Published in final edited form as:

Histopathology. 2018 April ; 72(5): 766–776. doi:10.1111/his.13444.

Observations on the Origin of Ovarian Cortical Inclusion Cysts in Women Undergoing Risk-Reducing Salpingo-Oophorectomy

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Abstract

Aims—Evidence suggests that **up to 70%** of high-grade serous ovarian carcinomas (HGSCs) **potentially** arise from fallopian tube fimbriae, and that many of the remaining **cases** arise from within the ovary in cortical inclusion cysts (CICs) with a Müllerian phenotype (Müllerian-CICs). It has been proposed that Müllerian-CICs arise either from metaplasia of mesothelial ovarian surface epithelium (OSE) entrapped within the ovary after ovulation, or from normal **tubal** cells entrapped post-ovulation. However, this proposal is controversial. We therefore conducted a study of CICs in women, most of them BRCA1/2 mutation carriers, undergoing risk-reducing salpingo-oophorectomy at our institution from 2000–2014.

Methods and results—We used immunohistochemistry for Paired Box gene 8 (PAX8), a Müllerian marker, and calretinin, a mesothelial marker to classify CIC cells. In 499 CICs from 59 women, 72.3% were positive for PAX8 (PAX8+): 10% of CIC cells positive; 43.5% positive for **calretinin** (CALRETININ+). The proportion of PAX8+ CICs increased from 62.9% in premenopausal to 80.5% in postmenopausal patients. The proportion of **CALRETININ+** CICs decreased from 52.6% to 35.6%, respectively. There was significant overlap of PAX8 and **calretinin** positivity: 82 (16.4%) CICs were PAX8+/CALRETININ+; 43 (40.2%) of these 82 demonstrated PAX8+/CALRETININ+ in the same cells.

Conclusions—These results, and the increased ratio of PAX8+ to **CALRETININ+** CICs from premenopausal to postmenopausal, show that many PAX8+ CICs likely arise from metaplasia of OSE-derived CICs. The proportion of PAX+/CALRETININ– CICs arising from OSE-derived

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Conflicts of Interest: There are no conflicts of interest declared by any of the authors

AUTHOR CONTRIBUTIONS

K.J. Park, A. Jotwani, P. Patel, M.C. Pike performed the research

M.C. Pike, N. Kauff designed the study

I. Linkov performed immunohistochemical studies

P. Patel, M.C. Pike analyzed data

K.J. Park, P. Patel, M.C. Pike wrote the paper

CICs is unclear, but our results strongly support the proposal that many Müllerian-CICs arise from OSE via metaplasia.

Keywords

Inclusion cysts; Ovarian surface epithelium; BRCA; Tubal metaplasia

INTRODUCTION

High-grade serous carcinoma (HGSC) accounts for ~70% of epithelial ovarian cancers (1), with the ovarian surface epithelium (OSE) traditionally being the favored cell of origin (2, 3). It was postulated that OSE entrapped in ovarian cortical inclusion cysts (CICs) after ovulation underwent metaplastic transformation to Müllerian-type epithelium (Müllerian-CICs). However, it is now believed that a high proportion of HGSCs arise from Müllerian fallopian tube fimbriae (4–8) based on a study reporting that this was the most common site of occult lesions in BRCA1/2 mutation carriers undergoing risk-reducing salpingo-oophorectomy (RRSO); a subsequent study identified occult tubal cancers in ~70% of ovarian HGSCs (4, 7). The site of origin of **those cases without tubal involvement** is controversial, but evidence suggests that a significant proportion arises within the ovary itself (5, 9, 10) from Müllerian-CICs, through metaplasia of entrapped OSE, or from **tubal** cells entrapped within the ovary post-ovulation (2, 5, 10).

The OSE is embryologically derived from coelomic epithelium, which is contiguous with the mesothelial lining of the peritoneal cavity and differs from the Müllerian-CICs thought to give rise to HGSCs (11). By immunohistochemistry (IHC), OSE is generally positive for calretinin and negative for Paired Box gene 8 (PAX8) (12); the secretory cells of the **fimbria** are negative for **calretinin** and positive for PAX8; the ciliated cells are negative for both (13). Banet and Kurman (14) used IHC to characterize CICs as originating from either fallopian tube fimbriae (PAX8+) or OSE (**CALRETININ+**), and estrogen receptor (ER) and progesterone receptor (PR), because of their potential roles in CIC development. Based on a study of 37 cysts from 29 patients, they concluded that Müllerian-CICs derive from implanted fallopian tube epithelium, not as a result of metaplasia in cysts derived from invagination of the OSE (14).

