Genetic and Molecular Changes in Ovarian Cancer

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REVIEW

Genetic and molecular changes in ovarian cancer

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ABSTRACT

Epithelial ovarian cancer represents the most lethal gynecological malignancy in the developed world, and can be divided into five main histological subtypes: high grade serous, endometrioid, clear cell, mucinous and low grade serous. These subtypes represent distinct disease entities, both clinically and at the molecular level. Molecular analysis has revealed significant genetic heterogeneity in ovarian cancer, particularly within the high grade serous subtype. As such, this subtype has been the focus of much research effort to date, revealing molecular subgroups at both the genomic and transcriptomic level that have clinical implications. However, stratification of ovarian cancer patients based on the underlying biology of their disease remains in its infancy. Here, we summarize the molecular changes that characterize the five main ovarian cancer subtypes, highlight potential opportunities for targeted therapeutic intervention and outline priorities for future research.

KEYWORDS

ovarian cancer; molecular genetics; histological subtypes; molecular subgrouping; ovary

Introduction

Ovarian cancer (OC) represents the most lethal gynecological malignancy in the developed world, with over 21,000 cases diagnosed, accounting for over 14,000 deaths per year in the United States alone. The vast majority of ovarian cancers are of epithelial origin, which are typically diagnosed at advanced stage. The current standard of care for epithelial ovarian cancer comprises maximal cytoreductive surgical resection and platinum-taxane combination chemotherapy.

A number of clinical parameters influence outcome in OC patients. Age at diagnosis, FIGO stage, disease grade and the presence of ascites are independent factors affecting progression-free-survival (PFS) and overall survival (OS) in OC patients. Suboptimal debulking surgery, leaving macroscopic residual disease, also has a significant impact on patient survival.

Epithelial OC has historically been grouped according to histology and is currently divided into five main subtypes: high grade serous (HGS), endometrioid, clear cell (CC), low grade serous (LGS) and mucinous OC. It is now recognized that these subtypes have distinct developmental origins: HGS OC predominantly arises from the epithelium of the distal fallopian tubes, while CC and endometrioid OC are associated with endometriosis. LGS OC is thought to progress in a step-wise fashion from serous cystadenoma or adenofibroma to serous borderline tumor, and then to LGS OC. These histological subtypes display distinct molecular landscapes at both the genomic and transcriptomic level.

In the face of mounting evidence for the discrete developmental origins and molecular pathogenesis of OC subtypes, there is now a growing appreciation that these five histologically-defined groups represent separate disease entities, and that there is a need for stratification in both the clinical and research setting.

In keeping with the argument that they are different diseases, these subtypes display different levels of chemosensitivity. CC, mucinous and LGS OC are highly platinum resistant, while HGS OC is often platinum sensitive in the first-line setting. Despite the tendency to display therapy resistance, LGS OC is associated with superior clinical outcome compared to HGS, displaying a more indolent disease course, even when diagnosed at advanced stage. Endometrioid and CC OC also display generally superior clinical outcome when compared to HGS, which is likely due to their propensity for diagnosis at earlier stage.

However, histological subtype alone does not account for the significant clinical heterogeneity seen in OC. Indeed, HGS OC patients matched for disease grade and stage show differential therapy sensitivity, PFS and OS, strongly
implicating a molecular basis for the clinical heterogeneity within these histologically-defined groups. Extensive molecular characterization of HGS OC has therefore been undertaken to identify subgroups defined by their genomic and transcriptomic characteristics, in the hope of finding a molecular basis for differential clinical outcome and to identify opportunities for targeted therapeutic intervention and treatment stratification.

Approximately one fifth of OC is associated with inherited pathogenic variants in the germline, commonly in BRCA1 or BRCA2 which account for around 75% of hereditary disease. The molecular and clinical implications of these defects are discussed below. While BRCA-associated disease is the most common form of hereditary OC, defects in other DNA repair associated genes have also been identified. These include genes that, like BRCA1 and BRCA2, are involved with double stranded DNA repair, such as BARD1, CHEK2, RAD51, PALB2 and BRIP1.

