Histogenesis of endometriosis-associated ovarian cancer

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ABSTRACT
Patients with endometriosis carry the potential risk for ovarian cancer later in life, but the histogenesis of endometriosis-associated ovarian cancer (EAOC) still remains unclear. This review outlines recent advances in the understanding of the genetic background and histogenesis of EAOC. This article reviews the English-language literature between 2000 and 2017. The oxidative stress, inflammation, immune response and hormone activity are mainly associated with deregulated pathways contributing to the carcinogenesis of EAOC. Ovarian endometrioid carcinoma (EC) and clear cell carcinoma (CCC) respond similarly to inflammatory and oxidative stimuli and share a common genetic abnormality. Several markers of histogenesis have been used to assess whether EC and CCC arise from the same clone or different precursor cells. Expression of various histogenesis markers may be epigenetically regulated in distinct EAOC subtypes. Secretory cells and ciliated cells in endometriosis are candidates for cells of origin of EC and CCC, respectively. In conclusion, EAOC may arise from the different precursor cells that undergo similar genetic changes.

KEYWORDS: endometriosis, malignant secretory cells, transformation, ciliated cells, epigenetics.

INTRODUCTION
Endometriosis is a common, chronic inflammatory disease that affects ~10% of women at reproductive age [1]. Endometriosis is defined as the ectopic implantation of endometrial glands and stroma outside the uterine endometrium [2, 3]. The common symptoms of this disease are dysmenorrhea, dyspareunia, chronic pelvic pain and/or infertility [1, 4]. Epidemiological studies have demonstrated that endometriosis is a risk factor for some types of malignancies, especially for uterine and ovarian endometrioid carcinoma (EC), clear cell carcinoma (CCC), low-grade serous carcinoma and seromucinous neoplasms [5, 6]. Endometriosis was found in approximately 20% of EC and CCC patients and present adjacent to the tumor [7, 8]. Approximately 1% of ovarian endometrioma gives rise to endometriosis-associated ovarian carcinoma (EAOC) [8]. Elegant studies have focused on the pathogenesis of EAOC [9, 10]. EAOC is thought to develop in at least two distinct environmental phases. CCC develops primarily during obvious oxidative stress associated with elevation of antioxidant content, while EC occurs during an estrogenic mode of action due to the observed induction of estrogen receptor (ER) [10, 11]. Both tumors frequently occur in perimenopausal and/or early postmenopausal women. The origin of the precursor cells has been attributed to normal endometrium, fallopian tube epithelium, or cortical inclusion cyst (CIC) with Müllerian metaplasia [12-15]. The eutopic and ectopic endometrium are composed of at least two cell types with unique properties, secretory cells and ciliated cells. Presence of endometriosis is associated with an increased risk of ovarian cancer later in life, but the histogenesis of EAOC still remains unclear. Two existing theories or postulations are eligible for consideration: 1) the pluripotent stem cells may possess the ability to replenish EC and CCC, securing multilineage differentiation and
reconstitution, and 2) the EAOCH dichotomy is probably due to the origin of cancer from different precursor cells in endometriosis. Here we seek to present the current state of knowledge regarding the genetic background and histogenesis of EAOC.

**MATERIALS AND METHODS**

Systematic review of the literature was done using electronic search in the PubMed/Medline databases. The current study aimed to summarize the current status of knowledge regarding the genetic background and histogenesis of endometriosis-associated ovarian cancer. A PubMed (http://www.ncbi.nlm.nih.gov/pubmed) search of the relevant literature published between 2000 and 2017 was performed. The search strategy included a combination of the keywords: endometriosis, endometriosis-associated ovarian cancer, genetic background, histogenesis, secretory cells, and ciliated cells in the titles or abstracts of articles. English-language publication search results from PubMed and references within the relevant articles were analyzed. To minimize selection bias, screening of the studies was independently performed by four of the co-authors (SM, KI, EN and MK) after agreeing on the selection criteria.