We conducted a study of CICs from the ovaries of 59 patients (mainly BRCA1/2 mutation carriers) undergoing RRSO at Memorial Sloan Kettering Cancer Center (MSKCC) from 2000–2014 **with the hypothesis that metaplasia of the OSE to Mullerian type epithelium does occur and may be an alternative pathway toward serous carcinogenesis**. Digital microscopy and computer image analysis were used to standardize cell count, and optimize concordance with visual inspection.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board (IRB) at MSKCC. Women undergoing RRSO from 2000–2014, who signed an IRB-approved release permitting use of their tissue for research, were eligible. Criteria for exclusion included: pregnancy or exogenous estrogen, progestin, or antiestrogen therapy within 6 months of RRSO; history of

cancer (except non-melanoma skin cancer). Cases were also excluded if the fallopian tube or ovary showed early malignancy. We obtained the BRCA1/2 status of patients signing an IRB-approved release permitting use of their germline genetic information.

Subject Identification and Tissue Collection

We identified ovarian tissue samples from entirely sampled ovaries and fallopian tubes using the “sectioning and extensively examining the fimbriated end” (SEE-FIM) protocol (15). For premenopausal patients at time of RRSO, we collected material only if endometrial sampling was performed, to estimate the menstrual cycle phase (16). For patients identified as postmenopausal, we confirmed this against their endometrial sample (if available). No discrepancies were found.

CICs

We focused only on cysts completely enclosed within the ovarian parenchyma, irrespective of proximity to the ovarian surface. Fallopian tube (FT)-like CICs were defined as columnar and/or cuboidal cells resembling those of the **tube**, or flattened cells with visible cilia; OSE-like CICs were defined as flattened or low cuboidal cells resembling OSE, without evidence of cilia.

IHC

One ovarian block containing the greatest number of cysts was obtained for each patient, chosen by inspecting all available hematoxylin and eosin (H&E) slides. Adjacent 3 μ m sections were cut, deparaffinized, and hydrated. IHC stains were performed: PAX8 (rabbit PAb at 1:1000, Proteintech, Rosemount, IL, USA), **calretinin** (rabbit PAb at 1:100, Zymed, San Francisco, CA, USA), ER α (ER; Clone SP1 at 1:2 from ready-to-use, Ventana Medical Systems, Hoffmann-La Roche, Nutley, NJ, USA) and PR A/B (PR; Clone 1E2 at 1:2 from ready-to-use, Ventana Medical Systems) on Ventana platform discovery XT. MSKCC standard methods include antigen retrieval CC1 (mild) regimen for **calretinin** and PAX8 antibodies, CC1 (standard) regimen for ER and PR antibodies; primary Ab-s incubation on Discovery XT platform, 60 min; secondary biotinylated anti-Mouse and anti-Rabbit Ab-s incubation on Ventana platform, 60 min. The Ventana DAB MAP kit was used to complete the staining procedure.

Digital Scanning and Computer Analysis

Slides were digitized using the Aperio® scanning system. Computer images were analyzed to obtain counts of total cells in individual CICs, and number of cells that were positive for various IHC markers. The cell layer lining each cyst was outlined (enclosed) using the Aperio® Annotations tool at 20 $\times$ -magnification; enclosed areas were analyzed using the Aperio® IHC Nuclear Algorithm. Figure 1 shows a PAX8 slide before and after outlining. If a cyst was too large to fit on the visual panel in Aperio® at 20 $\times$, the cell layer was selected in multiple parts; individual parts were analyzed separately and summed, to obtain a result for the entire cyst. The Aperio® IHC Nuclear Algorithm allows for adjustment of three parameters: average radius, curvature threshold, nuclear size. These were optimized across

all stains in a subset of cases to achieve close agreement with the counts, as made by one of the authors (KJP). The visual output is shown in Figure 1.

CICs were deemed positive for PAX8, **calretinin**, ER and PR if 10% or more of the cells showed nuclear (for PAX8, ER, PR) or nuclear and cytoplasmic (for **calretinin**) staining (definition proposed by Banet and Kurman) (14).

