Published in final edited form as:

Gynecol Oncol. 2009 October ; 115(1): 108–111. doi:10.1016/j.ygyno.2009.06.032.

Epidemiologic Correlates of Ovarian Cortical Inclusion Cysts (CICs) Support A Dual Precursor Pathway to Pelvic Epithelial Cancer

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Abstract

Objective—Many ovarian carcinomas are presumed to arise within ovarian cortical inclusion cysts (CICs). This study examined the frequency of ovarian CICs in relation to epidemiologic risk factors in women with BRCA1 and BRCA2 (BRCA+) mutations.

Methods—BRCA+ women who underwent risk-reducing bilateral salpingo-oophorectomy were studied (n=74). Fifteen demographic variables (e.g., age at time of surgery, age at first birth, age at menopause, body mass index (BMI), gravidity) from a review of the medical records and three pathologic variables (cystic and atretic follicles, corpora lutea) were recorded. Statistical associations were made using T-test or Chi Square analysis and logistic regression analysis for p-trend.

Results—Women whose ovaries contained 7 or more CICs were older at first birth (p=0.034), surgery (p=0.059), menopause (p=0.046) and had a higher BMI (p=0.034) than those with <7 CICs. Regression analysis revealed a significant association between CICs and increasing BMI (p=0.01).

Conclusions—CICs correlate with greater body mass index, similar to low-grade serous and endometrioid tumors and in contrast to high grade serous carcinoma and its putative precursor in the fallopian tube. A model is presented for ovarian and tubal pathways to pelvic cancer that are linked to different microscopic precursors with distinct epidemiologic correlates.

Keywords

Ovarian cancer; cortical inclusions; mullerian; endosalpingiosis; tubal ligation

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Conflict of interest: The authors declare that there are no conflicts of interest.

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Introduction

Primary ovarian carcinomas comprise a subset of malignancies that require a pre-existing epithelial source in the ovary, from which the tumor can arise via neoplastic transformation. The precise origin of this epithelium is controversial; one hypothesis argues that the mesothelial lining of the ovarian surface undergoes a Mullerian metaplasia; another proposes that the same epithelium is derived from the fallopian tube or uterus via passive transport. Intra-cortical envelopment of this epithelium is presumably facilitated by open wounds in the ovarian surface following ovulation or via peri-ovarian adhesions.^{1 2 3} Irrespective of the mechanism, the end result is a cortical epithelial inclusion cyst (or ovarian epithelial inclusion) within the ovarian cortex (CIC). CICs, via a poorly understood process of malignant transformation, presumably give rise to mucinous, endometrioid, and low grade serous carcinomas, and a proportion of high grade serous malignancies.⁴ The latter also can originate from the secretory cells of the distal fallopian tube and conceivably, from Mullerian inclusions elsewhere in the pelvic cavity.⁵

Epidemiologic studies of ovarian cancer have devoted considerable effort to ascertaining the risk factors for this disease. These studies consistently have identified a range of factors associated with an increased risk, including talc use, as well as factors associated with a reduced risk, including oral contraceptive (OC) use, tubal ligation, and increasing number of pregnancies. In general, these factors have pointed to ovulation as an important risk factor for ovarian cancer risk.⁶ However, relatively few studies have examined risk factors for the presence of CIC in the ovaries.^{7 8} Although it is not clear by how much the presence of CICs increases the risk of ovarian cancer, studying their etiology may help in determining what risk factors are associated with the early phases of ovarian carcinogenesis. Presumably, in the absence of CICs or ovarian endometriosis, the risk of their corresponding malignancies would be significantly diminished.

The purpose of this report is to describe a series of women with BRCA1 or BRCA2 mutations (BRCA+) who underwent prophylactic salpingo-oophorectomy. The presence of and epidemiologic risk factors for a putative precursor in the fallopian tube, which we now term the p53 signature (or p53-H2AX signature), were described previously in this group.⁹ In this study we examined the association of CICs with demographic variables obtained via chart review and will compare it to the prior study.