**RESULTS**

**A background for malignant transformation of endometriosis**

Although the pathogenesis and histogenesis of EAOC remain mysterious, the results of several studies support the hypothesis that the oxidative stress, inflammatory/immune response and hormone activity are associated with the malignant transformation of endometriosis [10, 16-21]. First, repeated episodes of hemorrhage occur in endometriosis throughout menstruation [10]. Old menstrual blood accumulated in ovarian endometrioma contains high concentrations of hemoglobin, heme and free iron [18, 22]. Lethal toxicity is initiated by the hemorrhage-induced reactive oxygen species (ROS) in endometriotic tissues. Excess DNA damage and mutations create increased cell death, but not carcinogenesis [10]. Such hemorrhage also induces macrophage-mediated inflammation process, including dysregulation of redox homeostasis, excessive cytotoxic ROS and DNA damage [18, 22]. Macrophages can polarize into M1 or M2 subpopulations [16]. M1-polarized macrophages generating pro-inflammatory mediators and ROS show antitumor activity.

Upon tissue damage, most of the tumor-associated macrophages display the immunosuppressive M2 phenotype [10]. Endometrial-like epithelial glands surrounded by immune stroma can escape immune destruction [2, 3]. Chronic inflammation promotes alternatively activated M2-polarized tumor-associated macrophages to produce a variety of anti-inflammatory cytokines and resolve inflammation, which contributes to tissue remodeling, regeneration, angiogenesis, tumorigenesis and progression [16]. Three of the known inflammation-related pathways in endometriosis are the canonical nuclear factor kappa-B (NF-κB) pathway, the MAPK pathways (ERK1/2, p38 and JNK) and the PI3K-AKT-mTOR pathway [17]. These pathways are responsible for a wide array of signaling events underlying tumorigenesis, stimulating cell proliferation and inhibiting apoptosis. Upon oxidative stress and inflammatory microenvironment, Nrf2 (NFE2-related factor2) and heme oxygenase-1 (HO-1) trigger the upregulation of antioxidant defense genes [10, 18]. Oxidative stress markers are downregulated in EAOC when compared to endometriosis [19]. The hemorrhage-induced, ROS-mediated cell death could be prevented by upregulation of a variety of antioxidant genes. The environmental change regulates the delicate balance between beneficial and harmful stress response, and preserves the beneficial effects of anti-inflammation and regeneration. Mild oxidative stress or sublethal dose of ROS may protect endometriotic tissues from oxidative damage, which leads to a likely precursor that may later progress into cancer [10]. The previous review [10] supports the hypothesis that there are at least two phases of malignant transformation of endometriosis: the initial wave of the iron-induced cellular and DNA damage by promoting ROS formation would be followed by the second wave of subsequent antioxidant production by recruiting macrophages endowed with protumoral activities. Antioxidants in protumoral microenvironment cause abnormal cell proliferation and malignant transformation of endometriosis to EAOC via preventing cell death by scavenging ROS [18]. Recent studies summarize important aspects on the dual role of oxidative stress response in the
The absence of ESR in EAOC may indicate a decisive turning point in CCC initiation, progression and aggressiveness. Taken together, these data support the postulation that the oxidative stress/inflammation, immune response and hormone activity are deregulated functions and act in a network contributing to the carcinogenesis of EAOC.

Common genetic abnormalities in EAOC

Topics addressed include a summary of genetic mutations and origin of epithelial ovarian cancer. An elegant review has highlighted the etiology and pathogenesis of epithelial ovarian cancer [9]. The gene expression profile-based clustering divided ovarian cancer into two groups, the type 1 (EC and CCC) and type 2 (high grade serous carcinoma). The new revised and expanded findings are based on the widely-accepted dualistic model of ovarian carcinogenesis [9]. To better understand the underlying molecular mechanism of EAOC, we review an integrated genomic analysis associated with somatic mutations and amplifications. Recent microarray, targeted sequencing and whole genome studies have identified that somatic mutations of ARID1A, PIK3CA, PTEN, KRAS, CTNNB1, BRAF, PPP2R1A and MLH1 were found across EAOC [9, 26-29]. Inactivation of tumor suppressor genes ARID1A and PTEN and additional activation of oncogenic KRAS and PI3K drive progression to EAOC [27]. Inactivating mutations in ARID1A have been found in a broad spectrum of cancer types, with high frequency in CCC [26]. Both atypical endometriosis and adjacent EAOC had loss of ARID1A immunoreactivity [29]. In contrast, loss of ARID1A protein expression was not found in the endometriotic epithelial cells that were not adjacent to the tumor [29]. Therefore, inactivating mutations in ARID1A have been found in a broad spectrum of cancer types, with high frequency in CCC [26]. Both atypical endometriosis and adjacent EAOC had loss of ARID1A immunoreactivity [29]. In contrast, loss of ARID1A protein expression was not found in the endometriotic epithelial cells that were not adjacent to the tumor [29]. Therefore, inactivating mutations in ARID1A have been found in a broad spectrum of cancer types, with high frequency in CCC [26]. Both atypical endometriosis and adjacent EAOC had loss of ARID1A immunoreactivity [29]. 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treatment of advanced ovarian CCC with ARID1A mutation and PI3K-AKT pathway activation [28]. Temsirolimus shows a potential therapeutic benefit for these patients [31]. ARID1A loss and KRAS activation followed by PTEN loss are found across EAOC and are suggested as major pathogenic mechanisms for EAOC.