Statistical Methods

The percentage of cells positive for PAX8, **calretinin**, ER and PR was calculated for each CIC. A cyst was analyzed for PAX8 and **calretinin** only if it was present on both the PAX8 and **calretinin** slides. A cyst was analyzed for ER and PR only if it was present on the PAX8, **calretinin**, ER and PR slides.

RESULTS

We identified 499 CICs from 59 patients, each CIC successfully stained for PAX8 and **calretinin**. Twenty-three of the 59 patients were BRCA1, 29 BRCA2, 4 BRCA negative, 3 of unknown BRCA status. The breakdown by BRCA status, associated total numbers of CICs, and PAX8/**calretinin** results, are shown in Table 1. Of 499 cysts, 361 (72.3%) were PAX8+; 217 (43.5%) **CALRETININ**+

Most FT-like CICs were PAX8+ and **CALRETININ**–; most OSE-like CICs were PAX8–/**CALRETININ**+. However, this was not invariably the case. Figure 2 shows a PAX8–/**CALRETININ**– FT-like CIC; Figure 3 shows a PAX8+/**CALRETININ**– OSE-like CIC. The intensity of calretinin positivity in the CICs was similar to that of OSE (strong intensity) when the entire cyst was positive. In the partially positive CICs, the calretinin intensity ranged from similarly intense to slightly less intense, but most cysts had robust staining. For these reasons, and because many CICs showed both FT-like and OSE-like areas, we ignored the distinction between OSE-like and FT-like when presenting our results.

Table 2 shows PAX8 and **calretinin** staining results for premenopausal, postmenopausal, and all patients. The combined results demonstrate that 3 cysts were PAX8– and **CALRETININ**– (<10% cells staining). Two hundred and seventy-nine (55.9%) cysts stained for PAX8 only; 135 (27.1%) stained for **calretinin** only.

The proportion of PAX8+ cysts increased from 62.9% (146/232) in premenopausal to 80.5% (215/267) in postmenopausal women, while the proportion of **CALRETININ**– cysts decreased from 52.6% (122/232) to 35.6% (95/267) (Table 2). The proportion of PAX8+ only cysts increased from 46.1% (107/232) to 64.4% (172/267); the proportion of **CALRETININ**– only cysts decreased from 35.8% (83/232) to 19.5% (52/267).

Eighty-two (16.4%) of the 499 cysts were both PAX8+ and **CALRETININ**–: 16.8% (39/232) premenopausal and 16.1% (43/267) postmenopausal women had PAX8+/**CALRETININ**– CICs. Figure 4 shows a PAX8+/**CALRETININ**– cyst, in which all non-ciliated cells are PAX8+ but only a portion are **CALRETININ**– (~80% overlap of PAX8+ and **CALRETININ**– cells); ciliated cells are PAX8– and **CALRETININ**–. Figure 5 shows

a PAX8+/CALRETININ+ cyst, in which nearly all cells are positive for both (~95% overlap of PAX8+ and CALRETININ+ cells).

Thirty-three of the 82 (40.2%) cysts that were both PAX8+ and CALRETININ+ showed positive staining for both in the same cells; the other 49 (59.8%) cysts had mutually exclusive PAX8+ and CALRETININ+ cells (no overlap). Cysts with joint staining in the same cells contained many cells that were PAX8+ only (Figure 4). In CICs with no overlap, more cells were PAX8+ only versus CALRETININ+ only.

In some instances, the staining patterns in ovarian surface cells were similar to those associated with the CICs. Figure 6 shows one case in which the surface epithelium comprised OSE-like cells adjacent to FT-like cells. All cells were strongly CALRETININ+, but only the OSE-like cells were PAX8+, contrary to expectation. Furthermore, only the PAX8-/CALRETININ+ cells on the surface expressed ER and PR, suggesting that the metaplastic process can occur without formation of CICs, and that this property is not exclusive to fully formed cysts in the ovary. One hundred and eighty-nine of the 282 (67%) PAX8+/CALRETININ- cysts showed 90% of cells staining for PAX8; these showed few ciliated cells.