Lynch syndrome, caused by inherited defects in genes involved in single stranded mismatch DNA repair (MMR), most commonly predisposes individuals to bowel and endometrial malignancy, but these patients are also at increased risk of OC. The most commonly affected genes in this syndrome are MLH1, MSH2, MSH6 and PMS2, and Lynch syndrome patients account for around 10%-15% of hereditary OC.

Li-Fraumeni syndrome, caused by an inherited TP53 mutation, accounts for much of the remaining identified hereditary OC cases (around 3%).

### Genetic and molecular changes in HGS OC

HGS OC accounts for approximately 70% of OC. Of these, only a minority are confined to the ovary at diagnosis. Despite response rates to first-line platinum-based chemotherapy of around 80%, the majority of patients experience disease recurrence which accrues resistance to platinum, and prognosis for advanced stage disease remains poor with a five-year survival of around 30%.

#### DNA sequence

The most frequent molecular defect in HGS OC at the genomic level is almost ubiquitous TP53 mutation. The majority of these mutations are missense variants, however around 30% are frameshift, nonsense or splice junction variants which result in complete loss of p53 protein, commonly referred to as 'p53 nulls'. Despite this canonical cancer-associated defect, HGS OC does not generally display the classical activating oncogenic mutations typical of other solid tumor types. Instead, extensive somatic copy number changes - rooted in chromosome instability and defective DNA repair - scar the genomic landscape.

Around half of HGS OC have identifiable germline, somatic or epigenetic defects in the homologous recombination DNA repair (HRR) pathway, the flagship defects being germline or somatic BRCA1 or BRCA2 mutations which together account for approximately 20% of cases (Figure 1). Around 8% and 6% of HGS OC patients harbor germline BRCA1 and BRCA2 defects, respectively.
while somatic changes in each occur in approximately 4% and 3% of cases. HRR-deficiency provides a rationale for the use of PARP inhibitors (PARPi), inducing synthetic lethality via inhibition of DNA single stranded break repair mechanisms and induction of error-prone non-homologous end joining (NHEJ) in HRR-deficient tumor cells. These agents have proven clinically effective, particularly in the BRCA-mutated HRR-deficient population.

Historically, analyses have grouped BRCA1- and BRCA2-defective patients together and have demonstrated improved sensitivity to cytotoxic chemotherapies and superior clinical outcome in this group, despite their propensity to develop visceral metastases and to present with HGS histology. However, it has recently emerged that the clinical implications of BRCA1 and BRCA2 defects are distinct, with BRCA1 carriers experiencing only short-term survival advantage while the survival benefit in BRCA2 carriers persists 10 years from diagnosis. Notably, BRCA1 can also be epigenetically inactivated, with around 11% of HGS OC showing BRCA1 promoter hypermethylation. However, BRCA1-methylated patients are not thought to experience the same survival benefit as those with other BRCA defects, and may even experience reduced disease-free intervals and inferior OS.

Structural and copy number changes

HGS OC is characterized by substantial genetic heterogeneity and these tumors display large numbers of structural genomic changes. As such, identifying structural variants that represent driver events in tumorigenesis has presented a significant challenge. Such structural changes are now known to be an important mechanism of tumor suppressor gene inactivation in HGS OC, most notably affecting the RB and MAPK/PI3K signalling pathways.

The TCGA investigators identified defects in RB1 (8% deletion, 2% mutation), NF1 (8% deletion, 4% mutation) and PTEN (7% deletion, <1% mutation) of their HGS OC cohort. However, recent whole genome analysis of HGS OC has revealed that RB1 and NF1 are frequent targets of previously unidentified gene breakage events, affecting 20% and 17.5% of cases, respectively, underscoring the role of large structural rearrangements in tumor suppressor inactivation in OC.

Approximately 6% of HGS OC displays amplification of the EMSY gene, which encodes a BRCA2-inhibiting protein implicated in DNA repair regulation, as well as in chromatin remodelling and wider transcriptional control. These tumors are thought of as likely HRR-defective, and it has therefore been suggested that EMSY-amplified OC may respond well to PARPi therapy. However, the question of whether these tumors are truly HRR-deficient remains controversial and the efficacy of PARPi use within this group remains to be established.

