functions in egg shell formation.

[b]GEMM or surgical models have been described, but mice do not spontaneously develop endometriosis.

[c]Hen tumor histotypes have been described based on histomorphology, but they have not been rigorously interrogated for immunohistochemical and genetic similarities with their corresponding human ovarian cancer histotypes.

N/A, not applicable.