Three hundred and three of the 499 cysts were present on subsequent ER/PR slides; positive ER and PR reactivity was observed in 249 (82.2%) and 254 (83.8%), respectively (Table 3). These proportions increased from premenopausal to postmenopausal women: ER 74.3% to 89.3%, PR 73.6% to 94.3% (Table 3). ER and PR positivity was widespread in PAX8+ and CALRETININ+ cells (Figures 2 and 5).

Analysis of CIC results from the four patient subgroups (BRCA1, BRCA2, BRCA-, BRCA status not known) showed no statistically significant differences between groups; the proportion of CICs showing joint PAX8 and calretinin positivity did not differ.

DISCUSSION

The majority (~65%) of PAX8+ CICs showed 90% of cells staining for PAX8; few ciliated cells were seen. This differs from the fallopian tube, where ciliated cells are common.

In this study, 72.3% of CICs were PAX8+, 43.5% were CALRETININ+, with significant overlap between the two antibodies: 82/499 (16.4%) CICs were PAX8+/CALRETININ+; 43 of these 82 (40.2%) were PAX8+/CALRETININ+ in the same cells. The proportion of PAX8+ and CALRETININ+ CICs is similar to the 83.8% and 32.4%, respectively, reported by Banet and Kurman (14). They also reported that 8/37 (21.6%) cysts were positive for both PAX8 and calretinin, but they did not provide results for dual staining in the same cells. The only other published reports noting the frequency of PAX8 and calretinin in CICs provide no figures on dual staining in the same cyst (17, 18). However, a study of PAX8 expression in OSE examined protein expression using IHC, immunofluorescent cytochemistry and PAX8 mRNA expression in normal ovaries and cell cultures of OSE, and IHC to examine calretinin and e-cadherin expression (19). The authors identified PAX8 IHC positivity in OSE (44%) and CICs (83%), in both flat and cuboidal/columnar cells, with no correlation between cell morphology and PAX8 positivity. In the 4 cases where

calretinin was also performed, 1 showed co-expression of PAX8 and **calretinin** in the OSE. In another study evaluating expression of PAX2 and PAX8 in benign Müllerian epithelium and malignancies, Ozcan et al reported that 63% of OSE and 100% of CICs expressed PAX8 (20), with positivity in both flat and columnar cells of both OSE and CICs; our findings are similar. In fact, we found OSE adjacent to FT-like cells on the ovarian surface with unexpected immunohistochemical staining patterns with only the OSE being positive for PAX8, ER and PR, but not the FT-like cells. This may partially account for primary peritoneal HGSCs with diffuse peritoneal tumor dissemination but no dominant mass in either the fallopian tube or ovary. The authors suggest that the discrepancy in PAX8 positivity among some studies (21, 22) may be due to variability in the polyclonal nature of the PAX8 antibody, and to differences in technique between laboratories.

Similarly, benign and malignant mesothelium has been shown to express PAX8, with up to 18% of malignant mesothelioma and 32% of benign mesothelium being PAX8 positive in one study (23). This does not necessarily suggest that Müllerian epithelium has implanted onto these surfaces, but does support the possibility of phenotypic fluidity in the peritoneal lining in benign and malignant lesions. In our study, the proportion of PAX8+ CICs increased from 62.9% in premenopausal to 80.5% in postmenopausal women; the proportion of **CALRETININ**+ CICs decreased from 52.6% to 35.6%, respectively. The existence of CICs with co-expression of PAX8 and **calretinin** and the increase in the proportion of **PAX8+ CICs from pre- to post-menopause supports the assumption** that many PAX8+ CICs likely arise from metaplasia of OSE-derived CICs. An analysis of PAX8+/**CALRETININ**+ cysts shown in Figures 1 and 4 suggests that metaplasia is initially seen with expression of **calretinin** and PAX8 in the same cells, progressing to expression of PAX8 with loss of **calretinin** expression. **This does not discount the possibility that some of the dual staining cysts may be a result of tubal implantation;** however, if Müllerian-CICs were exclusively derived from implanted fallopian tube epithelium, **one might speculate that** the proportion of PAX8+ cells to decrease, or remain unchanged from pre- to post-menopause when ovulation ceases. However, our findings contradict this. We postulate that the best explanation for this is metaplasia of the ovarian surface from PAX8-/**CALRETININ**+ OSE to PAX8+/**CALRETININ**- Müllerian (**tubal**) epithelium. The proportion of PAX8+/**CALRETININ**- CICs arising from OSE-derived CICs is unclear, but Banet and Kurman's conclusion (14) that Müllerian-CICs are "derived from implanted fallopian tube epithelium...rather than as a result of metaplasia occurring in cysts that are derived from invagination of the OSE" appears unwarranted. Their argument was partially based on findings of very low levels of ER and PR staining (14). However, we identified positive expression of ER and PR in ~83% of CICs; the proportion increased from ~74% in premenopausal to ~90% in postmenopausal women. ER and PR positivity was widespread in **CALRETININ**+ and PAX8+ cells. We have no explanation for this discrepancy.

