Ovarian Cancer: A Heterogeneous Disease

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Abstract

Ovarian cancer encompasses a collection of neoplasms with distinct clinicopathological and molecular features and prognosis. Despite there being a variety of ovarian cancer subtypes, these are treated as a single disease. Tremendous efforts have been made to characterize these subtypes and identify tumoral pathways and potential biomarkers for therapeutic strategies. As in other cancer types, tumor heterogeneity appears to be very high across subtypes and within a single tumor, representing a major cause of treatment failure. We describe the morphological and molecular heterogeneity among ovarian cancers and discuss recent advances in our understanding of intratumor heterogeneity.

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Introduction

Globally, ovarian cancer affects 239,000 patients and causes 152,000 deaths every year [1]. It remains the leading cause of death among gynecological cancers in most

developed countries. The World Health Organization (WHO) classifies epithelial ovarian carcinomas (EOC) into several morphological categories according to cell type: serous carcinomas (SC), mucinous carcinomas (MC), endometrioid carcinomas (EC), and clear-cell carcinomas (CCC), transitional-cell Brenner tumors, mixed, and undifferentiated type [2]. These subtypes are distinct in etiology, morphology, molecular biology and prognosis, but are treated as a single entity. The standard treatment is cytoreductive surgery and platinum/taxane combination chemotherapy. The response rate to first-line therapy is around 80–90%, but most patients relapse and develop chemotherapy resistance and the 5-year survival rate is <35% [3, 4]. Heterogeneity represents a key feature of these tumors, explaining, in part, the lack of successful treatment. With the development of molecular tools such as deep sequencing, along with RNA sequencing, epigenomics, and proteomics, we are gaining further insight into the complexity of heterogeneity within these subtypes and within individual patient tumors.

A dualistic model was proposed to classify EOC into 2 groups, type I and type II, that progress along 2 different tumorigenic pathways [5]. Along with molecular data, clinicopathological features were integrated. Type I EOC include low-grade (LG)SC, low-grade EC, MC,

Table 1. Features of the 5 major subtypes of ovarian carcinoma

	Low-grade SC	High-grade SC	MC	EC	CCC
Frequency	<5%	70%	2–3%	10%	5–10%
Immunophenotype	CK7+, WT1+, ER+	CK7+, CK20, PAX8+, WT1+	CK7+, CK20–, ER–, PR–, WT1–	CK7+, PAX8+, CK20–, WT1–	napsin A+, WT1–, p53–, ER–
Precursor lesion	low-grade malignant potential lesions	STIC	borderline mucinous lesions	endometriosis	endometriosis
Molecular abnormalities	<i>KRAS</i> , <i>BRAF</i> [22, 23]	<i>TP53</i> , <i>BRCA1/2</i> [16, 17]	<i>KRAS</i> , <i>HER2</i> [5, 33–36]	<i>ARID1A</i> , <i>PTEN</i> [38]	<i>ARID1A</i> , <i>PIK3CA</i> [39, 51, 52]
Prognosis	intermediate	poor	good	favorable	intermediate

SC, serous carcinoma; MC, mucinous carcinoma; EC, endometrioid carcinoma; CCC, clear-cell carcinoma; STIC, serous tubal intraepithelial carcinoma; ER, estrogen receptor; PR, progesterone receptor.

and CCC, and are considered to be of a low grade (except for CCC) and well-differentiated tumors. They exhibit somatic mutations of *KRAS*, *BRAF*, or *ERBB2* genes, lack *TP53* mutations and are thought to arise in a stepwise process from neoplastic/borderline lesions. They are relatively indolent and are diagnosed at an early stage. Type II EOC include high-grade (HG)SC, undifferentiated carcinoma, and carcinosarcoma. These tumors show frequent/recurrent mutations in specific oncogenes including a pathognomonic mutation of *TP53*. Serous tubal intraepithelial carcinomas (STIC) may represent the precursor lesion [6]. They are diagnosed at an advanced stage, have aggressive behavior, and disseminate rapidly.

A classification system is required to improve the clinical management and therefore survival rate of patients. However, this 2-tier system appears to be oversimplified, since tumors are lumped together that are not morphologically or genetically related. Even on a clinical level, it is important to note that type I tumors diagnosed at a late stage have a poor prognosis like type II tumors [7]. MC and CCC are type I tumors, but have been associated with poorer outcomes than the other subtypes within this group. Bamias et al. [8] evaluated the prognostic significance of this classification by comparing the median overall survival in 568 patients with ovarian cancer of both types. They reported a high prognostic heterogeneity in the type I but not in the type II tumors. The overall survival did not differ significantly in the 2 types, as demonstrated in other studies [9].