Of the non-HRR-deficient HGS OC cases, a significant proportion display amplification of CCNE1, encoding the cell cycle checkpoint regulator cyclin E1. Approximately 14% of HGS OC harbors this abnormality, which has been proposed as a novel therapeutic target. It has been suggested that CCNE1 amplification and BRCA1/2 dysfunction occur mutually exclusively, and thus CCNE1-targeted therapies may represent a valuable treatment option in patients who are not candidates for PARPi therapy. Furthermore, CCNE1 amplification has been implicated in intrinsic platinum resistance, and may represent a therapeutic target for sensitization of disease that is intrinsically resistant to cytotoxic agents.

Gene expression

Because HGS represents the majority of OC, gene expression
Heterogeneity

As the emergence of chemoresistant recurrent disease represents the primary cause of mortality in HGS OC patients, intratumor heterogeneity, facilitating selection of pre-existing chemoresistant subclones during treatment, is of great interest. Expansion of these clones represents an avenue for rapid emergence of recurrent disease with therapy resistance. Such intratumoral heterogeneity is a prominent feature in HGS OC. Indeed, quantitatively assessment of intratumoral heterogeneity may prove clinically informative in HGS OC, with patients suffering from highly heterogeneous disease showing shorter PFS and OS.

Existence of genetically and phenotypically diverse subclonal populations within primary HGS disease also present a critical avenue for the failure of novel and existing targeted therapies. Accordingly, the design of novel targeted therapeutic strategies must be centered towards carcinogenic driver mutations at the ‘trunk’ of the evolving cancer genome.

Molecular changes in acquired therapy resistance in HGS OC

The majority of HGS OC patients have a good clinical response to primary platinum-based chemotherapy. As a result, characterizing mechanisms of acquired resistance in the recurrent disease setting is of great clinical relevance. Reversion of BRCA1 and BRCA2 mutations via secondary genomic events that restore open reading frames, returning HRR proficiency, has been proposed as one mechanism of reducing sensitivity to conventional therapies. Accordingly, these BRCA-reverted tumors may well also show decreased sensitivity to targeted PARPi therapy.

Further proposed mechanisms of acquired resistance include upregulation of AKT signalling, promoting cell survival, and increased expression of the ABCB1 drug efflux protein via promoter hijacking. Paclitaxel, commonly used in combination with platinum agents in first-line treatment of OC, is a known substrate of ABCB1, and increased expression therefore represents a viable mechanism for reduced accumulation of cytotoxic agents within ABCB1-expressing OC cells. These molecular events may well be clinically actionable in the hope of re-sensitizing disease to conventional therapies, although modulation of ABCB1 activity has not yet proven efficacious in advanced stage OC.

While some mechanisms of therapy resistance have been elucidated, identification of pathways involved in platinum-resistant recurrent OC remains in its infancy and existing studies have investigated a relatively small number of
relapsed disease samples. Extensive characterization of the molecular events underpinning subsequent treatment failure have largely been hindered by the failure to acquire multiple, temporally separated biopsies from the same patient throughout the course of their disease. Investigation of large cohorts of such paired samples will undoubtedly uncover further mechanisms of acquired disease resistance which may present further opportunities for therapeutic intervention.

Epigenetic and microRNA dysregulation in HGS OC

OC research thus far has largely been dominated by analyses at the exomic sequence and transcriptional level. However, progress in defining the epigenomic and microRNA landscapes of OC has been made in recent years.

Promoter hypermethylation and associated gene silencing of BRCA1 is perhaps the most canonical epigenetic defect in HGS OC. While these tumors are considered HRR-deficient and this molecular event appears to occur mutually exclusively with germline or somatic BRCA1/2 mutation, BRCA1-methylated patients may not experience the classical BRCA-associated clinical benefit, as discussed above. Accordingly, this epigenetic defect may be of limited clinical interest.

The TCGA investigators reported over 150 genes with increased DNA methylation and associated reduction in gene expression, including BRCA1. Clustering of HGS OC samples within their dataset revealed four subtypes based on differential methylation which overlapped significantly with their transcriptionally-defined groups.

