Abstract. The present review focuses on the current status of molecular pathology in high-grade serous cancer (HGSC) and preneoplastic conditions. This article reviews the English-language literature on HGSC, precursor, fallopian tubal epithelium, secretory cells, ciliated cells, secretory cell expansion, secretory cell outgrowth (SCOUT), p53 signature, serous tubal intraepithelial carcinoma (STIC), DNA damage and immunohistochemistry in an effort to identify the precursor-carcinoma sequence in HGSC. The majority of HGSC originates from the fimbriated end of the fallopian tube secretory epithelial cells, while the small part of this disease may develop from ovarian cortical inclusion cyst (CIC). A series of morphological changes from normal fallopian epithelium to preneoplastic to neoplastic lesions were concomitant with the multistep accumulation of molecular and genetic alterations. Recent studies provide a stepwise progression of fallopian tubal epithelium to precursor lesions to carcinoma, with the aid of a ‘secretory cell-SCE-SCOUT-p53 signature-STIC-HGSC sequence’ model. Immunohistochemical markers, including p53, STMN1, EZH2, CCNE1, Ki67 and γ-H2AX, were gradually increased during the SCOUT-p53 signature-STIC-HGSC sequence. Conversely, PAX2 expression was decreased during the early phase of SCOUT development. Potential genes and proteins are involved in the evolutionary trajectory of the precursor-cancer lineage model. In the present review we examined detailed aspects of the molecular changes involved in malignant transformation from fallopian tube epithelium to HGSC. A precursor condition originating in ‘field cancerization’ may gain a growth advantage, leading to HGSC.

1. Introduction

The lifetime risk of developing ovarian cancer is 16.59% for women with germline BRCA mutations and only 1.4% for women without germline mutations (1). Recent molecular genetics and morphologic characteristics revealed that ovarian cancer is divided into two categories, designated types 1 and 2 (2). Type 1 tumors exemplify the classically held view of a stepwise progression (adenoma-carcinoma sequence), which comprise endometriosis-associated ovarian cancer (AOEC), such as clear cell carcinoma and low-grade endometrioid carcinoma, as well as mucinous carcinoma and low-grade serous carcinoma. According to this model, it is generally accepted that the malignant lesion originates from pre-existing adenomas. Type 1 cancer develops through a particular sequence of somatic mutations or genomic alterations (ARID1A, PIK3CA, PTEN, KRAS, BRAF, CTNNB1, and PPP2R1A), with rare mutations in BRCA1, BRCA2, and TP53 (2). By contrast, type 2 tumors arise from the normal epithelium to precursor lesions and finally to high-grade serous endometrioid carcinoma, malignant mixed mesodermal tumors (carcinosarcomas), and undifferentiated carcinoma.

High-grade serous cancers, including ovarian, tubal and pelvic cancers, are more aggressive and typically present in advanced stages, indicating that the evolutionary trajectory of type 2 cancer progression is rapid. High-grade serous cancer (HGSC) may develop from multiple extra-ovarian origins, including the fimbrial end of the fallopian tube and peritoneum (2). The small part of HGSC may originate from ovarian cortical inclusion cyst (CIC) (2). The majority of this disease may be the result of a stepwise process, from fallopian tubal epithelium to serous tubal intraepithelial carcinoma (STIC) to finally HGSC (1-13). However, we cannot rule out a 'parallel' evolution of synchronous precursor and cancer.

The present review focused on the current status of molecular pathology that initiates HGSC and preneoplastic...
conditions. The current review presents a stepwise model that incorporates both molecular alterations and the histopathology of precursor lesions.

**General.** The present study aimed to summarize the current status of molecular pathology in HGSC and preneoplastic conditions. A PubMed search of the relevant literature published between 2005 and 2017 was performed. The search strategy included the combination of the following key words: high-grade serous cancer (HGSC), precursor, fallopian tubal epithelium, secretory cells, ciliated cells, secretory cell expansion (SCE), secretory cell outgrowth (SCOUT), p53 signature, STIC, DNA damage and immunohistochemistry 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 two of the co-authors (K.I. and E.I.) after agreeing on the selection criteria.

