JOURNAL OF GYNECOLOGIC ONCOLOGY

Review Article

Check for updates

Correspondence to
Ikuo Konishi
Department of Obstetrics and Gynecology, National Hospital Organization Kyoto Medical Center, 1-1 Fukakusa Mukaihata-cho, Fushimi-ku, Kyoto 612-8555, Japan.
Email: konishi@kuhp.kyoto-u.ac.jp

ORCID iDs
Ikuo Konishi
https://orcid.org/0000-0002-4284-9569
Kaoru Abiko
https://orcid.org/0000-0001-9648-158X
Takuma Hayashi
https://orcid.org/0000-0002-7525-2048
Koji Yamanoi
https://orcid.org/0000-0002-1240-5422
Ryusuke Murakami
https://orcid.org/0000-0001-5007-5674
Ken Yamaguchi
https://orcid.org/0000-0002-7669-1405
Junzo Hamanishi
https://orcid.org/0000-0002-7750-0623

Peritoneal dissemination of high-grade serous ovarian cancer: pivotal roles of chromosomal instability and epigenetic dynamics

Ikuo Konishi [ID],[1,2,3] Kaoru Abiko [ID],[1] Takuma Hayashi [ID],[2] Koji Yamanoi [ID],[3] Ryusuke Murakami [ID],[3] Ken Yamaguchi [ID],[3] Junzo Hamanishi [ID],[3] Tsukasa Baba [ID],[4] Noriomi Matsumura [ID],[5] Masaki Mandai [ID],[3] Kyoto Study Group for Ovarian Cancer Research

[1]Department of Obstetrics and Gynecology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan
[2]Clinical Research Center, National Hospital Organization Kyoto Medical Center, Kyoto, Japan
[3]Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan
[4]Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, Morioka, Japan
[5]Department of Obstetrics and Gynecology, Kindai University Faculty of Medicine, Osakasayama, Japan

ABSTRACT

Epithelial ovarian cancer remains the lethal gynecological malignancy in women. The representative histotype is high-grade serous carcinoma (HGSC), and most patients with HGSC present at advanced stages with peritoneal dissemination. Since the peritoneal dissemination is the most important factor for poor prognosis of the patients, complete exploration for its molecular mechanisms is mandatory. In this narrative review, being based on the clinical, pathologic, and genomic findings of HGSC, chromosomal instability and epigenetic dynamics have been discussed as the potential drivers for cancer development in the fallopian tube, acquisition of cancer stem cell (CSC)-like properties, and peritoneal metastasis of HGSC. The natural history of carcinogenesis with clonal evolution, and adaptation to microenvironment of peritoneal dissemination of HGSC should be targeted in the novel development of strategies for prevention, early detection, and precision treatment for patients with HGSC.

Keywords: High-Grade Serous Ovarian Cancer; Peritoneal Dissemination; Chromosomal Instability; Epigenetic Dynamics; Cancer Stem Cell-Like Properties; Clonal Evolution

INTRODUCTION

Epithelial ovarian cancer is the leading cause of death from gynecologic cancer in developed countries, and consists of five main histological types such as high-grade serous carcinoma (HGSC), low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma [1]. Among them, HGSC is the most common histotype and often occurs in the sixth and seventh decades (mean age 63 years) [1,2]. Nearly all patients present with tumor of advanced stages, International Federation of Gynecology and Obstetrics (FIGO) Stage III or IV. Early detection of HGSC is clinically difficult, as Horiuchi et al. reported in 2003 the natural history of HGSC development based on the sequential

Tsukasa Baba [iD]
https://orcid.org/0000-0003-0066-3747
Noriomi Matsumura [iD]
https://orcid.org/0000-0002-4512-7975
Masaki Mandai [iD]
https://orcid.org/0000-0003-4428-8029

clinicopathological study using transvaginal ultrasonography (TVUS). There had been no apparent abnormalities in the adnexal regions 2 to 12 months prior to the diagnosis, but laparotomy showed all cases to have advanced stage of tumor [3]. In addition, a large randomized clinical trial in the United Kingdom (UK Collaborative Trial of Ovarian Cancer Screening; UKCTOCS) has demonstrated that screening program by TVUS with or without preceding CA125 does not increase the rate of early stage of ovarian cancer, and not decrease the mortality rate [4]. Regarding the treatment, primary debulking surgery followed by standard chemotherapy with taxane and platinum is effective in more than 80% of patients, who enjoy the complete remission after the first-line treatment. However, most patients will have recurrence and die due to peritoneal carcinomatosis, and 5-year survival rate of optimally debulked patients is approximately 50% [1,2].

Since the most important factor for poor prognosis of HGSC is peritoneal dissemination, novel development of precision treatment targeting the disease process is needed (**Fig. 1**). However, the central player responsible for peritoneal dissemination has not so far been identified in the genomic abnormalities. In 1889, Paget proposed first the "seed and soil" theory, and then, its pathogenesis has been discussed with the interactions among cancer cells, peritoneal mesothelial cells, endothelial cells, immune cells, and cancer-associated fibroblasts (CAFs) under the specific microenvironment [5]. Recently, genetic, epigenetic, and chromosomal analyses on HGSC have been disclosing the extreme genomic instability, as well as the transcriptional and epigenetic adaptation of HGSC cells to various microenvironments. Cancer stem cell (CSC)-like properties have been characterized. In addition, the histogenetic origin of HGSC and its clonal evolution have recently been explored. In this narrative review, being based on those studies, we present our hypothesis that chromosomal instability prepares the CSC-like properties, and epigenetic adaptation facilitates the progression of HGSC.

Our study was approved by the Ethics Committee at National Hospital Organization Kyoto Medical Center (No. 22-018).
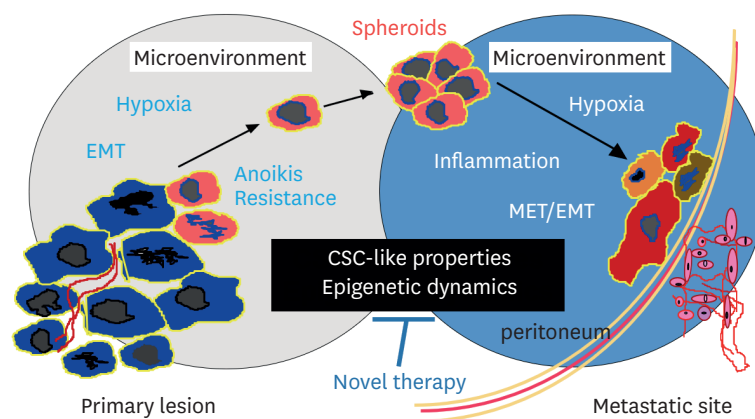
Fig. 1. Peritoneal dissemination of HGSC cells.
Peritoneal dissemination is a unique metastatic process, characterized by the interactions between cancer cells and other cells under the specific microenvironment. HGSC cells acquire CSC-like properties, and metastasize using epigenetic dynamics in the microenvironment. These processes should be targeted by novel therapy (original illustration by Dr. Akiko Horiuchi).
CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; HGSC, high-grade serous carcinoma; MET, mesenchymal-epithelial transition.

Table 1. Approved drugs and possible candidates targeting chromosomal instability, CSC-like properties, and microenvironment of peritoneal dissemination of HGSC

Target	Approved drugs	Candidate drugs
Chromosomal instability		
HRD	PARP inhibitors (olaparib, rucaparib, niraparib)	
CSC-like properties		
Hedgehog signal		Hedgehog inhibitor (sonidegib)
Wnt signal		Wnt inhibitor (ipafricept)
ALDH		ALDH inhibitor (disulfilam)
EpCAM		Anti-EpCAM Ab (catumaxomab)
Microenvironment of peritoneal dissemination		
VEGF	Anti-VEGF Ab (bevacizumab)	VEGFR inhibitors (apatinib, cediranib)
S100A4/RhoA		Statins (simvastatin)
Inflammation		Anti-inflammatory drugs (aspirin, NSAIDs)
		Anti-IL-6R Ab (tocilizumab)
Immune evasion	Immune checkpoint inhibitor (pembrolizumab for MSI-H)	Immune checkpoint inhibitors (nivolumab, pembrolizumab, avelumab)
EMT/Histone deacetylation		HDAC inhibitor (resminostat)
EMT/TGF-β signal		TGF-β inhibitors (vactosertib, ABBV151)

ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; HDAC, histone deacetylase; HGSC, high-grade serous carcinoma; HRD, homologous recombination deficiency; MSI-H, microsatellite instability-high; NSAID, non-steroidal anti-inflammatory drug; PARP, poly-(ADP-ribose) polymerase; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

GENOMIC ALTERATION CHARACTERISTICS OF HGSC

In 2011, The Cancer Genome Atlas (TCGA) Research Network reported a comprehensive genetic and epigenetic changes of HGSC [6]. Nearly all cases harbored mutations in *TP53*, although the frequency of each somatic mutation other than *TP53* is less than 5%, confirming that mutation of driver oncogene is extremely rare in HGSC. Elevated DNA methylation and reduced expression implicated 168 genes as epigenetically silenced in HGSC. Aberrations of the homologous recombination (HR) pathway genes include *BRCA1* germline mutation (8%), *BRCA1* somatic mutation (3%), *BRCA2* germline mutation (6%), *BRCA2* somatic mutation (3%), *BRCA1* promoter methylation (10%), *CDK12* mutations (3%), and other HR-related gene mutations (20%). Accordingly, approximately 50% of HGSC cases are characterized by homologous recombination deficiency (HRD). Currently, HRD is used as the scientific basis for clinical application of poly-(ADP-ribose) polymerase (PARP) inhibitors for patients with HGSC (**Table 1**).

