Insight updating of the molecular hallmarks in ovarian carcinoma

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ABSTRACT

Background and purpose: Ovarian cancer (OC) is the deadliest gynaecologic cancer characterised by a high heterogeneity not only at the clinical point of view but also at the molecular level. This review focuses on the new insights about the OC molecular classification.

Materials and methods: We performed a bibliographic search for different indexed articles focused on the new molecular classification of OC. All of them have been published in PubMed and included information about the most frequent molecular alterations in OC confirmed by omics approaches. In addition, we have extracted information about the role of liquid biopsy in the OC diagnosis and prognosis.

Results: New molecular insights into OC have allowed novel clinical entities to be defined. Among OC, high-grade serous ovarian carcinoma (HGSOC) which is the most common OC is characterised by omics approaches, mutations in *TP53* and in other genes involved in the homologous recombination repair, especially *BRCA1/2*. Recent studies in HGSOC have allowed a new molecular classification in subgroups according to their mutational, transcriptional, methylation and copy number variation signatures with a real impact in the characterisation of new therapeutic targets for OC to be defined. Furthermore, despite the intrinsic intra-tumour heterogeneity, the advances in next generation sequencing (NGS) analyses of ascetic liquid from OC have opened new ways for its characterisation and treatment.

Conclusions: The advances in genomic approaches have been used for the identification of new molecular profiling techniques which define OC subgroups and has supposed advances in the diagnosis and in the personalised treatment of OC.

1.   Introduction

Ovarian cancer (OC) is the second most common gynaecologic cancer in developed countries and is a major cause of morbidity and mortality among gynaecological malignancies [1]. These tumours have been subdivided into epithelial and non-epithelial. Regarding non-epithelial tumours, there are two types, germ cell and sex-cord stromal tumours that represent 10—15% of all OCs [1]. However, the vast majority of OCs (about 90%) are epithelial tumours [2]. These tumours start in the epithelial surface layer covering the ovary or in the distal fallopian tube [3].

Based not only on the histopathology characteristics but also on some molecular features, epithelial ovarian carcinomas may be classified in five main types [4]: high-grade serous ovarian carcinomas (HGSOCs), which are the most common (accounting for about 70% of the cases) and fatal type of all the histological subtypes, endometrioid ovarian carcinoma (EOC, 10%), ovarian clear cell carcinoma (OCCC, 10%), mucinous ovarian carcinoma (3%) and low-grade serous ovarian carcinomas (LGSOC, <5%) [2,3] (Figs. 1—5). Currently, the standard of care for EOC consists in a cytoreductive surgery followed by a platinum-based chemotherapy. Despite most patients initially respond to treatment, many (more than 75% of patients with HGSOC) develop resistance within one year and subsequently relapse [5]. From a diagnostic point of view, different biomarkers have been used and some of them have also an important role in prognosis, prediction and treatment. However, the knowledge of common genetic alterations in these tumours have provided context for interpreting 'omics' investigations, which have allowed to decipher new biomarkers with a predictive and/or prognostic role in this pathological scenario [6]. In this review, we will focus on epithelial ovarian tumours which are one of the most frequent gynaecological tumours. We have also remarked the molecular hallmarks driving OC heterogeneity and summarised how these advances might lead to better clinical management of patients with OC.