In this study, without using this dual point of view, we will explore the phenotypical and molecular heterogeneity of EOC by describing the specificities of the main subtypes. We discuss recent advances in our understanding

of the mechanisms underlying intratumor heterogeneity (ITH), a major cause of treatment failure and drug resistance in patients with these tumors.

The Wide Variety of EOCs

Some of the main features of the 5 histotypes are listed in Table 1. Morphological characteristics of the subtypes are depicted in Figure 1.

Serous Carcinomas

SC account for 75–80% of epithelial carcinomas and are subdivided into HGSC and LGSC. Histologically, HGSC and LGSC show some phenotypical similarities but develop from distinct molecular pathways [2, 10].

High-Grade SC

HGSC comprise 85–90% of SC. They usually occur in elderly patients. They are detected at an advanced stage and are responsible for the majority of cancer deaths. Morphologically, HGSC are composed of ciliated, columnar cells that form papillae, solid masses, or slit-like spaces with high-grade nuclear atypia and >12 mitoses/10 HPF. Immunohistochemistry is positive for CK7, PAX8, WT1, but negative for CK20; p53 is generally mutated, resulting in overexpression, or, in the case of a null mutation, can be completely negative. Surprisingly, large-scale genomic analysis of HGSC has uncovered only a few recurrently mutated genes, such as *TP53* and *BRCA1/BRCA2*, involved in at least 96 and 22% of the cases, respectively. Six other genes were statistically re-

Fig. 1. Histological slides of the main subtypes of epithelial ovarian cancer. HE-saffron. **a** High-grade serous carcinoma. $\times 10$. **b** Low-grade serous carcinoma. $\times 20$. **c** Clear-cell carcinoma. $\times 10$. **d** Mucinous carcinoma. $\times 20$. **e** Endometrioid carcinoma. $\times 20$.

current but with a low frequency, $<10\%$ [10]. Homologous recombinations are defective in around 50% of HGSC [12]. HGSC show a relatively high number of somatic copy number variations and structural variations with >100 recurrent amplifications and deletions identified [13–15]. Distinct clinical features and several morphological patterns of HGSC have been described and linked to specific genomic mutations, such as *BRCA1*/

BRCA2, which confer a better prognosis, but this needs to be validated [16, 17].

By applying gene expression profiling on 285 serous and endometrioid tumors of the ovary, Tothill et al. [18] identified 6 subtypes and showed that LGSC and HGSC clustered differently. The subtypes were correlated with distinct biology and prognosis. Four of them represented higher-grade cancers and showed the contribution of

both tumor and host-tissue cells. One subtype harbored a mesenchymal expression pattern and another showed a high expression of genes related to immune response. Their paper emphasized the relevance of the microenvironment. However, they used only 1 sample per tumor in each patient, which may not have been representative of the whole tumor. The prognostic significance of intraepithelial tumor-infiltrating T cells has been studied in ovarian cancers. Zhang et al. [19] identified intratumoral (later named intraepithelial) CD3+ T cell infiltration to be an independent prognostic factor in 186 advanced ovarian cancers. Clarke et al. [20] showed an association between intraepithelial T cells, serous ovarian cancer histology, and *BRCA* mutation status; they confirmed that intraepithelial CD8+ tumor-infiltrating lymphocytes (TIL) correlate with disease-specific survival. Interestingly, Emerson et al. [21] sequenced the immune repertoire of multiple tumor biopsies from each patient as well as from the peripheral blood, and showed that the immune response within ovarian carcinoma tumors is spatially homogeneous. Therefore, 1 biopsy might indeed be sufficient to investigate the TIL population within an entire tumor.

Low-Grade SC

In contrast to HGSC, LGSC are infrequent, representing 10–15% of SC and 2% of all ovarian carcinomas. They are diagnosed at a younger median age. They are slow-growing tumors with a 10-year survival rate of 50% [22]. Morphologically, they resemble HGSC but with no atypia and <12 mitoses/10 HPF. The immunohistochemical profile is similar to HGSC (e.g., positive for CK7, WT1, and estrogen receptor [ER]) but contrary to HGSC, LGSC never demonstrate aberrant p53 expression. On a molecular level, LGSC are genomically stable and harbor somatic mutations in *KRAS* and *BRAF* in approximately half of cases; these mutations are mutually exclusive [22, 23]. Although patients with LGSC may present with low-grade tumor recurrence, some cases of recurrence like HSGC or concomitant HGSC and LGSC have also been reported [24]. A thorough examination of the tumor is recommended in order to screen areas for HGSC.