In one of the few other studies of PAX8 and calretinin expression in CICs, DastranjTabrizi et al. showed the effect of oral contraceptive pills (OCPs) on the development of ovarian cysts and the quality of the cells that line them. When compared to patients on OCPs <5 years or other forms of contraception, those on OCPs >5 years had significantly fewer CICs and fewer number of CICs lined by FT-like epithelium (18). In addition, PAX8 was positive in

the vast majority of the CICs stained, irrespective of the tubal or flat nature of the lining cells. Only 4.8% of their cysts were calretinin positive; however, the authors do not comment on any dual staining. Based on these findings, they conclude that progesterone has a suppressive effect in the development of CICs and prevents tubal metaplasia in these cysts. This concept provides an intriguing and potential mechanism for the increased PAX8+ CICs in our postmenopausal patients, with the loss of progesterone's protective effect after menopause.

The existence of cellular metaplasia in multiple organ sites is well established, and is a common phenomenon of the Müllerian tract in both neoplastic and non-neoplastic settings. **Unopposed estrogen frequently causes tubal metaplasia in endometrial neoplasms with progesterone therapy frequently converting neoplastic glands to normal.** Tubal/ciliated, mucinous, squamous and eosinophilic metaplasias are often seen histologically, typically representing cellular reaction to injury or irritation. Squamous metaplasia in the cervical transformation zone in response to the acidic vaginal environment; tubal metaplasia in the endocervix after biopsy, or in the endometrial glands of endometriosis; mucinous metaplasia in endometrioid adenocarcinomas, are examples. These are so common that they do not necessarily warrant mention in a pathology report. Examples in other organ systems include intestinal metaplasia in Barrett's esophagus and squamous metaplasia in the urinary bladder, each of which is the basis for development of adenocarcinoma and squamous cell carcinoma, respectively. In the Müllerian tract, benign lesions and tumors can show heterologous metaplasia in the form of intestinal-type mucinous cells expressing intestinal markers (24). A particularly interesting example of mesothelial to Müllerian metaplasia is that which occurs in epithelial adrenal cysts which are lined by mesothelial and/or FT-like cells that express the associated markers calretinin/WT1 and PAX8/ER respectively (25, 26). Since adrenal glands do not naturally have any acinar structures with epithelial differentiation, the presence of epithelial lined cysts in these organs is thought to be a result of entrapped mesothelium during embryogenesis. In one case report, a 31 year old woman had an adrenal cyst lined by mesothelial and ciliated FT-like cells where the Müllerian cells stained with PAX8, WT1, EMA and PR but not calretinin, while the mesothelial cells were positive for calretinin, WT1 and D2-40 but also showed weak focal positivity for PAX8, EpCAM and EMA (26). Another case series by the same authors described 2 adrenal cysts lined by what appeared to be pure mesothelial cells lacking Müllerian differentiation which expressed both mesothelial (calretinin, WT1, D2-40) and Müllerian (PAX8, ER and EMA) markers diffusely or focally in various combinations (25). Interestingly, more than half the patients in their series were obese or overweight, though it is not specified if the 2 patients with epithelial cysts (the other cysts in the series were non-epithelial) were included in this group. One of those cysts occurred in a 59 year old male, supporting the metaplasia hypothesis over Müllerian implantation (25). Therefore, the notion that mesothelial OSE cells undergo tubal metaplasia is not farfetched; it is probably a common occurrence. HGSCs could very well arise from these metaplastic cysts.