Further to DNA methylation analysis, the TCGA investigators reported three HGS OC subtypes defined by their differential microRNA expression profiles, with one of these subtypes displaying superior OS. Numerous studies have now found associations between expression of specific microRNAs and clinical parameters, including disease stage, histological subtype and chemoresistance.

Improved understanding of how miRNA and epigenomic dysregulation contribute to OC tumorigenesis will undoubtedly further understanding of disease biology, and may well reveal opportunities for therapeutic intervention.

Key future research foci for HGS OC

With characterization of HGS OC at the DNA sequence level having made substantial advances, understanding the clinical implications of each of these molecular events is a clear long term research goal. In particular, the importance of NF1 and RB1 disruption - whether by deletion, mutation or recently identified gene breakage - are of great interest, as together these represent a significant proportion (approximately one third) of HGS OC.

While those patients with germline BRCA1 and BRCA2 mutations have been fairly well characterized in terms of improved sensitivity to platinum and efficacy of PARP inhibitors, the clinical implications of genetic events in other HRR pathway components - including EMTY amplification - remain to be extensively investigated. Further consideration of BRCA1 and BRCA2 defects as distinct entities, and comparison with BRCA1 promoter hypermethylation, will also help address whether all HRR pathway abnormalities convey equal clinical implication - although evidence thus far would suggest otherwise. Because of the relative rarity of non-BRCA HRR pathway aberrations, large retrospective cohorts of molecularly characterized patients with rich clinical annotation will be required.

A key step for future research will be to establish panels of cell lines that represent the spectrum of molecular changes that are now known to occur in HGS OC. This will provide an invaluable pre-clinical resource for investigating novel therapeutic strategies in the context of underlying molecular biology of disease, and will likely uncover potential new biomarkers for sensitivity to both targeted and conventional therapies.

The acquisition and molecular characterization of paired chemosensitive primary and chemoresistant recurrent disease specimens represent an immediate research priority. Sampling from recurrent disease, end stage disease and even post-mortem sampling from rapid autopsy will undoubtedly shed more light upon the molecular mechanisms underpinning therapy-resistant recurrent disease, to which patients ultimate succumb.

In terms of transcriptional profiling of HGS OC, significant advances have been made in finding clinically relevant subgroups. Unsupervised analyses have identified molecular subgroups with distinct clinical outcomes and supervised approaches have produced gene expression signatures predictive of survival. However, a real consensus in subgrouping remains to be established. Reproducibility has in part been hindered by limited sample numbers in training datasets in some studies, differential histological composition of datasets, as well as technical factors such as the diversity of gene expression platforms used and the use of fresh-frozen versus formalin-fixed paraffin-embedded (FFPE) tumor material. The culmination of these pitfalls is...
that molecular subgrouping is ultimately not currently used to guide management of patients in the clinic. Demonstrating subgroup-specific actionable molecular biology and drug sensitivities in the research setting will be a crucial step in demonstrating the need for such stratification in the clinic.

Given numerous reports of the impact of tumoral-immune response on PFS and OS, understanding if and how these above mentioned molecular features of OC influence the interaction with the host immune system will also be of interest\textsuperscript{86,87}. Indeed, recent data have shown that \textit{BRCA1/2}-mutated OCs display higher levels of tumor-infiltrating lymphocytes, indicating these tumors may be more immunogenic, consistent with the survival advantage experienced by this patient group\textsuperscript{88}. Such analyses may well prove informative in relation to the efficacy of immune checkpoint inhibitor therapy, and establishing biomarkers of tumor immunogenicity may help stratify patients who are likely to benefit from these, and other, immunotherapeutic strategies.

**Genetic and molecular changes in non-HGS OC**

Endometrioid, CC, LGS and mucinous subtypes account for nearly all remaining OC cases, representing around 10%, 10%, <5% and <5% of OC cases, respectively. These rarer subtypes represent distinct disease entities from both HGS OC and from one another. Collectively, and conversely to HGS OC, they harbor activating oncogenic mutations more typical of solid tumors but do not display high rates of \textit{TP53} mutation\textsuperscript{89,90}.