A recent review also demonstrated that EAOC includes similar patterns of pathway functional regularity and Gene Ontology term gene sets in both groups of tumors, EC and CCC [2]. The coexisting deregulated pathways involved in hormone response, inflammatory response, innate immune response and oxidoreductase activity play the key roles in the malignant transformation of EAOC [2]. Taken together, the gene expression profiles in EC may be matched to those in CCC.

EAOC has at least two subtypes originating from either EC or CCC, which show different pathologic and clinical features, characterized by unique morphologies and responses to treatment. EC is distinguished from CCC due to different morphologies, but both indeed represent common genetic mutational profiles [32, 33]. Furthermore, the overlapping mutational profiles of endometriosis adjacent to the tumor and EAOC also suggest that they share a common genetic origin and are genetically related [34]. It appears that EAOC shares similar molecular signatures through possible contribution of the environmental influences necessary for their initiation and progression [2].

Candidate cell of origin for EAOC

We next try to provide insights into the candidate cell of origin for EAOC. EAOC has a benign counterpart, endometriosis, suggesting that endometriosis and EAOC share a common origin [23]. Endometriosis and EAOC tumors are not only found within the ovary, but are also present outside the ovary. This finding supports the view that endometriosis can be a primary site of origin for a majority of EAOC tumors [23]. Although the etiology of endometriosis is still unclear, at least three models are considered: endometriosis arises from the normal endometrium (retrograde menstruation theory), the fallopian tube epithelium (tubal origin theory), or possibly from cortical inclusion cyst (CIC) with Müllerian metaplasia (secondary Müllerian theory) [12-15]. First, endometriosis probably develops from the peritoneal seeding of viable late secretory and menstrual endometrial cells during retrograde menstruation [13]. Second, the current paradigm is that fallopian tube-derived ovarian epithelial inclusions have been implicated as a cell source for endometriosis [12, 15]. When the ovarian surface epithelium (OSE) is damaged during repetitive ovulation, tubal epithelial cells implant upon the ovarian surface, suggesting that CICs are derived from fallopian tubal epithelial cells [12, 35]. Finally, ovarian cortical inclusion cyst (CIC) epithelial cells develop from invagination of ovarian surface epithelium (OSE) and subsequently differentiate into the remaining endometriosis [13]. Evidence suggests that endometriosis arises from regurgitated endometrial cells, fallopian tubal epithelial cells, or invagination of the OSE.