With regard to mRNA expression profile, TCGA report showed the four subtypes of HGSC, i.e., immunoreactive, differentiated, proliferative, and mesenchymal [6]. Subsequent studies reported that patients with the immunoreactive showed the best survival, then the differentiated, followed by the proliferative, and the mesenchymal one the worst [7,8]. Our analysis on the pathological features along with mRNA expression profile found that the differentiated is histologically well-differentiated grade 1 carcinoma (**Fig. S1A**), and the proliferative is poorly differentiated grade 3 carcinoma (**Fig. S1B**). Thus, they represent the conventional histological grades [9]. In contrast, the mesenchymal shows desmoplastic stroma (**Fig. S1C**), and the immunoreactive is characterized by many lymphocytes surrounding the cancer nest (**Fig. S1D**). Therefore, the latter two represent the total gene expressions of cancer including those from the microenvironment [9]. These data demonstrated that prognosis of the patients is influenced not only by tumor grade but also by microenvironment of cancer.

Widespread copy number (CN) alterations have occurred in the chromosomes of HGSC, as TCGA analysis identified 8 regional CN gains and 22 losses [6]. There are no specific patterns in either numerical or structural abnormalities. Recently, however, Macintyre et al. [10] reported that CN aberrations can be classified into the seven CN Signatures, being correlated

with the biological behavior and the outcome of patients. Signature 1 is correlated with RAS/MAPK signaling. Signature 2 frequently shows *CDK12* mutation and poor survival of patients. Signature 3 shows HRD with frequent *BRCA1/2* mutations and better survival of patients. Signature 4 is characterized by high CN states and mutation of *MYC*, *CDK12*, and *CCNE1*. Signature 5 suggests chromothriptic-like events. Signature 6 shows extremely high CN states with amplification of *CCNE1*, *CCND1*, *CDK2*, *CDK4*, or *MYC*. Signature 7 is related with non-BRCA HRD and good patient survival [10]. Thus, amplification of driver oncogene is present in some cases, but is not the ubiquitous phenomenon, suggesting that loss of tumor suppressor genes may be more important in the development of HGSC.

PATHOLOGY AND EPIGENETICS IN PERITONEAL DISSEMINATION OF HGSC

Peritoneal dissemination is a unique metastatic process, which consists of detachment of cancer cells from the primary lesion, floating in the ascitic fluid, attachment to the peritoneal mesothelium, invasion into the submesothelial space, and remodeling of the tumor microenvironment including angiogenesis (**Fig. 1**). After colonization in the metastatic sites, cancer cells show both mesenchymal-epithelial transition (MET) and epithelial-mesenchymal transition (EMT). From the first metastatic lesions, cancer cells metastasize again to other peritoneal or retroperitoneal sites. Thus, the peritoneal dissemination is a dynamic process in the peritoneal cavity, and HGSC cells encounter the different microenvironments according to the above sequential steps of the metastasis. Pathological findings of peritoneal dissemination have been reported mainly in advanced cases at Stage IIIC with high-volume disease [11]. In order to observe each step in detail, as tried similarly in a previous report [12], we examined closely the microscopic slides of patients at earlier cases, stage IIIA or IIIB, in which peritoneal dissemination is just starting or under early construction.

1. Detachment of HGSC cells and hypoxia

In HGSC having peritoneal dissemination, there are many spheroids floating in the ascitic fluid and approaching to the peritoneal mesothelial cells (**Fig. 2A**), indicating that HGSC cells metastasize in the form of spheroids [11-13]. HGSC is histologically characterized by papillary or solid proliferation of cancer cells (**Fig. S1A and B**). HGSC cells at the tip of papillary lesion or in the center of solid growth, being apart from the blood supply, are exposed to hypoxia (**Fig. 1**), as Imai et al. [14] demonstrated that these cells exhibit an increased expression of hypoxia-inducible factor-1α (HIF-1α). Interestingly, HGSC cells under hypoxia simultaneously show the loss of E-cadherin expression [14]. Hypoxia attenuates the expression of E-cadherin via upregulation of the transcription factor SNAIL, along with the increase in cell motility and invasive capacity of HGSC cells, suggesting that EMT program is working (**Fig. 3**) [14]. Since ascitic fluids contain half the soluble oxygen as blood [15], HGSC cells continue to be exposed to hypoxia, but survive with anoikis resistance (**Fig. 1**). Hypoxia stimulates the HIF-1α-responsive signaling of target genes, such as *GLUT-1*, *VEGF*, *EPO*, *CXCR4*, *OCT4*, *SOX2*, *NANOG*, and *KLF*, which are crucial regulating glucose metabolism, angiogenesis, inflammation, migration, and self-renewal of cancer cells [15,16]. In 1997, we first reported that elevated expression of vascular endothelial growth factor (VEGF) is an important prognostic factor, and that high levels of VEGF are also found in both serum and ascites of the patients [17]. Since 2014, molecular-targeted therapy with anti-VEGF antibody has been used as the maintenance treatment for better survival and quality of life of patients with advanced and recurrent ovarian cancer (**Table 1**).
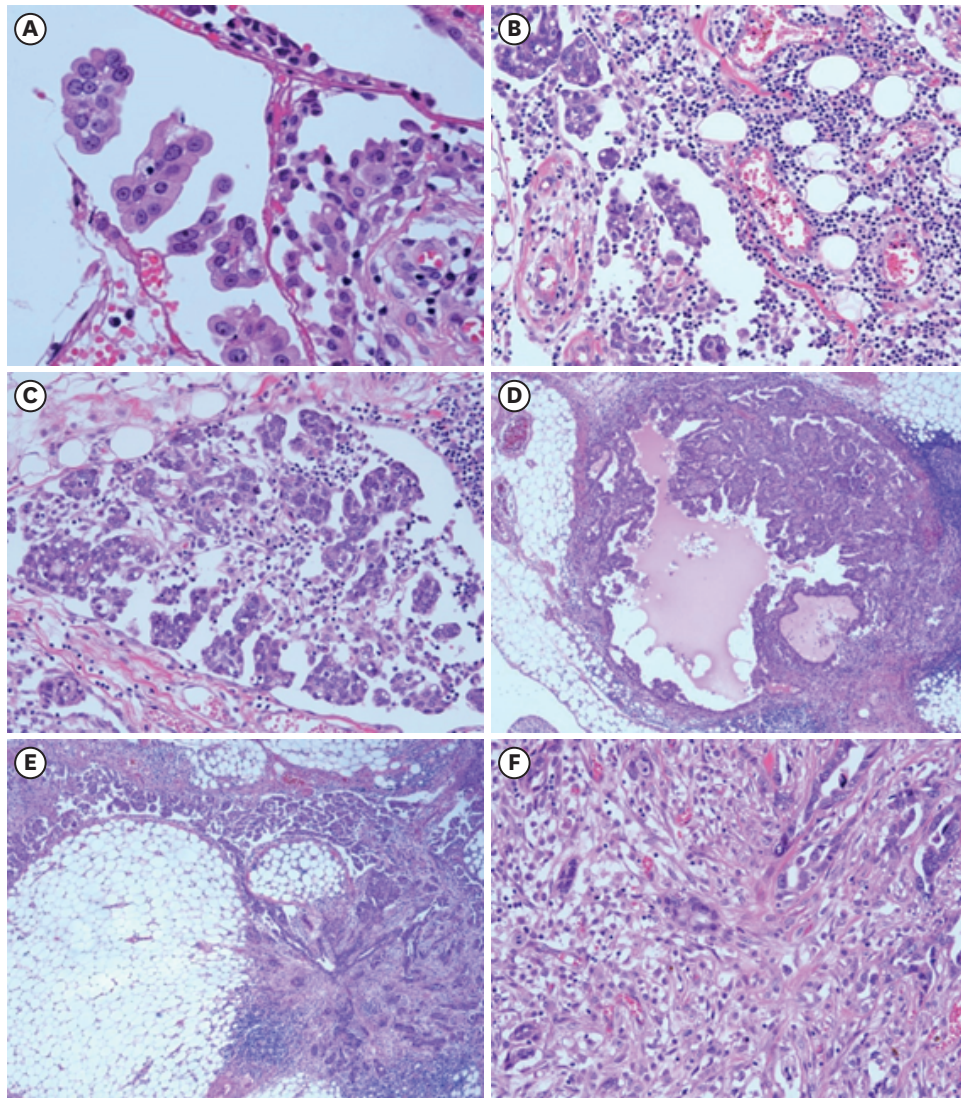
Fig. 2. Phenotypic plasticity of HGSC cells in peritoneal dissemination.
(A) Spheroids of HGSC cells are floating in the ascitic fluid, and are attaching to the mesothelial layer of greater omentum. (B) Then, spheroids of HGSC cells are embedded in the prominent inflammation with angiogenesis mimicking the granulation tissue of wound healing. (C) Colonization and proliferation of HGSC cells start at the disseminated sites, and (D) cancer cells show less-invasive features resembling those in the ovarian tumors. (E) HGSC cells at the metastatic sites show colonization (left), whereas other cells exhibit the invasive features with EMT (right). (F) In EMT lesions, HGSC cells exhibit spindle-like shape, being associated with desmoplastic reaction. HGSC cells are intermingled with CAFs, immune cells, and endothelial cells in the desmoplastic stroma (original magnification: A, ×200; B, C, F, ×100; D, E, ×40).
CAF, cancer-associated fibroblast; EMT, epithelial-mesenchymal transition; HGSC, high-grade serous carcinoma.