### 2. Histopathology of precursor lesions

We describe all possible origins of preneoplastic cellular alterations to HGSC, and discuss their pros and cons.

**SCE.** The first lesion is SCE. The oviduct comprises glands and a luminal epithelium which is composed of secretory cells and ciliated cells. The number of tubal secretory cells increases with age (4). An increase of secretory cells was observed in high-risk individuals and sporadic serous cancer cases (4). Although SCE may be a sensitive marker for early serous carcinogenesis in patients with coexisting HGSC, SCE is prevalent in both fimbria and ampulla tubal segments in fallopian tubal regions. Furthermore, animal experiments revealed an increase in the number of SCE and a decrease in the number of ciliated cells after hCG administration, indicating that SCE may serve a range of the multiple physiological roles of fallopian epithelial functions (14). Therefore, SCE is not directly linked to a precancerous lesion.

**SCOUT.** The second lesion is SCOUT, which contains a linear stretch of 30 or more fallopian epithelial cells of secretory type (5,13,15). SCOUT was observed in 60-90% of the HGSC group and 20-70% of the normal control group (3,12,15). This lesion was associated with older age and prevalent in both proximal and distal tube, but more common in the fimbrial end (12). The left vs. right location site of SCOUT did not correlate with the location of the primary serous cancer (12). Thus, this lesion may be a surrogate marker of HGSC.

**p53 signature.** The third lesion is p53 signature, which is defined as a linear expansion of >12 of morphologically normal epithelium with p53 overexpression (12). This lesion shares identical p53 mutations and other genomic changes with HGSC, but lacks excessive cell proliferation (16). p53 signature is seen predominantly in continuity with STIC, localizes to the same (fimbria) region as STIC and shares preeplastic properties with HGSC including p53 mutations (5,17). p53 signature is frequently identified in serous cancer (66%) and may promote p53-driven preneoplastic transformation (12). However, controversy exists as to the incidence and clinical significance of p53 signature. This lesion is rare in non-serous cancer (18). Conversely, it can occasionally be found even in non-cancer patients (12).

**STIC.** The last is STIC, which is characterized by the presence of a discrete lesion, single or multiple, and located in the fimbriated end of the tube. STIC lesions display epithelial stratification and mitotic figures, demonstrating atypical histologic changes (13,19). The epithelium of STIC showed strong p53 positivity and harbors clonal TP53 mutations (12). It has been suggested that STIC is found in 11-68% of HGSC patients (12,20,21) as well as up to 60% of sporadic cancer cases and in 0.6-10% of the carriers of patients with hereditary cancer (22). Conversely, the frequency of fallopian tube precursors in benign gynecologic diseases was approximately 20% of SCOUT, 10% of p53 signature and <4% of STIC cases (1,2). STIC was exclusively observed in patients with HGSC (3) and more common in the ipsilateral side of dominant HGSC (12).

This alteration is considered as a precursor or an early event in the oncogenesis of HGSC. Thus, STIC may be a malignant lesion with metastatic potential to ovarian HGSC (21,23). Another possibility is that STIC is regarded as an intraepithelial metastasis from HGSC to the fallopian tube (24).

In the present study, we characterized histopathological alterations recognized currently in the fallopian tube epithelium, including a ‘SCE-SCOUT-p53 signature-STIC-HGSC sequence’ model (1-13). SCE and SCOUT are not considered to be preneoplastic lesions. SCOUT, p53 signatures, and STIC are frequently identified in HGSC. p53 signature is a low-grade preneoplastic condition, whereas STIC is considered as a true high-grade preneoplastic lesion, with a significant risk for HGSC development. HGSC may exhibit a continuous spectrum of a variety of lesions ranging from normal to precursor, premalignant, and finally malignant lesions.