Methods

This study was approved by the Institutional Review Board at Brigham and Women's Hospital. The study was conducted on a population for which one or both ovaries had been removed for ovarian cancer risk reduction (BRCA1 or BRCA2 mutation carriers). The pathologic and demographic variables assessed are summarized in Table 1.

A cohort of 74 women with heritable BRCA1 or BRCA2 mutations who underwent prophylactic salpingo-oophorectomy were studied. All histologic sections from ovaries that had been entirely submitted for histologic analysis were reviewed and in each histologic section, the number of CICs were tallied. The criteria for a CIC was the presence of a pseudostratified Mullerian epithelium, or, if a monolayer or cuboidal lining, the presence of cilia. Attention was paid to contiguous sections to identify CICs that might be repeatedly sampled and to estimate as precisely as possible the absolute number per ovarian pair.

We assessed the association of CICs with several risk factors traditionally linked with ovarian cancer that were abstracted from associated medical records. The continuous variables included age at first childbirth, age at the time of prophylactic salpingo-oophorectomy, age at

menopause, body mass index (BMI), gravida, age at menarche, oral contraceptive (OC) use, parity, cystic and atretic follicles. The categorical variables included type of BRCA mutation (1 or 2), history of chemotherapy, personal history of breast cancer, type of menopause, history of smoking, use of tamoxifen, and presence of corpus luteum. This population was the subject of a prior study of fallopian tube precursors (p53 signatures).⁹

To evaluate univariate associations with CICs and risk factors, we used Student's *T*-test and Chi-square tests for continuous and categorical variables, respectively. Because CICs are common and found in virtually all ovaries, the women were divided into two outcome categories, including those with less than 7 CICs versus 7 or more (this cut-off was based on the mean number of CICs). We used linear regression with the outcome being square root-transformed CIC numbers (to improve normality) by categories of the risk factors; beta coefficients were squared to obtain values on the original scale. Tests for trend were conducted for continuous exposures using a linear term and the Wald test. Statistical significance was set at the $p \leq 0.05$ level, and all analyses were conducted using SAS 9.0 (Cary, NC).

Results

This population consisted of 74 women with BRCA1 or BRCA2 mutations. In seventy-two cases both ovaries were removed; in two cases, a single ovary was available due to a prior oophorectomy. In 69 of 72 cases, the ovaries were separately identified on the report. In the other three cases, both ovaries were combined in the histologic sections. The total number of CICs in each of the 72 cases was tallied, with the mean number of sections per ovary being 3.9 (range of 1 to 12). The median number of CICs per case was computed at 7. Based on this figure, patients were divided into those with less than 7 CICs and ≥ 7 CICs per ovary pair.

In univariate analyses, women with ≥ 7 versus < 7 CICs had an older age at first childbirth ($p=0.03$), older patient age at surgery ($p=0.06$), older age at menopause ($p=0.05$), and higher BMI ($p=0.03$) (Table 2). No significant associations were observed for parity, OC use, cystic or atretic follicles, presence of corpora lutea, BRCA mutation type, history of chemotherapy, personal history of breast cancer, type of menopause, smoking history, and prior use of tamoxifen (Tables 2 and 3). Overall, in the linear regression analysis most risk factors were not associated with CIC numbers ($p>0.10$). However, BMI was positively associated with an increasing number of CICs ($p\text{-trend}=0.01$) such that women with a BMI < 25 had an average of 2.60 CICs and women with a BMI > 30 had an average of 7.4 CICs.