S100A4 (metastasin) is a small Ca-binding protein of the S100 family, and is secreted in the form of extracellular vesicles, which stimulates invasiveness and upregulates matrix metalloproteinases (MMPs) [18]. In 2006, Kikuchi et al. [19] first demonstrated that an increased expression of S100A4 is significantly associated with poor prognosis of ovarian cancer patients, and that HGSC cells secrete S100A4 as well as express the receptor for S100A4. Transcription of *S100A4* gene in HGSC cells is upregulated by hypoxic condition, and is associated with EMT and increased invasive capacity in vitro, suggesting its pivotal role as autocrine/paracrine factor for the tumor progression [19]. Interestingly, elevated expression of S100A4 is correlated with hypomethylation of *S100A4* gene in ovarian cancer cases. In addition, chronic hypoxia in vitro is shown to induce the hypomethylation of CpG sites in the
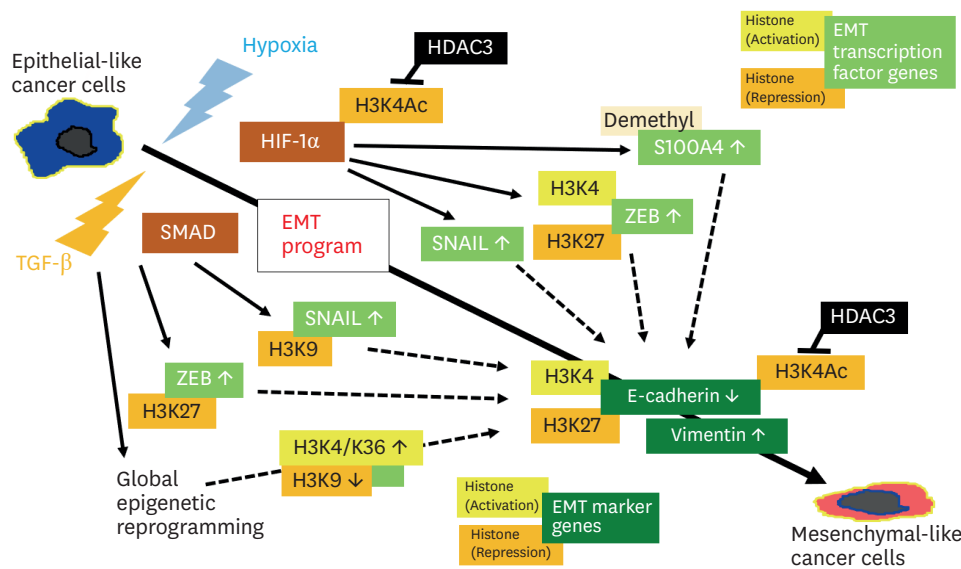
Fig. 3. Epigenetic regulation of EMT program under hypoxia or TGF-β stimulation in cancer.
EMT program proceeds by activation of EMT transcription factors such as SNAIL and ZEB, followed by the change in the expression of EMT marker genes such as E-cadherin and vimentin, mainly via modification of various histones.
EMT, epithelial-mesenchymal transition; TGF-β, transforming growth factor-β.

first intron of *S100A4* gene [20]. Accordingly, the epigenetic mechanism is actually involved in the adaptation to hypoxic microenvironment during progression of HGSC cells (**Fig. 3**). Since high levels of serum *S100A4* transcripts have recently been identified, it could be the useful tumor marker of ovarian cancer [18,21]. Intracellular signaling of S100A4 is transferred via RhoA [22], which mediates the autocrine/paracrine stimulation of not only S100A4 but also insulin-like growth factor (IGF), hepatocyte growth factor (HGF), and lysophosphatidic acid (LPA). Statins have been known to inhibit the signal transduction of RhoA, via inhibition of mevalonate pathway, and is shown to suppress the peritoneal dissemination of ovarian cancer cells in mouse model [23]. Thus, statins have received attention as a promising drug for the suppression of peritoneal dissemination of HGSC (**Table 1**) [24,25].

2. Implantation of HGSC cells and inflammation

The second step of peritoneal dissemination is an approach of HGSC spheroids to the peritoneum (**Figs. 1, 2A**), followed by attachment to the mesothelial cells with arrangement of the microenvironment. Just before cell-to-cell attachment, HGSC cells induce the inflammatory reaction leading to the leakage of fibrinogen, followed by the cell anchorage in the fibrin networks [12]. HGSC cells also produce various molecules such as HGF, transforming growth factor-β (TGF-β), and interleukin-1β (IL-1β), and induce the transformation of normal mesothelial cells into the mesenchymal-like mesothelial cells, which promotes the adhesion of cancer cells on the peritoneum, as well as enhances angiogenesis via VEGF production [26]. Interestingly, we found that the spheroids of HGSC are embedded in the inflammatory tissues resembling the granulation of wound healing (**Fig. 2B**). Malignant ascites have been reported to contain elevated levels of various cytokines, such as tissue factor, tumor necrosis factor-α (TNF-α), IL-6, IL-8, and LPA [11-13,26,27]. Pro-inflammatory prostaglandins may also be important, since the expression of COX-1 and COX-2 is reportedly increased in ovarian cancer [28]. Thus, the inflammatory microenvironment produced by various cytokines and prostaglandins seems to be beneficial for the survival of HGSC cells in the disseminated sites [11-13,15,16,26-28].

Recently accumulating evidence indicates that chronic inflammation plays an important role in the progression of cancer, via inflammation-mediated epigenetic alterations of cancer cells [27,29]. In ovarian cancer, epigenetic modification of histone such as suppression of H2B ubiquitylation has been shown to induce the upregulation of TNF-α and IL-6 along with increased migration of HGSC cells [30]. Inflammation in the peritoneal dissemination might be one of appropriate targets for preventing the metastasis of ovarian cancer, and current or post-diagnosis use of anti-inflammatory drugs like aspirin has been reported to contribute to better survival of patients (**Table 1**) [31]. Immunologic aspects including the evasion of ovarian cancer cells from the host immune mechanism are also very important, especially in the modern era of use of immune checkpoint inhibitors, and have already been reviewed elsewhere [32].