**Endometrioid OC**

Of the OC histological subtypes, endometrioid OC represents the group with most favorable clinical outcome: they have a tendency to be diagnosed at earlier stage versus HGS OC and are generally sensitive to platinum in the first line setting\textsuperscript{27,28}. Endometrioid OC is associated with endometriosis, and their gene expression profiles bear resemblance to that of endometrial tissue\textsuperscript{81,16,91}. Together, endometrioid and CC OC represent the majority of Lynch syndrome-associated OC cases\textsuperscript{92-94}.

Historically, endometrioid OC has been subclassified into either low or high grade disease. However, it is now recognized that high grade endometrioid OC more closely resembles HGS OC both molecularly and clinically, while low grade endometrioid OC represents a more distinct ‘true endometrioid’ OC subtype\textsuperscript{95}.

Mutations deregulating the PI3K pathway are common in this latter subtype: around 20% of cases harbor \textit{PTEN} tumor suppressor gene mutations and around 30% display activating \textit{PIK3CA} mutations\textsuperscript{96-98}. Around 30% of endometrioid OC displays mutations in the chromatin-remodelling associated gene \textit{ARID1A}, a suggested tumor suppressor gene\textsuperscript{99,100}. A minority also harbor somatic mutations in the \textit{PPP2R1A} gene, encoding a subunit of protein phosphatase 2A\textsuperscript{101}.

Endometrioid OC also commonly displays activated Wnt signalling, with around half showing \textit{CTNNB1} mutation\textsuperscript{96,97,102}.

**Clear cell OC**

As with endometrioid ovarian cancer, CC OC is associated with endometriosis and has a tendency to be diagnosed at earlier stage versus HGS OC\textsuperscript{22,29,30}. However, CC OC frequently displays intrinsic platinum resistance and advanced stage CC OC remains a great clinical challenge, with inferior PFS and OS compared to advanced stage HGS OC\textsuperscript{22,30}. CC OC accounts for the majority of non-endometrioid cases associated with inherited MMR deficiency\textsuperscript{93,94}.

Like endometrioid OC, CC carcinomas harbor defects in \textit{PTEN} (in around 10% of cases), \textit{PIK3CA} (in around 50% of cases), and \textit{ARID1A} (in around 50% of cases), consistent with the shared molecular pathogenesis and developmental origins of these carcinomas\textsuperscript{97,99,103-106}. Similarly, a minority display somatic \textit{PPP2R1A} mutation\textsuperscript{101}. However, unlike endometrioid OC, around a third of CC tumors show amplification of chr20q13.2 and do not generally harbor Wnt-activating \textit{CTNNB1} mutations\textsuperscript{97,107}.

**Mucinous OC**

Mucinous OC was once thought to account for a significant number of OC cases, but it is now recognized that few are true primary mucinous OC, while the rest represent metastases from other malignancies, most commonly from the gastrointestinal tract\textsuperscript{45,108}. In comparison to HGS OC, mucinous OC tends to present at earlier stage\textsuperscript{109,110}. However, this subtype frequently displays platinum resistance in the first line setting, and advanced stage mucinous OC is associated with particularly poor OS\textsuperscript{24,111}.

The molecular pathogenesis of mucinous OC remains relatively poorly understood, and extensive characterization of this subtype has largely been hindered by its low prevalence compared to other histological OC subtypes.
However, KRAS mutation and HER2 gene amplification are known common events in mucinous OC, with around 50% and 20% of cases displaying these defects, respectively\(^ {109,112}\).

**LGS OC**

LGS OC is characterized by young age at diagnosis, indolent disease course, and prolonged OS versus HGS OC, even when diagnosed at advanced stage\(^ {25}\). Over 60% of LGS OC harbor MAPK pathway-activating KRAS or BRAF mutations, accounting for around 30% of cases each, but almost invariably do not harbor TP53 mutations\(^ {113-115}\). Furthermore, KRAS mutation may be associated with more aggressive, recurrent disease versus BRAF-mutated LGS OC\(^ {116,117}\).