Others argue that implantation of fallopian tube cells on the ovarian surface—not metaplasia—is the sole cause of endosalpingiomatic cysts in the ovary (27). However, this fails to account **not only for the aforementioned adrenal cysts, but also** for the numerous reports of

Müllerian cysts and tumors arising in men (in genital organs and lymph nodes) (28–32). Some propose that Müllerian metaplasia of the peritoneum is the source of endometriosis, due to expression of BerEP4, ER and PR in mesothelial cells of the ovary and peritoneum (33). **As previously mentioned**, mesotheliomas **and benign mesothelium** reportedly express Müllerian markers such as PAX8, PAX2, ER and PR (23, 27, 34–36), suggesting a dynamic balance between mesothelial and Müllerian cells in the peritoneum (21).

We used digital microscopy and computer analysis to determine the cell count in ovarian CICs and found that, contrary to previous reports, metaplasia occurs in entrapped OSE, from PAX8-/CALRETININ+ mesothelial cells to PAX8+/CALRETININ– FT-like cells; these could account for the **proportion** of HGSCs that arise in the ovary without an associated fallopian tube neoplasm. Incidentally, we noted that the same metaplastic phenomenon can be observed on the ovarian surface. This has been previously reported (19), and could explain the occurrence of primary peritoneal HGSC diffusely involving the peritoneum in the absence of a dominant adnexal mass. HGSC, a Müllerian neoplasm, is derived from Müllerian-type epithelium.

One question that is frequently posed is, if ovarian CICs are a potential source of HGSC, then why are intraepithelial carcinomas analogous to serous tubal intraepithelial carcinoma (STIC) never seen in these cysts? Is there a mechanism by which carcinomas that originate in the ovary are different from those arising in the fallopian tube? While there are no definitive answers to these questions yet, some have observed that the tumor microenvironment could have a significant role in the development of cancers at different sites. Karnezis et al. have proposed that the microenvironments of the ovary and fallopian tube play distinct roles in tumor initiation, progression and/or growth, with the fallopian tube having an inhibitory effect and the ovary having a nurturing one (37). They cite examples of metastatic tumors and those arising in endometriosis that preferentially grow in the ovary as compared to other pelvic sites, often sparing the fallopian tube mucosa. One could postulate that the ovarian stroma is so stimulatory for tumor growth, that the window between intraepithelial and invasive carcinoma is too short to be able to detect the pre-invasive lesions. Obviously, further studies are needed to address these hypotheses.

The origin of Müllerian-type epithelium outside of the fallopian tube is likely multifactorial. We have shown that this includes metaplasia of coelomic-derived mesothelial cells to FT-like Müllerian cells.

Acknowledgments

The authors wish to thank all of the patients who permitted use of their tissues for research. We also wish to thank the anonymous donor who provided funding to Dr. Kauff for this work.

FUNDING

This study was supported by a grant from the National Cancer Institute (R21 CA181923; PIs M.C. Pike, N. Kauff).

This study was also funded in part through the NIH/NCI Support Grant P30 CA008748.

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Figure 1. Cyst selection and annotation in an ovary slide using Aperio® Annotation

(a) PAX 8 whole slide imaging; (b) PAX8 with Aperio selections; the various colors represent how much of each cyst was evaluable on one Aperio visual panel. The small cysts could fit in one panel and were annotated in a single color. Larger cysts were broken down into multiple parts represented by different colors in each cyst; (c) PAX8 $\times 200$ magnification; (d) **calretinin** $\times 200$ magnification; (e) PAX8 with yellow and blue delineation of cells lining the cyst $\times 400$ magnification; (f) **calretinin** cyst with orange and turquoise delineation of cells lining the cyst $\times 400$ magnification; (g) PAX8 with Aperio® IHC Nuclear Algorithm output; red marks positive nuclei, blue marks negative nuclei $\times 400$

magnification; (H) **calretinin** with Aperio® IHC Nuclear Algorithm output; red marks positive nuclei, blue marks negative nuclei $\times 400$ magnification.

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Figure 2. FT-like CIC that is PAX8-/CALRETININ+

(a) H&E showing cuboidal cells and a few ciliated cells $\times 400$ magnification; (b) PAX8 is negative $\times 400$ magnification; (c) **calretinin** is positive $\times 400$ magnification; (d) ER is weakly positive in most cells $\times 400$ magnification; (e) PR is weakly positive in most cells $\times 400$ magnification.