3. Colonization of HGSC cells and EMT

The third step is the colonization of HGSC cells at the disseminated sites after obtaining an appropriate blood supply (**Fig. 2C**). Here, HGSC cells show the pathologically less-invasive features (**Fig. 2D**), and a reverse phenomenon of MET occurs in such lesions, as the expression of E-cadherin has reportedly been increased again at metastatic sites [33]. In most cases, however, HGSC cells re-start the invasion into the stroma, being associated with desmoplastic reaction (**Fig. 2E**), which consists of CAFs, immune cells, endothelial cells, and the extracellular matrix (**Fig. 2F**). HGSC cells exhibit the pathologic finding of EMT, which is characterized by the transformation into spindle-like shape of cancer cell. Such EMT feature is typically observed in the mesenchymal subtype of HGSC (**Fig. S1C**), and also prominent in the huge metastasis to the greater omentum, being clinically called as "omental cake" (**Fig. S2A**) [34]. Our analysis comparing the gene expressions between the ovarian and omental tumors identified the activation of TGF-β pathway in the omental metastasis [35]. This is consistent with that, among a variety of growth factors, TGF-β signaling plays a fundamental role in EMT [36,37].

EMT of cancer cells is known to be regulated by both transcriptional and epigenetic mechanisms (**Fig. 3**) [38,39]. Various factors such as hypoxia and TGF-β trigger the EMT program via the intracellular signaling cascades including HIF-1α and SMAD, followed by activation of EMT transcription factors such as SNAIL and ZEB. Then, these transcription factors act on the promoter of EMT marker genes such as E-cadherin and vimentin [36,39]. Epigenetic regulation of the EMT program has been investigated mainly on the modifications of chromatin-associated histones, such as H3K27, H3K9, and H3K4, in the promoter of both EMT transcription factor genes and marker genes [38]. For example, hypoxia influences the expression of EMT markers via change of acetylation levels of H3K4Ac by activation of histone deacetylase 3 (HDAC3) [39]. In ovarian cancer, expression of HDAC3 has reportedly been increased, and the suppression of HDAC3 in vitro resulted in the elevation of E-cadherin expression along with reduced cell migration of HGSC cells [40]. In the TGF-β-induced EMT, global epigenetic reprogramming occurs via histone modifications including the methylation of different histones such as H3K9, H3K4, and H3K36 [39,41]. In addition, EMT has been known to play an important role in the chemoresistance, and especially, an enhanced resistance to drugs has been shown in the hybrid EMT phenotype consisting of the mixture of epithelial-like cells and mesenchymal-like cells (**Fig. S2B**) [16,36]. In mouse model of peritoneal dissemination of ovarian cancer, treatment with inhibitor of TGF-β signaling is effective for better survivals [35]. Therefore, EMT in the peritoneal dissemination of HGSC cells under hypoxia or TGF-β action could be the target of novel treatment strategies (**Table 1**).

4. Phenotypic plasticity and CSC-like properties

As shown in the pathology of peritoneal dissemination (**Fig. 2A-F**), HGSC cells exhibit the extreme phenotypic diversity according to different microenvironments. Although genetic alterations had previously been considered as the driver for such plasticity [42], accumulating evidences indicate that epigenetic dynamics plays a pivotal role in adaptation to each microenvironment of the peritoneal dissemination [43]. Such phenotypic plasticity appears to represent the pluripotentiality of HGSC having CSC-like properties, and the microenvironments such as hypoxia, inflammation, and desmoplastic stroma are currently considered to function as niche, that is essential for the survival of CSCs [15,16]. Interestingly, there have been no specific genome alterations known for acquiring the CSC-like properties [44], and the possible role of epigenetics has recently been discussed [45,46]. CSCs are characterized by enrichment in the side population (SP) in the flow cytometry analysis [47], and also by the expression of CSC markers such as CD133, ALDH, CD44, CD117, and CD 326 (EpCAM) [15,16]. In ovarian cancer, the presence CSCs was implicated by successful isolation of a single tumorigenic clone derived from the ascites of a patient with HGSC [48], and have received much attention as the main cause of intratumoral heterogeneity leading to the tumor recurrence with chemoresistance [15,16,48-50]. Novel drugs targeting the CSC signaling such as Hedgehog and Wnt have been tried in ovarian cancer patients (**Table 1**).

CELL ORIGIN AND CLONAL EVOLUTION OF HGSC

Historically, the cell origin of epithelial ovarian cancer was proposed by Scully that it is originated in the ovarian surface epithelium (OSE), and growing toward various directions of mullerian metaplasia [51]. Possible OSE origin has been still under re-examination [52]. Since 2007, however, thorough examinations of the fallopian tubes obtained from women with germline *BRCA1/2* pathogenic variants have disclosed the presence of precursor lesion in the tubal fimbria, that is serous tubal intraepithelial carcinoma (STIC) with *TP53* mutation [53,54]. In addition, further studies revealed the epithelial foci of tubal secretory cells having *TP53* mutation and overexpression without cellular atypia. Such foci are designated as p53 signature, and considered to be the earliest event of carcinogenesis. Serous tubal intraepithelial lesion (STIL) or "dormant STIC" (Ki-67 labeling index <10%) is an intermediate lesion between p53 signature and "proliferative STIC" (Ki-67 labeling index ≥10%) [55].

Strikingly, we recently identified that, in a case of incidentally found STIC with micro-metastasis to the ovary, there are many spheroids of HGSC floating in the tubal fluid near or apart from the STIC (**Fig. 4**). There has been another report of spheroids floating in a case of STIC without ovarian or tubal HGSC [56]. These findings suggest that HGSC cells can be shed directly from STIC without the accompanying invasive tumors, and have the capacity of forming spheroids [55-57]. Generally, organization of the spheroids is known as one of the characteristic features of CSC [58], which shows the expression of Yamanaka transcription factors, such as OCT4, SOX2, KLF4, and C-MYC [59]. The spheroids derived from ovarian cancer cell lines also exhibit an increased expression of OCT4, SOX2, NANOG, and C-MYC, along with that of CSC markers such as CD133, ALDH, CD44, and CD117 [13,60,61]. Therefore, it is likely that STIC cells have already acquired the CSC-like properties during the precursor phase of HGSC. For the comprehensive understanding of peritoneal dissemination, it is essential to introduce the concept of CSC in the story on the clonal evolution of HGSC.
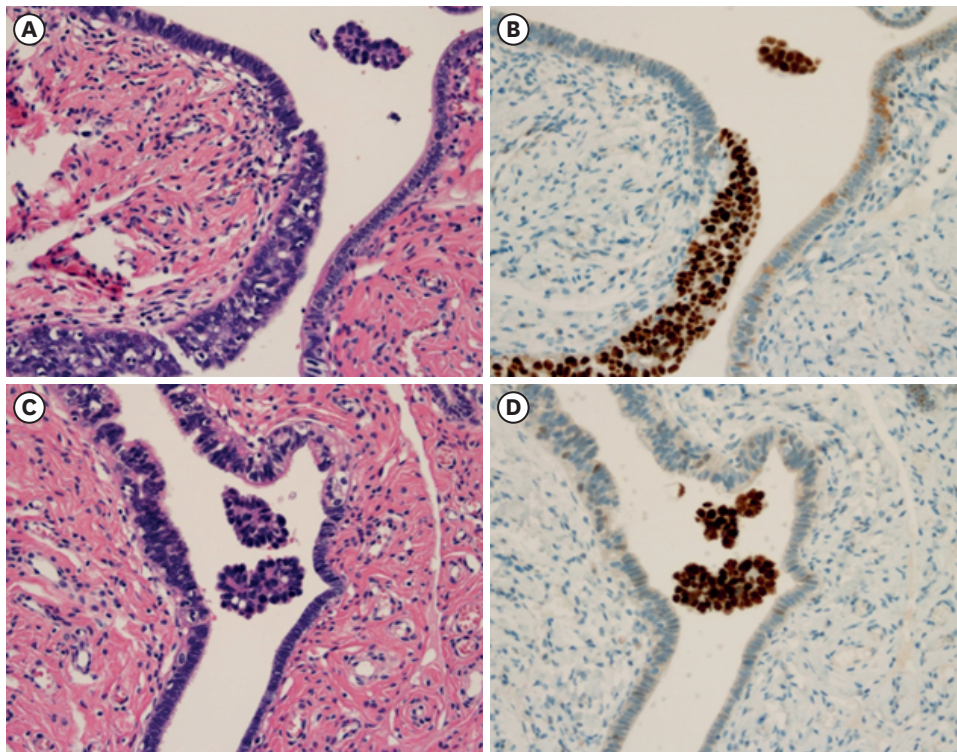
Fig. 4. Spheroids floating in the tubal fluid in the vicinity of STIC.
In a case of incidentally found STIC with micrometastasis to the ovary, HGSC spheroids are identified in the vicinity of STIC, floating in the tubal fluid, as shown in (A) H&E and (B) p53 immunostaining. Spheroids are also floating in the tubal fluid apart from STIC lesion, as shown in (C) H&E and (D) p53 immunostaining (original magnification ×200).
H&E, hematoxylin and eosin; HGSC, high-grade serous carcinoma; STIC, serous tubal intraepithelial carcinoma.