This review assesses whether EC and CCC arise from the same clone or different precursor cells. EC and CCC have a Müllerian phenotype, but possess unique morphologic features that differ from each other [36]. Several markers of histogenesis have been identified, including estrogen receptor (ER), cystathionine gamma lyase (CTH) and hepatocyte nuclear factor (HNF)-1β (Table 1). ER and CTH are markers for endometrial cells committed to the secretory cell lineage [32]. Expression of HNF-1β identifies cells undergoing clear cell lineage differentiation [37]. Immunohistochemical analysis has been used to distinguish secretory (ER-positive, CTH-negative, HNF-positive) from ciliated (ER-negative, CTH-positive, HNF-negative) cells, and these markers are thought to be involved in cellular determination [13, 23, 32, 37, 38]. Estrogen receptor (ER) was exclusively detected in the nuclei of the secretory epithelial cells, but not in ciliated cells and Müllerian-CICs [39]. ER immunoreactivity was frequently seen in EC, but rarely in CCC [25, 39]. CTH is a cytoplasmic enzyme in the trans-sulfuration pathway that converts cystathione derived from methionine into cysteine. CTH, a marker of ciliated cells, is expressed in CCC, whereas secretory cell marker (ER) is expressed in EC [32]. These data support the hypothesis that secretory cells and ciliated cells are the major sources for EC and CCC, respectively (Figure 1).
Table 1. An immunoprofiling of uterine endometrium/fallopian tube endometrium, cortical inclusion cyst with Müllerian metaplasia, endometriosis, endometrioid carcinoma and clear cell carcinoma using ER, CTH and HNF-1β.

<table>
<thead>
<tr>
<th>Uterine endometrium/fallopian tube endometrium</th>
<th>Cortical inclusion cyst with Müllerian metaplasia</th>
<th>Endometriosis</th>
<th>Endometrioid carcinoma</th>
<th>Clear cell carcinoma</th>
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<tr>
<td>Secretory cells</td>
<td>Ciliated cells</td>
<td></td>
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<tr>
<td>ER</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>CTH</td>
<td>Negative</td>
<td>Positive</td>
<td>NT</td>
<td>Negative</td>
</tr>
<tr>
<td>HNF-1β</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive*</td>
<td>Positive</td>
</tr>
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ER, estrogen receptor; CTH, cystathionine gamma lyase; and HNF-1β, hepatocyte nuclear factor-1β.

*: HNF-1β is negative for ciliated cell metaplasia in endometriosis.

Figure 1. The dichotomy in the histogenesis of EAOC. Secretory cells showed a high level of ER and HNF-1β expression. In contrast, ciliated cells showed a negative expression pattern with these two markers. Immunohistochemical markers distinguished EC (ER+, HNF-1β-) from CCC (ER-, HNF-1β+). Three models are suggested for the etiology of endometriosis: 1) endometriosis arises from the normal endometrium (retrograde menstruation theory), 2) the fallopian tube epithelium (tubal origin theory), or 3) possibly from cortical inclusion cyst (CIC) with Müllerian metaplasia (secondary Müllerian theory) [12-15].

However, other immunohistochemical studies showed the opposite finding [13]. HNF-1β gains further insight into the histogenesis of EAOC [40]. CCC cells showed strong nuclear staining for HNF-1β, but EC was HNF-1β-negative [40]. Atypical endometriosis might be a precursor of EAOC. Atypical endometriosis and adjacent CCC showed their expression, demonstrating that HNF-1β is a
molecular marker for clear cell histogenesis [40]. The late secretory and menstrual endometria are positive for HNF-1β staining, while Müllerian-CICs derived from OSE via metaplasia are negative for HNF-1β [13, 41]. The ectopic epithelia showing eosinophilic or hobnail metaplasia were positive for HNF-1β, whereas those showing ciliated cell metaplasia were negative for HNF-1β [40]. EC and CCC arise from the HNF-1β-negative ciliated cells and HNF-1β-positive secretory cells of endometriosis, respectively [13]. These data suggest that HNF-1β-negative EC may be derived from Müllerian-CICs or ciliated cells in endometriosis, while HNF-1β-positive CCC arises in secretory cells in endometriosis (Figure 1). A ‘two precursor cell’ theory has been plausible, although EAOC has debatable immunohistochemical profiles. The histogenesis of EAOC remains a matter of controversy.

DISCUSSION

This article reviews the recent progress in our understanding of evidence for the genetic background and histogenesis of EAOC.

First, the overlapping genetic alterations and mutational profiles of EC and CCC suggest that they share common genetic background. EC and CCC share remarkable genetic similarities in somatic genetic mutations of ARID1A, PTEN and KRAS genes [9, 26-29]. Despite common genetic abnormalities, EAOC tumors had specific gene signatures related to estrogen signaling and oxidative stress response, respectively.