**Key future research foci for non-HGS OC**

Recognition of non-HGS OC subtypes as distinct disease entities has been a critical step in OC research. Although mucinous, CC and endometrioid OC is more commonly diagnosed at early stage versus HGS OC, advanced stage cases present a significant clinical challenge\(^ {22,27-30,111}\). Clinical studies now need to be performed in a subtype-specific fashion in order to properly characterize the underlying biology within each of these histotypes at both the genomic and transcriptional level.

In particular, the characterization of non-HGS OC subtypes that display resistance to the platinum-based chemotherapies are needed, as these agents remain a cornerstone of OC treatment irrespective of histological subtype. These studies hope to uncover underlying disease biology that may be actionable through the use of novel or existing targeted therapies, with the aim of improving treatment either directly through selective cytotoxicity or indirectly through sensitizing disease to conventional therapies.

Our current understanding of the biology behind these rarer subtypes has already revealed avenues for potential implementation of targeted therapies: LGS and mucinous OC may well benefit from MAPK pathway inhibitors, while CC and endometrioid OC may benefit from agents targeting the PI3K pathway. Indeed, the MEK1/2 inhibitor selumetinib has shown promising results in patients with recurrent LGS OC\(^ {118}\). Targeting HER2 has already proven a successful treatment strategy in HER2-amplified breast cancer, and the use of therapies such as the monoclonal antibody trastuzumab may also prove useful in treating HER2-amplified mucinous OC\(^ {119}\). Investigating the potential therapeutic efficacy of these and other agents in genomically characterized disease models will be an important step toward therapy stratification within these rarer OC subtypes.

Perhaps the greatest challenge for single-histotype studies of non-HGS OC will be acquiring large cohorts of these rarer subtypes. Historic samples will need to undergo rigorous pathology review to ensure the accuracy of histotype assignment. Sample numbers will be a particular challenge for studies looking to identify transcriptionally-defined molecular subgroups by unsupervised analysis, as these investigations will require large training and validation cohorts. Indeed, acquiring uniformly staged, graded and treated cohorts of OC remains a challenge even in HGS disease.

**Conclusions**

OC is a substantial cause of morbidity and mortality in the developed world. A number of clinical features are known to affect PFS and OS rates in OC, including disease stage, grade and surgical outcome. The five main histologically-defined subtypes of OC are now recognized as separate diseases and display differences in stage at diagnosis, responses to platinum-based chemotherapies as well as OS.

However, substantial clinical heterogeneity remains even within these histological groups, particularly within HGS which represents the majority of OC. As such, the majority of the research effort thus far has focused on this subtype, elucidating clinically meaningful subgroups at both the genomic and transcriptomic level, despite extreme genomic heterogeneity. The challenge remains for these subgroups to be taken forward into the clinic, and we await a consensus on clinically meaningful transcriptomic subgroups that validate in the wealth of publicly available HGS OC gene expression data. At present, only BRCA1 status is routinely used clinically, with germline BRCA1 and BRCA2 genetic testing now in place at a number of centers as a biomarker for the use of PARPi therapy. Rarer genomic defects in HRR pathway components remains an area of great interest, and the field awaits data on whether these patients are truly HRR deficient and likely to benefit from PARP inhibition.

The clinical implications of more recently identified genomic defects, including NF1 and RB1 loss, remain to be established. Together with CCNE1 amplification, these defects account for much of HRR-proficient HGS OC, and finding novel therapeutic strategies to improve clinical outcome in these patients is an area of substantial unmet need.

Recurrent HGS OS with acquired chemoresistance is the ultimate cause of the majority of patient mortality. Therefore,
investigating its molecular drivers is an urgent research priority. The acquisition and molecular characterization of matched primary and recurrent samples promise to reveal opportunities for using novel therapeutic strategies and re-sensitizing to cytotoxic agents.

Non-HGS OC is characterized by more classical oncogenic mutations, and subtype-specific studies of endometrioid, CC, LGS and mucinous OC are now needed to further stratify these subtypes at both the transcriptomic and genomic level. The real challenge for these investigations will be acquiring sufficiently large cohorts to make meaningful conclusions that can pave the way for stratification of therapy, which will undoubtedly require international collaborative efforts.

**Conflict of interest statement**

No potential conflicts of interest are disclosed.

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