Clonal expansion and genomic evolution from p53 signature to STIC, and STIC to invasive HGSC of ovarian and peritoneal lesions has been demonstrated using whole-genome analyses of the microdissected cells from each lesion [62-64]. In p53 signatures, there are little somatic mutations other than *TP53* mutations. Then, various mutations have accumulated in the phase from "dormant STICs" to "proliferative STICs." With regard to the allelic imbalance, the frequency of LOH is very rare in the phase of p53 signature or "dormant STICs," and varies in the "proliferative STICs" without HGSC. In contrast, the "proliferative STICs" having ovarian or peritoneal HGSC tumors have already acquired various chromosomal aberrations, such as deletions and amplifications [62] or LOH in encompassing the tumor suppressor genes like *TP53*, *BRCA1*, *BRCA2*, and *PTEN* [63]. Interestingly, during or after the progression from STIC to ovarian or peritoneal tumors, additional somatic mutations or chromosomal CN aberrations are little and not ubiquitous [65-69], suggesting that genomic alterations in the later phase do not play an important role in the peritoneal dissemination. Thus, the basic set of genetic and chromosomal alterations necessary for the peritoneal metastasis in each case has already been established during the precursor phase of carcinogenesis (**Fig. 5**).

With regard to the timeline from such tubal precursor lesions to HGSC tumors, p53 signature and "dormant STIC" may take a prolonged time (2 decades or more) to develop into "proliferative STIC", whereas "proliferative STIC" may progress to ovarian carcinoma in a much shorter time (6 years) [64]. Another study also indicated that the average time between STIC and ovarian cancer is 6.5 years (1.4–10.7 years), and the time between the
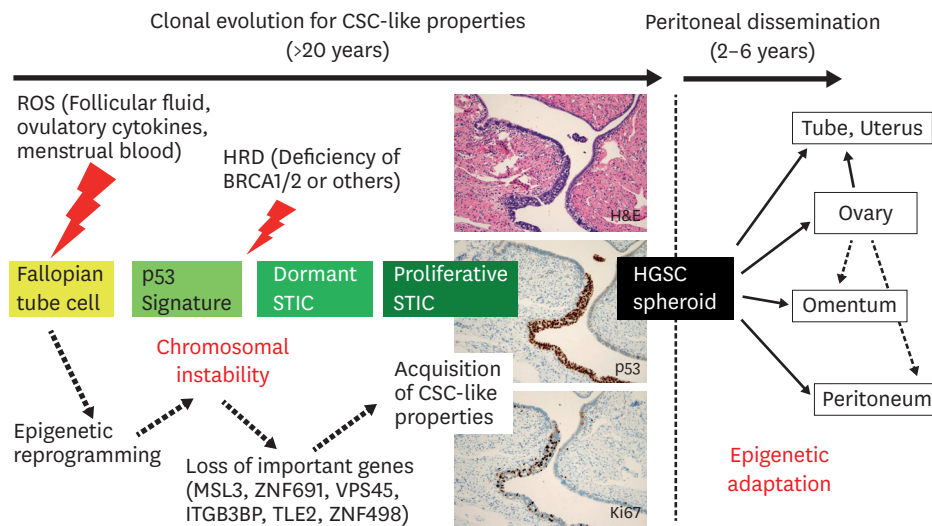
Fig. 5. Hypothetical model for development and progression of HGSC.
Under the influence of ROS from the ovary or menstrual blood, epigenetic reprogramming may occur in the fallopian tube cells. Chromosomal instability, especially loss of important genes, leads to the acquisition of CSC-like properties, during the precursor phase more than 20 years from p53 signature to proliferative STIC. After establishment of metastatic potential, HGSC cells leave the fallopian tube in the form of spheroids, and produce peritoneal disseminations in 2–6 years via epigenetic adaptation to microenvironment.
Histopathological pictures show H&E and immunostainings for p53 and Ki67 in the "proliferative STIC" (original magnification ×100).
CSC, cancer stem cell; H&E, hematoxylin and eosin; HGSC, high-grade serous carcinoma; ROS, reactive oxygen species; STIC, serous tubal intraepithelial carcinoma.

initiation of ovarian cancer and development of other metastases appears to be rather rapid [63]. However, other phylogenic analyses on the clonal evolution of HGSC during the peritoneal dissemination have revealed that, in many cases, HGSC cells metastasize to the peritoneum independently from ovarian carcinomas, indicating the presence of diverse metastatic trajectories [62,65-69]. Thus, peritoneal spreading might occur before or in parallel with ovarian metastases. All of these data indicate that the most important phase of HGSC tumorigenesis is the incubation-like period in the fallopian tube for more than 20 years, during which the precursor cells evolve to HGSC cells having CSC-like properties, and eventually obtained the capability of peritoneal dissemination (**Fig. 5**) [62-64].

CHROMOSOMAL INSTATBILITY AND MACROEVOLUTION OF HGSC

Genomic instability is one of the fundamental features of cancer and contributes to the intratumoral heterogeneity. The events from genomic instability are classified into the two classes, i.e., small range aberrations such as mutation of driver genes and tumor suppressor genes, and large range aberrations due to chromosomal instability leading to the production of aneuploid cells [70]. The latter has been designated as macroevolution of cancer [71], in which various chromosomal changes result in loss of tumor suppressor genes, chimeric fusion of oncogenes, and oncogene amplifications [72,73]. Phenomenon of macroevolution in cancer might be comparable to the creation of new species in animal and plant [74], and large genomic rearrangement is more effective for adaptation to serious change of the environment than single gene mutation [72-74]. In normal condition, aneuploid cells cannot survive due to reduced fitness under the selection pressures, although *TP53* deficiency has been known to permit the survival of aneuploid cells [75]. This is consistent with the timeline of HGSC tumorigenesis in which *TP53* mutation occurs earlier than chromosomal aberrations

[55,56]. Thus, HGSC is a typical example of macroevolution, being characterized by the drastic jump from the intraepithelial cancer cells directly into the aggressive cells creating peritoneal disseminations (**Fig. 5**). Such HGSC tumorigenesis is completely different from that of other solid cancers, showing the stepwise progression from intraepithelial tumor to locally invasive cancer and to aggressive cancer with metastatic potential. This is why early detection of HGSC using the imaging modalities is impossible, and novel development of strategies such as detection of biomarkers produced by HGSC spheroids is mandatory.

A promising hypothesis is that chromosomal instability causing the loss of key genes is responsible for its acquisition of CSC-like properties along with the capability of peritoneal dissemination of HGSC cells. To address this hypothesis, we performed a study of functional genomics screen using a shRNA library targeting 15,000 genes in HGSC cells, which have not yet obtained the capacity of anoikis resistance. A library of 81,000 shRNAs targeting 15,000 genes was transduced into OVCA420 cells, followed by incubation in soft agar and colony selection. shRNAs directed to *ABHD2*, *ELAC2* and *CYB5R3* caused reproducible anoikis resistance [76]. Furthermore, we also identified 6 novel genes, such as *MSL3*, *ZNF691*, *VPS45*, *ITGB3BP*, *TLE2*, and *ZNF498*, the suppression of which markedly increased the proportion of SP cells, exhibiting the capacity for spheroids formation, single cell clonogenicity, in vivo tumorigenicity, and chemoresistance [77]. These findings strongly suggests that the loss of such important genes due to chromosomal instability plays a central role in the acquisition of CSC-like properties of HGSC (**Fig. 5**). Another previous experiment using ovarian cancer cell line also demonstrated that the loss of chromosomal fragments leads to the phenotypic switching to metastasis [78].