### 3. Molecular pathogenesis

Differences in gene and protein expression between precursors and malignant lesions identify a continuous disease spectrum underlying HGSC. Investigators have characterized the stepwise changes of molecular profiles identified by genomic, proteomic and immunohistochemical approaches, including BCL2 (BCL2 apoptosis regulator), p73 (tumor protein p73), PAX2 (paired box 2), p53, PAX8 (paired box 8), H2AX (H2A histone family member X), STMN1 (stathmin 1), EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit), Ki67, ALDH1A1 (aldehyde dehydrogenase 1 family member A1), CTNNB1 (β-catenin), CCNE1 (cyclin E1), LAMC1 (Lamin γ1), and HMG2A (high mobility group AT-hook 2) genes and telomere shortening (Table I) (3,5,12,13,16,25-37).

**Normal fallopian epithelium.** Non-ciliated cells, such as secretory cells, in normal fallopian tubal epithelium were PAX2-positive, but ciliated cells were PAX2-negative (25). The protein encoded by the PAX2 gene induces epithelial invagination to form a tubular structure in Mullerian duct precursors of the coelomic epithelium. The fimbria is exposed to estrogen and progesterone in a cyclic manner. Estrogen can induce differentiation of fallopian epithelium to a ciliated...
phenotype (26). A member of the PAX family, PAX8, also plays a role in organogenesis of the Müllerian system and is a marker of fallopian tubal secretory cells and ovarian surface epithelium (OSE) (16,27). Other molecular characteristics identified in fallopian tube epithelium are as follows: Fallopian tubal epithelium comprises different phenotypic and molecular subtypes; secretory cells (BCL2+, PAX2+, PAX8+, HMFG2+, p73-, FOXJ1-, and acetylated tubulin-) and ciliated cells (BCL2-, PAX2-, PAX8-, HMFG2-, p73+, FOXJ1+, LhS28+, acetylated tubulin+, and Sall2+) (16,25-27). p73 is a homologue of the p53 and induces cell cycle arrest, conferring its tumor suppressive activity. HMGA2 is associated with the epithelial-mesenchymal process. A monoclonal antibody, LhS28, reacts with basal bodies of ciliated epithelial cells. BCL2 gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death. These specific molecules can function as promising markers to track a stepwise progression of fallopian tube epithelium to precursor lesions to carcinoma.

OSE/CIC. OSE did not exhibit tubal biomarkers, BCL2, p73, FOXJ1 and phospho-Smad2, while PAX8 was expressed by OSE (27). Steroid hormones convert mesothelial-derived OSE to Müllerian-type tissues including CIC (28). CIC is a heterogeneous group and can be divided into two types; ciliated (tubal-type, PAX8+ and p73±) or flat (OSE-type, calretinin+) (28). CIC, but not OSE, had heterogeneous p73 staining with a ciliated phenotype. BCL2 gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death. These specific molecules can function as promising markers to track a stepwise progression of fallopian tube epithelium to precursor lesions to carcinoma.

SCOUT. SCOUT has normal-appearing tubal epithelium without atypia (5). This lesion is identified by BCL2+, STMN1+, EZH2+, p53+, p73+, PAX2+, PAX8+, MIB1+, ALDH1A1+, and γ-H2AX- (5,13,25,31). The majority of SCOUT is typically associated with wild-type p53 expression (do not overexpress p53) and a loss of PAX2 and p73 expression (13,25). SCOUT may be a non-obligate precursor of HGSC. However, a small proportion of SCOUT is identified by p53+ and p73+ in fallopian tubes from women with inherited and sporadic HGSC (5). SCOUT, p53 signature, STIC and HGSC share a positive staining for STMN1 and EZH2 and negative staining for PAX2 (25). P53 signature, STIC and HGSC exhibit a negative staining for ALDH1A1 (31). STMN1 and EZH2 are cancer-associated genes and involved in cell cycle progression and proliferation.

p53 signature. In the p53 signature, abnormal p53 protein accumulation was observed by immunohistochemistry. The p53 signature contains an altered expression of multiple genes and pathways within histologically unremarkable precursor in benign tubal epithelium (13). This lesion was identified by p53+, BCL2+, STMN1+, PAX2- and a low Ki-67 proliferative index (3,5,13). Overexpression of p53 protein (usually associated with mutation) may serve as a useful diagnostic marker in the assessment of HGSC and its precursor lesions, suggesting an essential role for p53 mutation in early serous tumorigenesis.