Discussion

Carcinogenic pathways involving precursor lesions require multiple events to develop into invasive cancer; some risk factors may be important in precursor development, while others are important for progression of disease. For example, HPV infection, which initiates cervical carcinogenesis, is common, and presumably additional events or risk factors are required for development of malignancy since not all women with HPV develop cancer.¹⁰ Similarly, the descriptions of a candidate early precursor to serous cancer in the fallopian tube –the p53 signature – have emphasized its independence from genetic (BRCA+) risk factors, which may play a role in precursor progression.¹¹ It is well understood that CICs are extremely common, only periodically give rise to ovarian malignancy, and are not considered a “high risk” entity.¹² Earlier reports observed a higher than expected frequency of CICs associated with ovarian cancer risk, but larger systematic analyses have found no clear associations, including no link to p53 mutations or increased proliferative activity.^{7 12 13 14 15 16 17} However, because the presence of CICs are presumably required for most ovarian epithelial neoplasms to develop, it is important to determine their cause. We conducted these studies to identify potential risk factors for development of CICs.

The risk factors associated with CICs in univariate analysis in this study included older age at first birth, older age at surgery, and older age at menopause. These data, combined with a prior report, suggest a relationship between CICs and increasing age. The relationship to older age at first birth is less clear as reports have shown increased, decreased and no appreciable ovarian cancer risk associated with older age at first birth.^{18 19 20} It is of interest that age at first birth also has been associated with a putative serous cancer precursor (p53 signatures) in the distal fallopian tube.⁹ However, the precise relationship between age at first birth and ovulatory factors, which have been linked to CICs via repair of ovulation sites, is unclear.⁶

The novel association in this study showing statistical significance with linear regression for trend was the link between BMI and CICs. The relationship between BMI and ovarian cancer has been studied in detail.^{21 22 23 24 25 26 27} The results of these studies, while not always in agreement, highlight two variables that segregate with increased BMI and are relevant to the findings in this study. The first is the age of the population at risk for ovarian cancer. Several studies have shown a link between elevated BMI and ovarian cancer risk but only in premenopausal women.^{20 21 24} The implication is that hormonal factors related to obesity may influence ovarian cancer risk, but in only a younger population. The second is tumor histologic type, in that studies suggest stronger associations of BMI with risk of borderline serous neoplasms as well as endometrioid and clear cell carcinomas, both thought to originate in the ovary.^{19 24 28 29} This connection between obesity, age of tumor development and endometrioid cell type is similar for endometrioid carcinomas of the endometrium.³⁰

The above variables, either directly or indirectly, distinguish borderline serous tumors, and low grade serous and endometrioid carcinomas from high-grade serous carcinomas. First, high-grade serous carcinoma tends to occur in postmenopausal women, whereas borderline tumors are more common in premenopausal women, and endometrioid carcinomas occur over a wider age range. Second, serous carcinoma has not been associated with an elevated BMI and in one study has been inversely correlated to BMI.^{19, 31} Third, in a previous study from our group, a putative precursor to serous cancer in the distal fallopian tube (the p53 signature) was inversely associated with BMI ($p=0.06$).⁹

One limitation of this study is that the analyses were unadjusted for potential confounders due to the relatively small sample size. Future studies of risk factors for CICs with larger sample sizes are warranted. Further, because the study population was BRCA+, and thus removed any confounding by genetic risk, these results may not be generalizable to other women.

The observations in this and prior reports support a model in which two candidate precursor conditions in the ovary (CICs) and the tube (p53 signatures) are linked to ovulatory risk factors but diverge in others (e.g., BMI) (Figure 1). In this model, hormonal factors (related to obesity), as well as ovulation, influence the likelihood of ovarian CIC development, and conceivably may increase their risk of progression to neoplasia. Genotoxic injury to the distal fallopian tube epithelium, whether by ovulation or other factors, leads to early p53 mutations from which progression can occur, particularly in women with BRCA1 or BRCA2 mutations.^{11 32} Although this model does not explain all tumors arising in the ovary or fallopian tube, it addresses the endometrioid and serous spectrum and provides a framework for addressing their diverse pathogenesis from a perspective of their precursors. More detailed studies devoted to “microscopic epidemiology” are warranted to tease out additional risk factors and determine their place in the early phases of pelvic carcinogenesis.