Accumulating evidences indicate that chronic inflammation generating reactive oxygen species (ROS) has been implicated in the chromosomal instability [72,74]. In case of HGSC, its histogenetic origin, distal part of fallopian tubes, in women of reproductive age might be exposed continuously to severe stresses, such as high-level ROS from the follicular fluid [79] or from the menstrual blood regurgitating from the uterine cavity [80], and high-levels of cytokines during ovulation [27,81]. Risk of HGSC increases with increasing number of ovulation in the lifetime [82]. This is consistent with that early carcinogenic process of HGSC starts in young women ranging between 35 and 45 years at latest [64]. ROS often causes the DNA damage with double-strand break, and dysfunction of DNA repair pathway due to HRD may accelerate the chromosomal instability (**Fig. 5**) [70]. In addition, it has recently been hypothesized that epigenetic reprogramming precedes to various genetic or chromosomal alterations as the earliest step of carcinogenesis [83-85]. Initial epigenetic events for the reprogramming consist of global CpG hypomethylation and CpG hypermethylation of the specific genes. Such epigenetic alterations have been reported to occur in the normal-appearing fallopian tubes in women especially with *BRCA1/2* pathogenic variants [86], and in the tubal precursors of HGSC [87]. These findings might give us a hint about the novel development of method for the detection of pre-cancerous lesion as well as for the prevention of HGSC in the near future.

CONCLUSIONS

Evidences have been accumulating about the genomic mechanisms in the development and progression of HGSC, and our current hypothetical scheme is shown in **Fig. 5**. Studies on the clonal evolution of the precursor cells in the fallopian tubes to HGSC cells have been

disclosing the natural history of development. Epigenetic reprogramming and chromosomal instability, especially loss of the important genes, might lead to the development of HGSC cells with CSC-like properties via the precursor phase from p53 signature to STIC in the fallopian tube. Then, HGSC cells depart directly from STIC in the form of spheroids, and metastasize to the ovary and peritoneal cavity. During the peritoneal dissemination, addition of genetic mutations and chromosomal aberrations are minimal, whereas the epigenetic dynamics facilitates the phenotypic plasticity of HGSC for adaptation to various microenvironments. Clinical trials for new drugs targeting the peritoneal dissemination of ovarian cancer are in preparation or ongoing [16, 88-93] (**Table 1**). Further studies on the dynamic biology of HGSC cells are needed to develop the epoch-making methods for prevention, early detection, and treatment for HGSC.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIALS

Fig. S1
Histological features of the 4 molecular subtypes of HGSC.

Click here to view

Fig. S2
"Omental cake" in laparoscopy and EMT in pathology.

Click here to view

REFERENCES

1. International Agency for Research on Cancer. Tumours of the ovary. In: Female genital tumours. WHO classification of tumours. 5th ed. Lyon: International Agency for Research on Cancer; 2020. p. 31-167.

2. Seidman JD, Ronnett BM, Shih IM, Cho KR, Kurman RJ. Epithelial tumors of the ovary. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's pathology of the female genital tract. 7th ed. Cham: Springer; 2019. p. 841-966.

3. Horiuchi A, Itoh K, Shimizu M, Nakai I, Yamazaki T, Kimura K, et al. Toward understanding the natural history of ovarian carcinoma development: a clinicopathological approach. Gynecol Oncol 2003;88:309-17.
   PUBMED | CROSSREF

4. Menon U, Gentry-Maharaj A, Burnell M, Singh N, Ryan A, Karpinskyj C, et al. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet 2021;397:2182-93.
   PUBMED | CROSSREF

5. Mikuła-Pietrasik J, Uruski P, Tykarski A, Książek K. The peritoneal "soil" for a cancerous "seed": a comprehensive review of the pathogenesis of intraperitoneal cancer metastases. Cell Mol Life Sci 2018;75:509-25.
   PUBMED | CROSSREF

6. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609-15.
   PUBMED | CROSSREF

7. Verhaak RG, Tamayo P, Yang JY, Hubbard D, Zhang H, Creighton CJ, et al. Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest 2013;123:517-25.
   PUBMED | CROSSREF

8. Konecny GE, Wang C, Hamidi H, Winterhoff B, Kalli KR, Dering J, et al. Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. J Natl Cancer Inst 2014;106:dju249.
   PUBMED | CROSSREF

9. Murakami R, Matsumura N, Mandai M, Yoshihara K, Tanabe H, Nakai H, et al. Establishment of a novel histopathological classification of high-grade serous ovarian carcinoma correlated with prognostically distinct gene expression subtypes. Am J Pathol 2016;186:1103-13.
   PUBMED | CROSSREF

10. Macintyre G, Goranova TE, De Silva D, Ennis D, Piskorz AM, Eldridge M, et al. Copy number signatures and mutational processes in ovarian carcinoma. Nat Genet 2018;50:1262-70.
    PUBMED | CROSSREF

11. van Baal JO, van Noorden CJ, Nieuwland R, Van de Vijver KK, Sturk A, van Driel WJ, et al. Development of peritoneal carcinomatosis in epithelial ovarian cancer: a review. J Histochem Cytochem 2018;66:67-83.
    PUBMED | CROSSREF

12. Matsuoka A, Mizumoto Y, Ono M, Kagami K, Obata T, Terakawa J, et al. Novel strategy of ovarian cancer implantation: pre-invasive growth of fibrin-anchored cells with neovascularization. Cancer Sci 2019;110:2658-66.
    PUBMED | CROSSREF

13. Uno K, Iyoshi S, Yoshihara M, Kitami K, Mogi K, Fujimoto H, et al. Metastatic voyage of ovarian cancer cells in ascites with the assistance of various cellular components. Int J Mol Sci 2022;23:4383.
    PUBMED | CROSSREF

14. Imai T, Horiuchi A, Wang C, Oka K, Ohira S, Nikaido T, et al. Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells. Am J Pathol 2003;163:1437-47.
    PUBMED | CROSSREF

15. Jain S, Annett SL, Morgan MP, Robson T. The cancer stem cell niche in ovarian cancer and its impact on immune surveillance. Int J Mol Sci 2021;22:4091.
    PUBMED | CROSSREF

16. Terraneo N, Jacob F, Dubrovska A, Grünberg J. Novel therapeutic strategies for ovarian cancer stem cells. Front Oncol 2020;10:319.
    PUBMED | CROSSREF

17. Yamamoto S, Konishi I, Mandai M, Kuroda H, Komatsu T, Nanbu K, et al. Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. Br J Cancer 1997;76:1221-7.
    PUBMED | CROSSREF

18. Fei F, Qu J, Zhang M, Li Y, Zhang S. S100A4 in cancer progression and metastasis: a systematic review. Oncotarget 2017;8:73219-39.
    PUBMED | CROSSREF

19. Kikuchi N, Horiuchi A, Osada R, Imai T, Wang C, Chen X, et al. Nuclear expression of S100A4 is associated with aggressive behavior of epithelial ovarian carcinoma: an important autocrine/paracrine factor in tumor progression. Cancer Sci 2006;97:1061-9.
    PUBMED | CROSSREF

20. Horiuchi A, Hayashi T, Kikuchi N, Hayashi A, Fuseya C, Shiozawa T, et al. Hypoxia upregulates ovarian cancer invasiveness via the binding of HIF-1α to a hypoxia-induced, methylation-free hypoxia response element of S100A4 gene. Int J Cancer 2012;131:1755-67.
    PUBMED | CROSSREF

21. Link T, Kuhlmann JD, Kobelt D, Herrmann P, Vassileva YD, Kramer M, et al. Clinical relevance of circulating MACC1 and S100A4 transcripts for ovarian cancer. Mol Oncol 2019;13:1268-79.
    PUBMED | CROSSREF

22. Horiuchi A, Imai T, Wang C, Ohira S, Feng Y, Nikaido T, et al. Up-regulation of small GTPases, RhoA and RhoC, is associated with tumor progression in ovarian carcinoma. Lab Invest 2003;83:861-70.
    PUBMED | CROSSREF

23. Horiuchi A, Kikuchi N, Osada R, Wang C, Hayashi A, Nikaido T, et al. Overexpression of RhoA enhances peritoneal dissemination: RhoA suppression with Lovastatin may be useful for ovarian cancer. Cancer Sci 2008;99:2532-9.
PUBMED | CROSSREF

24. Kobayashi Y, Kashima H, Rahmanto YS, Banno K, Yu Y, Matoba Y, et al. Drug repositioning of mevalonate pathway inhibitors as antitumor agents for ovarian cancer. Oncotarget 2017;8:72147-56.
PUBMED | CROSSREF