Figure 3. OSE-like CIC that is PAX8+/CALRETININ-
(a) H&E $\times 100$ magnification; (b) H&E $\times 400$ magnification; (c) PAX8 $\times 100$ magnification;
(d) calretinin $\times 100$ magnification.

Figure 4. PAX8+/CALRETININ+ cyst with variable staining

(a) H&E slide of cyst lined by columnar FT-like cells with cilia adjacent to flat OSE-like cells $\times 100$ magnification; notice occasional ciliated cells in OSE-like stretch; (b) PAX8 is positive in all non-ciliated cells, including OSE-like cells; (c) **calretinin** is positive only in OSE-like cells and ciliated cells in this stretch are also negative.

Figure 5. OSE-like CIC PAX8+/CALRETININ+ in same cells

(a) H&E $\times 100$ magnification; (b) PAX8 diffusely positive $\times 100$ magnification; (c) **calretinin** diffusely positive $\times 100$ magnification; (d) ER positive in nearly all cells $\times 100$ magnification; (e) PR positive in ~60% of cells $\times 100$ magnification.

Figure 6. Ovarian surface epithelium where all cells are CALRETININ+ but only some of are PAX8+

(a) H&E $\times 200$ magnification; (b) PAX8 positive in flattened OSE-like cells and not cuboidal FT-like cells $\times 200$ magnification; (c) **calretinin** diffusely positive in all cells of ovarian surface $\times 200$ magnification; (d) ER and (e) PR show reactivity in the PAX8-/CALRETININ+ FT-like cells only $\times 200$ magnification.

Table 1

Numbers of Patients, Cysts and Overall CRT/PAX8 Positivity

	BRCA1	BRCA2	BRCA –	BRCA NK	Total
Patients	23	29	4	3	59
Cysts	148	266	53	32	499
PAX8+ (%)	94 (63.5%)	205 (77.1%)	49 (92.5%)	13 (40.6%)	361 (72.3%)
CRT+ (%)	73 (49.3%)	106 (39.8%)	16 (30.2%)	22 (68.8%)	217 (43.5%)

CRT – calretinin; NK – not known

Table 2**CALRETININ** and PAX8 Staining in Pre- and Postmenopausal Women (n=499 cysts)

Premenopausal		CALRETININ (%)		
PAX8 (%)		Neg	Pos	Total
	Neg	3	83	86 (37.1%)
	Pos	107	39	146 (62.9%)
	Total	110 (47.4%)	122 (52.6%)	232 (100%)
Postmenopausal		CALRETININ (%)		
PAX8 (%)		Neg	Pos	Total
	Neg	0	52	52 (19.5%)
	Pos	172	43	215 (80.5%)
	Total	172 (64.4%)	95 (35.6%)	267 (100%)
All		CALRETININ (%)		
PAX8 (%)		Neg	Pos	Total
	Neg	3	135	138 (27.7%)
	Pos	279	82	361 (72.3%)
	Total	282 (56.5%)	217 (43.5%)	499 (100%)

Neg is <10% and Pos is 10% of cells staining with marker

Table 3
ER and PR staining in relation to PAX8 and **CALRETININ** positivity (n=303 cysts)

Premenopausal	ER				PR			
	ER-	ER+	ER+%	PR-	PR+	PR+%		
CALRETININ+ *	13	29	69.0%	10	32	76.2%		
PAX8+ *	12	56	82.4%	13	55	80.9%		
P+/C+	12	22	64.7%	15	19	55.9%		
Total	37	107	74.3%	38	106	73.6%		
Postmenopausal	ER				PR			
	ER-	ER+	ER+%	PR-	PR+	PR+%		
CALRETININ+ *	1	33	97.1%	3	34	91.9%		
PAX8+ *	12	84	87.5%	4	90	95.7%		
P+/C+	4	25	86.2%	2	24	92.3%		
Total	17	142	89.3%	9	148	94.3%		
All	ER				PR			
	ER-	ER+	ER+%	PR-	PR+	PR+%		
CALRETININ+ *	14	62	81.6%	10	66	86.8%		
PAX8+ *	24	140	85.4%	19	145	88.4%		
P+/C+	16	47	74.6%	20	43	68.3%		
Total	54	249	82.2%	49	254	83.8%		

P+/C+ - positive for both PAX8 and **CALRETININ**

* Excluding cysts that stain positive for both PAX8 and **CALRETININ**