Precursor Lesions

Traditionally, it was thought that SC arise from the surface epithelium of the ovary, and that HGSC and LGSC are grades of the same spectrum, but an increasing number of studies suggest that they do not actually arise from the same precursor lesions. LGSC seems to develop

according to the following sequence: serous cystadenoma/adenofibroma, typical serous borderline tumors and noninvasive micropapillary SC, and, finally, invasive LGSC [25].

An HGSC precursor has been identified in the fallopian tube, especially in fimbriated-end STIC, but this is still a subject of debate [25]. The hypothesis is that tumor cells from STIC exfoliate from the fimbriae, and then implant and develop onto the ovary [26]. Some studies showed that STIC were observed in *BRCA* carriers, but they have also been identified in the fallopian tubes of 70% of patients with sporadic ovarian and serous peritoneal cancer [27, 28]. Importantly, these STIC lesions are not found in other subtypes such as MC or EC, suggesting that they are specific to SC.

These findings are “primordial” to have a better understanding of the biology of the disease and to help map out its heterogeneity.

Mucinous Carcinomas

MC, previously called intestinal-type carcinomas, are rare, accounting for only 2–3% of ovarian carcinomas. Patients are usually diagnosed at an early stage with an excellent prognosis after surgery. However, recurrent or metastatic MC have a poor prognosis [29, 30]. They are unilateral, large, multicystic tumors, filled with mucus and frequently containing solid areas. Morphologically, they are composed of cysts and glands of varying size with a confluent pattern and back-to-back glands. The cells are tall, columnar, and stratified, with a large cytoplasm containing mucin. Different patterns have been described: the expansile type is composed of confluent glands and a papillary pattern and has a very good prognosis whereas the infiltrative type has a destructive invasion pattern with desmoplastic stroma reaction and a higher risk of extraovarian spread [29, 31, 32]. On immunohistochemistry, MC show CK7 and CK20 positivity, but are usually negative for hormone receptors (progesterone receptor and ER) and WT1. PAX8 is positive in less than half of the cases. MC seem to arise from borderline mucinous neoplasms, and harbor a heterogeneous pattern with coexisting mucinous, benign, borderline, and adenocarcinoma areas. Diagnosis therefore implies exhaustive sampling. The most common molecular alterations are *KRAS* mutations, occurring in 75% of primary MC; these have been identified in benign and in borderline areas as well as in adjacent carcinomas, supporting the sequential tumorigenesis process going from

a low-grade malignant mucinous lesion to an MC [5, 33–36]. *HER2* amplification is also found in around 20% of MC. These genomic abnormalities are basically mutually exclusive [33].

Endometrioid Carcinomas

EC account for 10% of all ovarian carcinomas and are usually unilateral solid masses. These tumors present at a low grade and are associated with a good prognosis. Histologically, they are composed of glands resembling endometrial epithelium. They mostly exhibit a glandular architecture with squamous differentiation, but solid areas can be seen. The immunohistochemistry profile shows positivity for CK7, PAX8, and hormone receptors, and negativity for WT1 and CK20; p53 can be overexpressed in high-grade EC. There are 3 grades according to the FIGO (Federation of Gynecology and Obstetrics) system, based on the presence of solid areas and nuclear atypia.

This subtype is the most frequently seen in patients with Lynch syndrome. Sporadic cases showing microsatellite instability have been reported [37]. EC harbor some somatic mutations of *CTNNB1*, *PI3KCA*, *PPP2R1A*, *PTEN*, and *ARID1A* [38]. Adjacent endometriosis is often reported and shared *PTEN* and *ARID1A* mutations have also been identified in adjacent endometriosis cysts, suggesting that endometriosis might be a precursor of EC [39].