25. Feng JL, Dixon-Suen SC, Jordan SJ, Webb PM. Statin use and survival among women with ovarian cancer: an Australian national data-linkage study. Br J Cancer 2021;125:766-71.
PUBMED | CROSSREF

26. Mogi K, Yoshihara M, Iyoshi S, Kitami K, Uno K, Tano S, et al. Ovarian cancer-associated mesothelial cells: transdifferentiation to minions of cancer and orchestrate developing peritoneal dissemination. Cancers (Basel) 2021;13:1352.
PUBMED | CROSSREF

27. Savant SS, Sriramkumar S, O'Hagan HM. The role of inflammation and inflammatory mediators in the development, progression, metastasis, and chemoresistance of epithelial ovarian cancer. Cancers (Basel) 2018;10:251.
PUBMED | CROSSREF

28. Beeghly-Fadiel A, Wilson AJ, Keene S, El Ramahi M, Xu S, Marnett LJ, et al. Differential cyclooxygenase expression levels and survival associations in type I and type II ovarian tumors. J Ovarian Res 2018;11:17.
PUBMED | CROSSREF

29. Vezzani B, Carinci M, Previati M, Giacovazzi S, Della Sala M, Gafà R, et al. Epigenetic regulation: a link between inflammation and carcinogenesis. Cancers (Basel) 2022;14:1221.
PUBMED | CROSSREF

30. Hooda J, Novak M, Salomon MP, Matsuba C, Ramos RI, MacDuffie E, et al. Early loss of histone H2B monoubiquitylation alters chromatin accessibility and activates key immune pathways that facilitate progression of ovarian cancer. Cancer Res 2019;79:760-72.
PUBMED | CROSSREF

31. Merritt MA, Rice MS, Barnard ME, Hankinson SE, Matulonis UA, Poole EM, et al. Pre-diagnosis and post-diagnosis use of common analgesics and ovarian cancer prognosis (NHS/NHSII): a cohort study. Lancet Oncol 2018;19:1107-16.
PUBMED | CROSSREF

32. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Immunogy and immunotherapy in ovarian cancer. In: Katabuchi H, editor. Frontiers in ovarian cancer science. Singapore: Springer; 2017. p. 225-42.

33. Imai T, Horiuchi A, Shiozawa T, Osada R, Kikuchi N, Ohira S, et al. Elevated expression of E-cadherin and alpha-, beta-, and gamma-catenins in metastatic lesions compared with primary epithelial ovarian carcinomas. Hum Pathol 2004;35:1469-76.
PUBMED | CROSSREF

34. Motohara T, Masuda K, Morotti M, Zheng Y, El-Sahhar S, Chong KY, et al. An evolving story of the metastatic voyage of ovarian cancer cells: cellular and molecular orchestration of the adipose-rich metastatic microenvironment. Oncogene 2019;38:2885-98.
PUBMED | CROSSREF

35. Yamamura S, Matsumura N, Mandai M, Huang Z, Oura T, Baba T, et al. The activated transforming growth factor-beta signaling pathway in peritoneal metastases is a potential therapeutic target in ovarian cancer. Int J Cancer 2012;130:20-8.
PUBMED | CROSSREF

36. Loret N, Denys H, Tummers P, Berx G. The role of epithelial-to-mesenchymal plasticity in ovarian cancer progression and therapy resistance. Cancers (Basel) 2019;11:838.
PUBMED | CROSSREF

37. Deshmukh AP, Vasaikar SV, Tomczak K, Tripathi S, den Hollander P, Arslan E, et al. Identification of EMT signaling cross-talk and gene regulatory networks by single-cell RNA sequencing. Proc Natl Acad Sci U S A 2021;118:e2102050118.
PUBMED | CROSSREF

38. Tam WL, Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med 2013;19:1438-49.
PUBMED | CROSSREF

39. Lin YT, Wu KJ. Epigenetic regulation of epithelial-mesenchymal transition: focusing on hypoxia and TGF-β signaling. J Biomed Sci 2020;27:39.
PUBMED | CROSSREF

40. Hayashi A, Horiuchi A, Kikuchi N, Hayashi T, Fuseya C, Suzuki A, et al. Type-specific roles of histone deacetylase (HDAC) overexpression in ovarian carcinoma: HDAC1 enhances cell proliferation and HDAC3 stimulates cell migration with downregulation of E-cadherin. Int J Cancer 2010;127:1332-46.
PUBMED | CROSSREF

41. Cardenas H, Vieth E, Lee J, Segar M, Liu Y, Nephew KP, et al. TGF-β induces global changes in DNA methylation during the epithelial-to-mesenchymal transition in ovarian cancer cells. Epigenetics 2014;9:1461-72.
PUBMED | CROSSREF

42. Hoogstraat M, de Pagter MS, Cirkel GA, van Roosmalen MJ, Harkins TT, Duran K, et al. Genomic and transcriptomic plasticity in treatment-naive ovarian cancer. Genome Res 2014;24:200-11.
PUBMED | CROSSREF

43. Nath A, Cosgrove PA, Mirsafian H, Christie EL, Pflieger L, Copeland B, et al. Evolution of core archetypal phenotypes in progressive high grade serous ovarian cancer. Nat Commun 2021;12:3039.
PUBMED | CROSSREF

44. Mazzoldi EL, Pastò A, Pilotto G, Minuzzo S, Piga I, Palumbo P, et al. Comparison of the genomic profile of cancer stem cells and their non-stem counterpart: the case of ovarian cancer. J Clin Med 2020;9:368.
PUBMED | CROSSREF

45. Ciriello G, Magnani L. The many faces of cancer evolution. iScience 2021;24:102403.
PUBMED | CROSSREF

46. Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, et al. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene 2009;28:209-18.
PUBMED | CROSSREF

47. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci U S A 2006;103:11154-9.
PUBMED | CROSSREF

48. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res 2008;68:4311-20.
PUBMED | CROSSREF

49. Tao Y, Li H, Huang R, Mo D, Zeng T, Fang M, et al. Clinicopathological and prognostic significance of cancer stem cell markers in ovarian cancer patients: evidence from 52 studies. Cell Physiol Biochem 2018;46:1716-26.
PUBMED | CROSSREF

50. Motohara T, Katabuchi H. Ovarian cancer stemness: biological and clinical implications for metastasis and chemotherapy resistance. Cancers (Basel) 2019;11:907.
PUBMED | CROSSREF

51. Scully RE, Young RH, Clement PB. Surface epithelial-stromal tumors and serous tumors. In: Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. Washington, D.C.: Armed Forces Institute of Pathology; 1996. p. 51-79.

52. Zhang S, Dolgalev I, Zhang T, Ran H, Levine DA, Neel BG. Both fallopian tube and ovarian surface epithelium are cells-of-origin for high-grade serous ovarian carcinoma. Nat Commun 2019;10:5367.
PUBMED | CROSSREF

53. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. Am J Surg Pathol 2007;31:161-9.
PUBMED | CROSSREF

54. Kurman RJ, Shih IM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol 2010;34:433-43.
PUBMED | CROSSREF

55. Shih IM, Wang Y, Wang TL. The origin of ovarian cancer species and precancerous landscape. Am J Pathol 2021;191:26-39.
PUBMED | CROSSREF

56. Bijron JG, Seldenrijk CA, Zweemer RP, Lange JG, Verheijen RH, van Diest PJ. Fallopian tube intraluminal tumor spread from noninvasive precursor lesions: a novel metastatic route in early pelvic carcinogenesis. Am J Surg Pathol 2013;37:1123-30.
PUBMED | CROSSREF

57. Mei J, Tian H, Huang HS, Hsu CF, Liou Y, Wu N, et al. Cellular models of development of ovarian high-grade serous carcinoma: a review of cell of origin and mechanisms of carcinogenesis. Cell Prolif 2021;54:e13029.
PUBMED | CROSSREF

58. Ishiguro T, Ohata H, Sato A, Yamawaki K, Enomoto T, Okamoto K. Tumor-derived spheroids: relevance to cancer stem cells and clinical applications. Cancer Sci 2017;108:283-9.
    PUBMED | **CROSSREF**

59. Gheytanchi E, Naseri M, Karimi-Busheri F, Atyabi F, Mirsharif ES, Bozorgmehr M, et al. Morphological and molecular characteristics of spheroid formation in HT-29 and Caco-2 colorectal cancer cell lines. Cancer Cell Int 2021;21:204.
    PUBMED | **CROSSREF**