Second, EC and CCC likely arise from distinct histotypes of endometriosis with different cells of origin although it still lacks experimental proofs [13, 32, 33]. In order to explore the EAOC histogenesis, we focused on an immunoprofiling approach using some molecular markers, including ER and HNF-1β. Eutopic endometrium, fallopian tubal endometrium and ectopic endometrium are composed of secretory cells and ciliated cells [32]. ER, a sensitive secretory cell marker, was detected in EC tumor cells. The CCC tumor cells and ciliated cells showed a concordant ER-negative pattern. This study suggests that EC and CCC may originate from secretory and ciliated cell components, respectively [32, 33]. On the other hand, HNF-1β, a highly sensitive CCC cell marker, was detected in secretory cells, but not ciliated cells, which is indicative of a secretory cell origin of CCC [13]. Interestingly, two immunohistochemical studies showed the opposite finding [13, 32] (Figure 1).

We will discuss the possibility of this debated topic. Women with endometriosis are predisposed to develop EAOC tumors later in life [5-8]. Various epigenetic modifications produce cancer-predisposed cells from somatic cells that possess an epigenetic signature by the influence of aging and an environmental change. Epigenetic alterations, including DNA methylation and histone modification are thought to be involved in the pathogenesis of endometriosis and its malignant transformation [42, 43]. Ito et al. tried to characterize an epigenetic signature or switch in endometriosis and its malignant transformation [44]. The decision to characterize the epigenetic switch of HNF-1β may be triggered by a detrimental change in environmental conditions such as excess oxidative stress. DNA and histone methylations are modulated by the activities of methyltransferases and demethylases. Oxidative stress is involved in the expression of CpG demethylases, ten-eleven translocation (TET) and jumonji (JMJ) [44]. Expression of HNF-1β was inversely correlated with DNA methylation, and HNF-1β overexpression in CCC was associated with DNA hypomethylation [37]. Epigenetic switch (histone modifications and the DNA methylation) can activate HNF-1β signaling cascades. Glucose-6-phosphatase (G6PC) and glycogen synthase kinase-3β (GSK-3β) are putative targets of HNF-1β and controls gluconeogenesis and glycogenolysis, resulting in metabolic homeostasis that favors glycogen accumulation [45, 46]. Thus, acquisition of intracellular glycogen droplets by HNF-1β would induce a phenotype switch from ciliated cells to CCC cells [45, 47]. Depending on epigenetic alterations with aging and environmental damage, HNF-1β promoter hypomethylation may progress to give rise to immortal cells, which generates precursor cells of CCC. In close interaction with the changing environment, endometriotic cells may have an intrinsic high plasticity as they are capable of transforming into EC cells or CCC cells. Although HNF-1β could serve as a surrogate marker for CCC, the expression of a marker does not simply represent cell of origin due to epigenetic plasticity [33]. Taken together, two different cells
in endometriosis, secretory cells and ciliated cells, could be candidate precursors of EAOC. The biggest mystery is how and why the epigenetic switch will occur?

The current review focuses on immunoprofiling markers that can distinguish ciliated from secretory epithelial cells. However, scanning electron microscopy analyses confirmed three subtypes of epithelial cells, including secretory cells, ciliated cells and clear cells [48]. Small deposits or vacuoles of glycogen appear in clear cells of early and mid-secretory endometrium. The nature of the cytoplasmic clarity is at least in part owing to glycogen accumulation through its regulation by G6PC and GSK-3β [45, 46]. Since CCC is characterized by glycogen accumulation, clear cells can be candidate cells-of-origin for CCC [49]. Furthermore, inactivating mutations in ARID1A are relatively common in CCC and probably influence glycogen metabolism via downregulation of the mevalonate pathway [50]. These studies will enable us in the future to determine the origin of CCC.

CONCLUSION
In conclusion, secretory cells and ciliated cells are candidates for cells of origin of EAOC that undergo similar genetic changes. The precursor cells can respond similarly to inflammatory and oxidative stimuli within the pelvis, leading to shared genetic abnormalities. Expression of various histogenesis markers may be epigenetically regulated in distinct EAOC subtypes. Therefore, there is considerable controversy in the literature regarding the cells of origin. Additional studies are required to clarify the histogenesis of EAOC.

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