Acknowledgments

This work was supported by grants from the NCI (P50CA10500 [SPORC]: D. Cramer, PI), NCI 1R21CA124688-01A1 (CP Crum, PI), The Columbia Hospital For Women Research Foundation (CP Crum, PI), the Francis Ward Paine and

TSA Pemberton Funds from the Division of Women's and Perinatal Pathology, Brigham and Women's Hospital, and a gift in memory of Elizabeth Ford Smith.

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Ovarian Pathway

Mullerian inclusion
High BMI
Hormonal factors
Endometrioid or low
grade serous histology
k-ras, b-raf, PTEN,
beta-catenin mutations

Tubal Pathway

P53 signature
Low/normal BMI
Genotoxic injury
High grade serous
histology
p53 mutation

Figure 1.

Two pathways to pelvic epithelial carcinoma and their earliest precursors, both influenced by ovulatory related risk factors. On the left, the ovarian cortical inclusion cyst provides an epithelial (epithelial membrane antigen (EMA) positive) source for low grade serous and endometrioid malignancies. On the right, the p53 signature (identified by p53 nuclear localization) in the distal fallopian tube is a putative source for serous carcinomas. These pathways diverge in location, precursor or preexisting condition, initiating factors, cell type and mutations.⁴⁸⁹¹¹¹⁸⁻²⁶

Table 1

Variables analyzed in the study population

Continuous	Categorical
Age at first childbirth	BRCA1 or B RCA2
Age at surgery	History of Chemotherapy
Age at menopause	History of Breast Cancer
Age at menarche	History of Smoking
Oral contraceptive use	Tamoxifen Use
Body mass index (BMI)	Corpus Luteum
Cystic Follicles	Menopause Type:
Atretic Follicles	surgery or chemotherapy
Gravidity	
Parity	

Table 2
Relationship between Number* of Cortical Inclusion Cysts (CIC) and Continuously-Measured Ovarian Cancer Risk Factors.

Risk Factor	n	Overall Mean (SD)	Mean, CIC < 7	Mean, CIC ≥ 7	p-value, T-Test
Age at first birth, yr	57	29.2 (4.1)	28.7	31.7	0.03
Age at surgery, yr	74	48.6 (9.1)	47.7	52.7	0.06
Age at menopause, yr	74	44.7 (5.7)	44.1	47.3	0.05
Body Mass Index, kg/m ²	66	26.0 (5.5)	25.2	28.8	0.03
Gravidity	74	2.3 (1.7)	2.4	2.1	0.50
Age at menarche, yr	54	12.9 (1.5)	13.0	12.4	0.37
Duration of Oral contraceptive use, yr	57	3.4 (5.1)	3.6	1.8	0.33
Parity	74	1.9 (1.3)	1.9	1.6	0.41
Cystic Follicles	73	4.4 (6.4)	4.3	4.5	0.94
Atretic Follicles	74	5.5 (8.3)	5.7	4.5	0.60

* Comparing women with <7 versus ≥7 CICs (n=59 and 15, respectively).

Table 3

Relationship between Number* of Cortical Inclusion Cysts (CIC) and Categorical Ovarian Cancer Risk Factors.

Risk Factor RF	% Population w. RF	% of CIC < 7 w. RF	% of CIC ≥ 7 w. RF	p-value, Chi Square
<i>BRCA 1</i> mutation	54%	57%	40%	0.24
No history of Chemotherapy	63%	64%	53%	0.56
History of breast cancer	44%	42%	53%	0.45
Menopause Type: <i>Surgical</i>	80%	81%	67%	0.42
History of Smoking	12%	14%	7%	0.47
No history of Tamoxifen Use	88%	90%	79%	0.25
Presence of Corpus Luteum	49%	53%	33%	0.18

* Comparing women with <7 versus ≥7 CICs (n=59 and 15, respectively).