2.   Molecular features of ovarian carcinoma

During last years, existence of a wide number of cytogenetic, genetic and epigenetic variations has been reported in OC [7]. Epithelial ovarian tumours have classified into two major groups as per clinicopathological features and their genetic stability that is higher in LGSOC, EOC and OCCC (classified as type I) in comparison with HGSOC which shows high DNA instability (type II) [8—10] (Fig. 1). Type I tumours are predominantly diagnosed in early stages and generally are indolent tumours with poor contribution to OC deaths [11]. In contrast, type II tumours account for the highest rates of advanced stage and OC deaths [11,12]. At the genomic level, non-random chromosomal abnormalities and allele imbalance have been reported [13], such as rearrangement of 19q, which occurs in more than 60% of advanced OC and it is associated with poor clinical outcome, or gains in 14q32.33 related to platinum resistance [14]. In fact, somatic copy number amplification is a recurrent molecular alteration in high-grade ovarian carcinomas in contrast to the presence of somatic mutational activation of oncogenes which barely happen [15]. In this sense, type I tumours, with the exception of OCCC, frequently show mutations in regulators of the mitogen-activated protein kinase (MAPK) pathway (such as KRAS or BRAF) [10]. In addition, it has been revealed that many single nucleotide variant (SNV) are located on non-coding regions. Their effects might be associated with the risk to alter the activity of regulatory elements and consequently, they could impact in the gene expression profile of these tumours [16]. From the histological point of view, these copy number modifications are prevalent in serous subtype followed by endometrioid and clear cell tumours [7]. Furthermore, TP53 mutations detected in most of the sporadic serous ovarian carcinomas could favour a suitable environment that give rise to the loss of BRCA1 or BRCA2 function, as well as other DNA repair deficiency phenotypes [17]. In fact, specifically in HGSOCs, one of the most relevant issues is the presence of chromosomal instability and widespread somatic copy
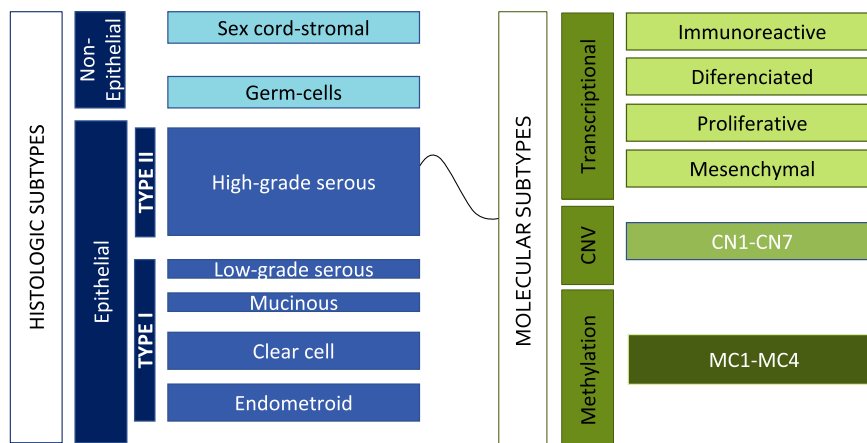

Fig. 1 — **Histological and molecular subtypes of ovarian cancer. Summary of the current ovarian cancer histological subtypes where the size of the squares reflects the incidence of each subtype. Molecular classification represents the different subtypes according to the transcriptional, copy number variation (CNV) and methylation studies.**

number alterations (SCNAs), probably as a consequence of the DNA repair disorders due to TP53 and BRCA1/2 mutations [18]. Inactivation of BRCA1/2 genes occurs in more than 65% of HGSOC [15]. In addition, mutations in FAT3, CSND3, NF1 and CDK12 RB1 are also frequently altered in these tumours. BRAF mutations are restricted to serous borderline carcinomas suggesting that these tumours do not progress to serous tumours. Loss of PTEN and activation in PIK3CA are frequently found in endometrioid and clear cell subtypes but not in serous or mucinous tumours. Inactivating mutations in ARID1A are frequent in ovarian clear cell carcinomas [19], and HER2 amplification have been reported in about 15–20% in both mucinous and clear cell carcinomas [10]. On the other hand, epigenetic events have been also reported in OC highlighting the hypermethylation of MLH1 in more than 50% of OC with platinum resistance phenotype [20], or ARMCX2 and COL1A, as well as DLEC1 epigenetic silencing associated to resistance or recurrence, respectively [21]. In summary, some of the most relevant molecular characteristics and their specific clinical relationship are indicated in Table 1. Similarly, the study performed by The Cancer Genome Atlas (TCGA) in almost 500 OC samples has allowed delineation of a comprehensive landscape of the genetic and genomic profile [22]. This specific context will be discussed in the following.

In terms of tumour origin, LGSOC, EOC and OCCC carcinomas are developed from well-established benign precursor lesions, whereas HGSOC may expand *de novo* from the tubular and/or ovarian surface epithelium [23]. During the past decade, different studies found new transformations in the fallopian tube [24]. These dysplastic lesions within the tubal epithelium have been classified as a 'serous tubal intraepithelial carcinomas' (STIC) [23]. Currently, an increasing consensus in the field points to that HGSOC arises from STIC. Both genetic and clinical remarks suggest that STICS may be precursor lesions, particularly in women with increased genetic risk [25].