Clear-Cell Carcinomas

The WHO defined CCC as a new subtype of EOC in 1973 [40]. CCC are rare, accounting for 3.7–12.1% of all EOC. Some studies showed that CCC have the worst prognosis of all EOC [41], even though Bamias et al. [8] were unable to identify any prognostic difference to HGSC. CCC present at a younger age than SC, and have a clear association with endometriosis [42–44]. These tumors usually present as a large pelvic mass and rarely occur bilaterally. They are often associated with thromboembolic complications and hypercalcemia, with a higher frequency of lymph node metastases than SC [45–47]. Histologically, CCC are composed of glycogen-laden, large, cuboidal, hob-nailed or flattened clear cells; they often display an admixture of growth patterns including solid, tubulocystic, or papillary [48]. An oxyphilic variant exists and shows eosinophilic rather than clear cytoplasm

[49]. Hyalinised stroma can be observed. In some cases, a benign or borderline clear-cell adenofibroma is present. They can present a diagnostic challenge and be mistaken for the clear-cell area of other subtypes such as HGSC or EC, although a mixed tumor combining EC and CCC can be identified.

Immunohistochemistry can be helpful. In contrast to SC, CCC do not usually express WT1, p53, and ER. They specifically express napsin A, in contrast to the other EOC [50]. Some studies have shown that the tumor suppressor *ARID1A* is mutated in almost half of CCC cases [39, 51]. Activating mutations in *PI3KCA* have also been reported [52]. Recent studies showed that CCC are resistant to platinum-based chemotherapy, but the management of CCC is the same as for other EOC [46].

Seromucinous Carcinomas

Seromucinous carcinoma is a new category that was added to the new WHO classification in 2014 [53]. Referred to as an endocervical type (Müllerian) of mucinous ovarian carcinoma, the term “seromucinous tumor” was first coined in 2002 by Shappell et al. [54]. These tumors are rare and occur across a wide age range. Most present as a unilateral mass, but they are sometimes bilateral, unlike MC. Diagnosed at an early stage, they have a good prognosis but patients presenting with advanced disease appeared to have a poor prognosis. Further studies are necessary to fully evaluate the behavior of this tumor entity.

Morphologically, they are composed of an admixture of architectural patterns and cell types, including serous, endocervical-type mucinous, endometrioid, and squamous cells [54, 55]. Adjacent benign and borderline seromucinous tumor components are often present. On immunohistochemistry, CK7, hormone receptors, CA125, CA19.9, and PAX8 are consistently positive. Some cases are positive for WT1. CK20 and CDX2 are generally negative. Seromucinous carcinomas and endometrioid neoplasms are often present in the same ovary, and both lesions seem to be associated with endometriosis. In addition, the tumor suppressor gene *ARID1A* has been reported in both lesions, suggesting that they are closely related [56, 57]. Nonetheless, seromucinous carcinoma is considered a distinct entity, which emphasizes the heterogeneous aspect of ovarian cancer.

Intratumor Heterogeneity

As shown in this study, EOC represent a variety of entities. It is therefore clear that they should be managed according to their specific features and not as a single disease. However, within each tumor of each subtype, ITH represents another degree of complexity and has even been blamed for the failure of treatment [58]. Morphologically, a single tumor often exhibits various patterns and shows heterogeneous immunostaining. There is increasing evidence that not every mutation will be found in every cancer cell within a tumor [52, 53].

Some study groups have evaluated ITH on a molecular level in EOC. Khalique et al. [59] analyzed several regions of tumor tissue from 16 patients with HGSC, and then used microsatellite analysis and single-nucleotide polymorphism analysis to evaluate the extent of ITH and identify the clonal relationship between these regions. Although the tumors exhibited similar morphology, all cases showed a high degree of ITH between patients and between samples. Bashashati et al. [60] also showed a wide range of genomic alterations in 31 samples from 6 patients with primary HGSC resected prior to treatment. Using exome sequencing, copy number analysis, target amplicon deep-sequencing and gene expression profiling, they analyzed samples from different sites including the fallopian tubes. Their study revealed that spatially distinct samples show large differences at the level of copy number. Except for *TP53*, some mutations, in the well-characterized genes like *PIK3CA*, *CTNNB1*, and *PDGFR*, were observed but were not present in all samples, emphasizing the fact that a tumor sample is not representative of the entire tumor. They established evolutionary trajectories from 2 histologically and spatially distinct samples of a tumor within the same patient, and found that these both arose from the fallopian tube. Their work is consistent with other reports showing that HGSC has diverse ITH and that high-throughput technologies are helping to generate phylogenetic trees in order to better understand the etiology and progression of tumors [61].