STIC. STIC exhibits a panel of immunohistochemical markers, TP53+, BCL2+, γ-H2AX+, STMN1+, EZH2+, PAX8+, Laminin γ1+, HMGA2+, MIB1+, Ki67+, CCNE1+ and PAX2- (3,12,25,32-37). The distribution of HMGA2 immunoreactivity overlapped with TP53 mutation-positive STIC (37). The finding of short telomeres and overexpression of CCNE1 in STIC may be the earliest molecular changes in

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+, positive staining; -, negative staining; +/-, weak staining; OSE, ovarian surface epithelium; SCE, secretory cell expansion; SCOUT, secretory cell outgrowth; STIC, serous tubal intraepithelial carcinoma; HGSC, high-grade serous cancer.
chromosomal instability and carcinogenesis (32,35,36). STIC may therefore be an immediate precursor of HGSC.

HGSC. Immunohistochemical studies demonstrated that HGSC exhibited p53+, PAX8+, BCL2+, HMG2A2+, STMN1+, EZH2+, PAX2+ and ALDH1A1- (3,12,25,27,29,31,33,34,37). Since PAX8 is frequently expressed by secretory cells of the normal fallopian tube and OSE, these cells are thought to be the origin of HGSC. ALDH1A1 was expressed in secretory and ciliated tubal epithelial cells and OSE, but was absent in p53 signature, STIC and HGSC (31). ALDH1A1 loss seems to be an early event in HGSC carcinogenesis, suggesting that ALDH1A1 may act as a tumor suppressor.

In this review, we have demonstrated detailed aspects of stepwise deterioration during HGSC progression from precursors to carcinoma. The immunostainings of p53, STMN1, EZH2, BCL2, CCNE1, Ki67 and γ-H2AX were significantly increased in a stepwise manner from SCOUT to p53 signature, STIC, and finally HGSC. Conversely, PAX2 and ALDH1A1 expression was decreased during the early phase of SCOUT development. The pathogenesis of HGSC may be centered on cell cycle deregulation, cell proliferation and anti-apoptosis. The current model of serous carcinogenesis can be stated as a set of two core predictions: HGSC is exemplified through stepwise cancer development with a particular sequence of molecular alterations, including PAX2, ALDH1A1, TP53, STMN1, EZH2 and BCL2; and the evolutionary trajectory of HGSC progression is rapid because secondarily acquired genetic alterations may occur independently.

4. Conclusion

Historically, HGSC was believed to originate from OSE cells that form CIC (2,23,27). CIC did not present before menarche and the number of CIC increased with age (28). Coexisting cell hyperplasia and papillary growth were observed in OSE of ovaries from aged women (30). A greater lifetime number of ovulatory cycles, incessant ovulation, leads to localized OSE injury and inflammation, which increases ovarian cancer risk (2,27,38). A previous study revealed that CIC correlates with low-grade serous and endometrioid tumors, but not HGSC (39). Descriptive evidence failed to show a direct link between morphologic changes and genic alterations in HGSC arising from CIC foci. CIC may originate from implantation of tubal epithelium when the OSE is disrupted at ovulation (28).

The Müllerian-type tubal epithelium results in the formation of CIC by a process of implantation of tubal tissue rather than by a process of metaplasia from OSE with mesothelium-derived lining (40). Thus, more recent evidence supports the idea that HGSC is exemplified through a stepwise progression of fallopian tubal epithelium to precursor lesions to carcinoma, with the aid of a 'secretory cell-SCC-SCOUT-p53 signature-STIC-HGSC sequence' model. Immunohistochemical markers, including p53, STMN1, EZH2, CCNE1, Ki67 and γ-H2AX, were gradually increased during the SCOUT-p53 signature-STIC-HGSC sequence. Conversely, PAX2 expression was decreased during early phase of SCOUT development.

In conclusion, the present review provides a descriptive molecular pathology in a serous carcinogenic sequence model. We summarize the current understanding of temporal and spatial changes of candidate markers in HGSC development.

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