2.1. TP53 and its controversial potential prognosis role in OC

As aforementioned, HGSOC represents most OC cases and is widely characterised by TP53 mutations, which happen in at least 96% of the cases [15,26]. TP53 is key a tumour suppressor gene involved in G2 checkpoint, mediating cell cycle arrest, senescence or apoptosis in response to different kinds of cellular stress, including DNA damage, activated oncogenes or hypoxia [27,28]. Most TP53 mutations are missense substitutions in contrast to other tumour suppressor genes that present mainly truncating mutations [29]. These types of mutations lead to nuclear protein accumulation with a diffuse and strongly positive immunohistochemistry (IHC) staining, whereas TP53 wild-type tumours present a focal positive staining (less than 50% of the cells, usually less than 10%). Nevertheless, approximately 30% of somatic TP53 mutations are nonsense, frameshift or splicing junction variants that lead to the complete absence of p53 protein, also known as null mutations (Fig. 2).

Although somatic mutations in the TP53 are the most frequent genetic alterations in HGSOCs [15,26,29,30], its prognosis value is a controversial issue nowadays. Numerous studies have correlated the TP53 mutational status with different clinical parameters such as overall survival or response to therapy, but these results remain conflicting [31]. This could be partially explained taking into account that the vast majority of studies have performed IHC to assess p53 alterations, a technique prone to misclassify an important number of cases because of the difficulty in distinguishing wild-type and null tumours [32,33]. Similarly, other analyses limited TP53 sequencing to those exons that encode the DNA binding domain or did not differentiate between biological consequences of these mutations [34]. However, several studies support that tumours with TP53 null mutations present a worse clinical

| Table 1 – Clinical and molecular features of the most common ovarian cancer types.					
	HGSOC	EOC	OCCC	MOC	LGSOC
Risk factors	BRCA1/2	Lynch syndrome	Unknown	Unknown	Unknown
Precursor lesions	STIC	Atypical endometriosis	Atypical endometriosis	Cystadenoma, borderline tumour	Serous borderline tumours
Pattern of spread	Very early transcoelomic spread	Usually confined to pelvis	Usually confined to pelvis	Usually confined to ovary	Transcoelomic spread
Frequently mutated genes	BRCA, TP53, NF1, RB1, CDK12	PTEN, ARID1A, PI3KCA	HNF1, ARID1A, PIK3CA, PTEN, CTNNB1	KRAS, HER2	BRAF, KRAS, NRAS, ERBB2
Chromosomal abnormalities	Rearrangement of 19q				
Gene copy number variation		Gain of JUNB, KRAS2, MYCN, ESR and CCND2; TPM3 amplification		ERBB2 amplification	
Chemosensitivity	High	High	Low	Low	Intermediate
Prognosis	Poor	Favourable	Intermediate	Favourable	Favourable
HGSOC: high-grade serous ovarian carcinoma, EOC: endometrioid ovarian carcinoma, OCCC: ovarian clear cell carcinoma, MOC: mucinous ovarian carcinoma, LGSOC: low-grade serous ovarian carcinoma, STIC: serous tubal intraepithelial carcinoma. Adapted from the study by Prat et al. [4].					
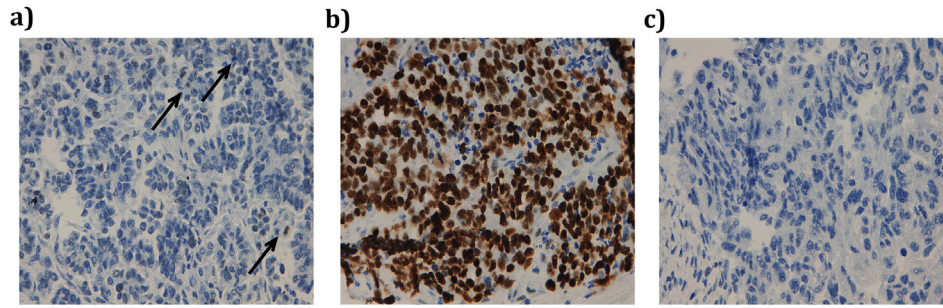
Fig. 2 — p53 immunostaining patterns. Immunohistochemical analyses of p53 in high-grade serous ovarian carcinomas; representative examples for the different patterns of p53 staining: (a) *TP53* wild-type: focal nuclear expression (some positive cells are marked with black arrows) in less than 50% of tumour cells. (b) *TP53* missense mutant: strong nuclear overexpression in more than 80% of tumour cells. (c) *TP53* null mutant: complete absence of expression. Magnification 40×. Images were kindly provided from tumour tissue samples at the Pathology Department, MD Anderson Cancer Center, Madrid.