Mechanisms of treatment resistance related to ITH have been investigated. Hoogstraat et al. [62] collected samples from primary and metastatic sites, based on 27 samples of 3 treatment-naïve HGSC cases. They combined whole-genome mate-pair sequencing, topographic mapping of somatic break points, and transcriptional profiling. Their analysis of genomic alterations influenced intratumor gene expression, and revealed the activation of different pathways in the metastatic lesions and primary tumors in the same patient, suggesting that tu-

mor lesions at different sites seems to evolve independently and adapt to the environment.

Another study quantified ITH and correlated with clinical characteristics and outcomes [63]. Heterogeneity was defined as the degree of clonal expansion between tumors, using an algorithm on high-resolution whole-genome copy number and selected genome-wide sequencing. It was applied on 135 tumor samples spatially and temporally collected from 17 women undergoing chemotherapy for HGSC. The evolutionary history of the disease for each patient was determined by calculating the minimum number of events (amplification or deletion) needed to transform one genomic profile to another. Wide differences in clonal expansions between patients and within samples of the same tumor were observed but minor changes were detected after treatment.

HGSC are likely to spread in a branching process (rather than a linear evolution) with varying rates of clonal expansion. These rates, reflecting the degree of heterogeneity, have been negatively correlated with survival. Some other subtypes have also been extensively studied, but LGSC show a lower degree of heterogeneity with fewer mutations, and they are genomically stable. This suggests that ITH may be most relevant to HGSC, certainly due to their plasticity as a consequence of high genomic instability.

Discussion

Heterogeneity represents a hallmark of many cancers including ovarian cancer. EOC are currently subdivided into histological subtypes. They often show complex morphology, with mixed patterns and contingents of different tumor grades. On a molecular level, deep sequencing has facilitated the establishment of catalogs of cancer genes and mutations, some of which are specifically implicated in the development of certain types of EOC, e.g., mutations in the *TP53* gene in HGSC and the *ARID1A* gene in CCC.

The dualistic model proposed by Kurman and Shih [5] integrated pathological, molecular, and clinical data; however, this 2-tier system is too simplified and is incomplete, gathering very distinct subtypes under the same label. Further studies are required to extend and sharpen this classification and characterize less common subtypes.

EOC biology has progressively unfolded, allowing the identification of biomarkers that could be used for targeted therapies. Although poly(ADP-ribose) polymerase

(PARP) inhibitors show significant clinical activity in women with *BRCA1* or *BRCA2* mutations, most targeted therapies in EOC have not demonstrated substantial results [64]. EOC are highly heterogeneous within each subtype, and multiregion genetic analysis (in the primary tumor and metastases) of the same patient reveals a high degree of variation. This has several implications for our understanding of the disease on a clinical level. While a linear clonal evolution model, consisting of a process of cycles of mutation and selection that will lead to the development of metastases, has been suggested, studies have provided another model of evolution consisting of a branching process represented by an evolutionary tree [65, 66]. This tree is constructed by comparing mutations between samples and applying algorithms. Within a tumor, some cells will display metastatic potential by acquiring additional mutations which vary from one site to another. They evolve independently from the primary clone, with a possible metastasis-to-metastasis spread. This demonstrates that a single biopsy is not representative of the entire tumor, that metastases may harbor targetable mutations that are not present in the initial tumor, and vice versa. Interestingly, a precursor clonal population in a fallopian tube in situ lesion was identified and gave rise to histologically and genetically different tumor populations, supporting the notion of STIC being a precursor lesion and showing that clonal expansion occurs at an early stage [60].

Quantitatively measuring tumor heterogeneity, as proposed by Schwarz et al. [63], could facilitate categoriz-

ing patients who might be responsive to targeted therapies. A patient who presents with a tumor that exhibits a low degree of heterogeneity might be eligible for such treatments. However, such a screening method is hardly applicable on a routine basis. The plasticity of EOC has been underlined by studies showing dynamic interactions between tumor cells within the microenvironment, depending on the site; this constitutes a relevant parameter to take into account in the development of treatment targets.

Conclusion

EOC are extremely heterogeneous, encompassing different entities with distinct clinicopathological features and genomic profiling. High-throughput “omics” technologies have helped to unravel the complexity of this disease and allow the discovery of tumorigenic pathways and biomarkers that are potentially targetable. ITH appears to be very high, representing a relevant parameter to consider for effective therapeutic strategies since it is a major cause of treatment failure. Nevertheless, from a practical point of view, the actual histological classification of subtypes should not be neglected but rather improved. Indeed, survival studies have shown that histological type appears to be the most important prognostic factor, with several trials having evaluated histology-specific chemotherapy agents (e.g., PARP inhibitors) for treating HGSC [8].

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