60. Liao J, Qian F, Tchabo N, Mhawech-Fauceglia P, Beck A, Qian Z, et al. Ovarian cancer spheroid cells with stem cell-like properties contribute to tumor generation, metastasis and chemotherapy resistance through hypoxia-resistant metabolism. PLoS One 2014;9:e84941.
    PUBMED | **CROSSREF**

61. Robinson M, Gilbert SF, Waters JA, Lujano-Olazaba O, Lara J, Alexander LJ, et al. Characterization of SOX2, OCT4 and NANOG in ovarian cancer tumor-initiating cells. Cancers (Basel) 2021;13:262.
    PUBMED | **CROSSREF**

62. Eckert MA, Pan S, Hernandez KM, Loth RM, Andrade J, Volchenboum SL, et al. Genomics of ovarian cancer progression reveals diverse metastatic trajectories including intraepithelial metastasis to the fallopian tube. Cancer Discov 2016;6:1342-51.
    PUBMED | **CROSSREF**

63. Labidi-Galy SI, Papp E, Hallberg D, Niknafs N, Adleff V, Noe M, et al. High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun 2017;8:1093.
    PUBMED | **CROSSREF**

64. Wu RC, Wang P, Lin SF, Zhang M, Song Q, Chu T, et al. Genomic landscape and evolutionary trajectories of ovarian cancer precursor lesions. J Pathol 2019;248:41-50.
    PUBMED | **CROSSREF**

65. Bashashati A, Ha G, Tone A, Ding J, Prentice LM, Roth A, et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol 2013;231:21-34.
    PUBMED | **CROSSREF**

66. Schwarz RF, Ng CK, Cooke SL, Newman S, Temple J, Piskorz AM, et al. Spatial and temporal heterogeneity in high-grade serous ovarian cancer: a phylogenetic analysis. PLoS Med 2015;12:e1001789.
    PUBMED | **CROSSREF**

67. Lee JY, Yoon JK, Kim B, Kim S, Kim MA, Lim H, et al. Tumor evolution and intratumor heterogeneity of an epithelial ovarian cancer investigated using next-generation sequencing. BMC Cancer 2015;15:85.
    PUBMED | **CROSSREF**

68. McPherson A, Roth A, Laks E, Masud T, Bashashati A, Zhang AW, et al. Divergent modes of clonal spread and intraperitoneal mixing in high-grade serous ovarian cancer. Nat Genet 2016;48:758-67.
    PUBMED | **CROSSREF**

69. Chien J, Neums L, Powell AF, Torres M, Kalli KR, Multinu F, et al. Genetic evidence for early peritoneal spreading in pelvic high-grade serous cancer. Front Oncol 2018;8:58.
    PUBMED | **CROSSREF**

70. Zhou J, Zhou XA, Zhang N, Wang J. Evolving insights: how DNA repair pathways impact cancer evolution. Cancer Biol Med 2020;17:805-27.
    PUBMED | **CROSSREF**

71. Vendramin R, Litchfield K, Swanton C. Cancer evolution: Darwin and beyond. EMBO J 2021;40:e108389.
    PUBMED | **CROSSREF**

72. Tijhuis AE, Johnson SC, McClelland SE. The emerging links between chromosomal instability (CIN), metastasis, inflammation and tumour immunity. Mol Cytogenet 2019;12:17.
    PUBMED | **CROSSREF**

73. Bach DH, Zhang W, Sood AK. Chromosomal instability in tumor initiation and development. Cancer Res 2019;79:3995-4002.
    PUBMED | **CROSSREF**

74. Ye CJ, Sharpe Z, Heng HH. Origins and consequences of chromosomal instability: From cellular adaptation to genome chaos-mediated system survival. Genes (Basel) 2020;11:1162.
    PUBMED | **CROSSREF**

75. Thompson SL, Compton DA. Proliferation of aneuploid human cells is limited by a p53-dependent mechanism. J Cell Biol 2010;188:369-81.
    PUBMED | **CROSSREF**

76. Yamanoi K, Matsumura N, Murphy SK, Baba T, Abiko K, Hamanishi J, et al. Suppression of *ABHD2*, identified through a functional genomics screen, causes anoikis resistance, chemoresistance and poor prognosis in ovarian cancer. Oncotarget 2016;7:47620-36.
    PUBMED | **CROSSREF**

77. Yamanoi K, Baba T, Abiko K, Hamanishi J, Yamaguchi K, Murakami R, et al. Acquisition of a side population fraction augments malignant phenotype in ovarian cancer. Sci Rep 2019;9:14215.
PUBMED | CROSSREF

78. Gao C, Su Y, Koeman J, Haak E, Dykema K, Essenberg C, et al. Chromosome instability drives phenotypic switching to metastasis. Proc Natl Acad Sci U S A 2016;113:14793-8.
PUBMED | CROSSREF

79. Bahar-Shany K, Brand H, Sapoznik S, Jacob-Hirsch J, Yung Y, Korach J, et al. Exposure of fallopian tube epithelium to follicular fluid mimics carcinogenic changes in precursor lesions of serous papillary carcinoma. Gynecol Oncol 2014;132:322-7.
PUBMED | CROSSREF

80. Mandai M, Yamaguchi K, Matsumura N, Baba T, Konishi I. Ovarian cancer in endometriosis: molecular biology, pathology, and clinical management. Int J Clin Oncol 2009;14:383-91.
PUBMED | CROSSREF

81. Sapoznik S, Bahar-Shany K, Brand H, Pinto Y, Gabay O, Glick-Saar E, et al. Activation-induced cytidine deaminase links ovulation-induced inflammation and serous carcinogenesis. Neoplasia 2016;18:90-9.
PUBMED | CROSSREF

82. Trabert B, Tworoger SS, O'Brien KM, Townsend MK, Fortner RT, Iversen ES, et al. The risk of ovarian cancer increases with an increase in the lifetime number of ovulatory cycles: an analysis from the Ovarian Cancer Cohort Consortium (OC3). Cancer Res 2020;80:1210-8.
PUBMED | CROSSREF

83. Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harb Perspect Biol 2016;8:a019505.
PUBMED | CROSSREF

84. Suvà ML, Riggi N, Bernstein BE. Epigenetic reprogramming in cancer. Science 2013;339:1567-70.
PUBMED | CROSSREF

85. Matei D, Nephew KP. Epigenetic attire in ovarian cancer: the emperor's new clothes. Cancer Res 2020;80:3775-85.
PUBMED | CROSSREF

86. Bartlett TE, Chindera K, McDermott J, Breeze CE, Cooke WR, Jones A, et al. Epigenetic reprogramming of fallopian tube fimbriae in BRCA mutation carriers defines early ovarian cancer evolution. Nat Commun 2016;7:11620.
PUBMED | CROSSREF

87. Pisanic TR 2nd, Asaka S, Lin SF, Yen TT, Sun H, Bahadirli-Talbott A, et al. Long interspersed nuclear element 1 retrotransposons become deregulated during the development of ovarian cancer precursor lesions. Am J Pathol 2019;189:513-20.
PUBMED | CROSSREF

88. Yang Y, Yang Y, Yang J, Zhao X, Wei X. Tumor microenvironment in ovarian cancer: function and therapeutic strategy. Front Cell Dev Biol 2020;8:758.
PUBMED | CROSSREF

89. Arildsen NS, Hedenfalk I. Simvastatin is a potential candidate drug in ovarian clear cell carcinomas. Oncotarget 2020;11:3660-74.
PUBMED | CROSSREF

90. Luo X, Xu J, Yu J, Yi P. Shaping immune responses in the tumor microenvironment of ovarian cancer. Front Immunol 2021;12:692360.
PUBMED | CROSSREF

91. Caminear MW, Harrington BS, Kamdar RD, Kruhlak MJ, Annunziata CM. Disulfiram transcends ALDH inhibitory activity when targeting ovarian cancer tumor-initiating cells. Front Oncol 2022;12:762820.
PUBMED | CROSSREF

92. Jin C, Yuan M, Bu H, Jin C. Antiangiogenic strategies in epithelial ovarian cancer: mechanism, resistance, and combination therapy. J Oncol 2022;2022:4880355.
PUBMED | CROSSREF

93. Wang T, Tang J, Yang H, Yin R, Zhang J, Zhou Q, et al. Effect of apatinib plus pegylated liposomal doxorubicin vs pegylated liposomal doxorubicin alone on platinum-resistant recurrent ovarian cancer: the APPROVE randomized clinical trial. JAMA Oncol 2022;8:1169-76.
PUBMED | CROSSREF