outcome compared with those in which *TP53* harbours mutations involving overexpression, not only in OCs but also in leukaemia and breast, colorectal and head and neck cancers [35—38]. Conversely, a recent analysis using TCGA data sustains that *TP53* wild-type tumours could have a worse prognosis than mutant tumours, although it is important to note that no differentiation between missense and null mutations was considered [39]. In fact, when this type of mutations is separated, *TP53* null subgroup shows an intermediate clinical behaviour between the wild-type and mutant groups, with significant and nearly equivalent differences between these categories as is shown in Fig. 3 [40]. Despite the important role of *TP53* in controlling proliferation and maintaining the integrity and stability of the genome, key mutations identified at this gene are not directly 'druggable'. However, *TP53* mutations can be used as a biomarker to predict patient response to chemotherapy [41,42]. Indeed, the type of *TP53* mutations defines the

biological consequence related to an increase in the response or in the resistance to the treatment [43]. The accurate mutant identification can lead to determination of the correct treatment avoiding high chemoresistance rates in these patients. Different approaches have been studied to obtain a potential treatment; among them, the destabilisation of mutant p53 using US Food and Drug Administration (FDA)—approved HSP90 and deacetylase inhibitors has been explored [44,45].

2.2. *BRCA1* and *BRCA2* *therapeutic prognosis markers in OC*

Although OC is more frequent in advancing ages and a rare disease in pre-menopausal women, there are different risk factors that increase the probability of the development of OC. Among them, specific genetic factors have been considered a sustainable heritable risk component, and there is a threefold
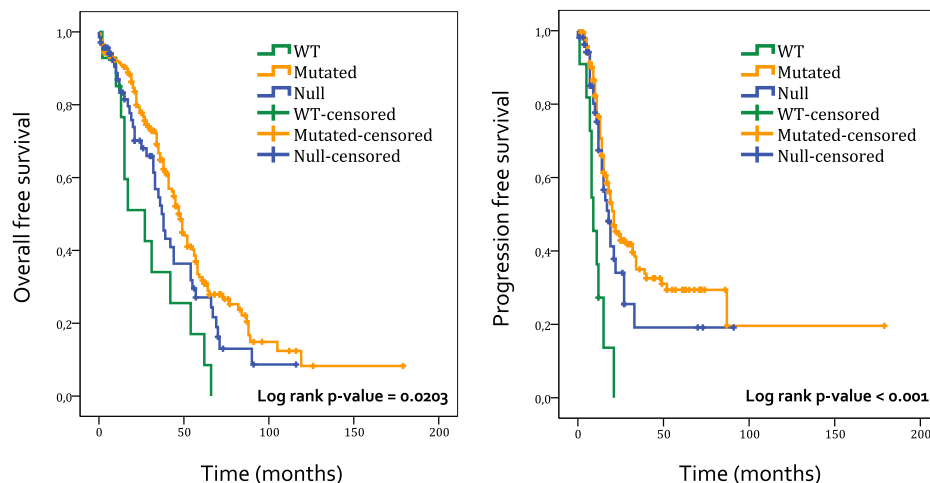
Fig. 3 — *TP53* mutational status meta-analysis in TCGA ovarian cancer cohort. Patients included in the TCGA study were subdivided into three subgroups depending on *TP53* mutational status: mutated (orange), null (blue) and wild-type (green) carcinomas. The Kaplan-Meier plots show the association between *TP53* mutational status and overall and progression-free survival. Significant correlation was performed using a logrank test. *p*-value lower than 0.05 was considered statistically significant (*p < 0.05). TCGA, The Cancer Genome Atlas.

rise in risk of developing OC in women who have a first-degree relative OC-affected [46]. In fact, germline mutations in *BRCA1* and *BRCA2* genes are present in most patients with hereditary ovarian carcinomas. Furthermore, the inactivating *BRCA1/2* mutations have also been observed, as somatic alterations, in around of 15% of ovarian tumours, especially in HGSOC [15,47]. *BRCA1* and *BRCA2* are essential components of the homologous recombination repair (HRR) of DNA double-strand breaks. Despite their implication in ovarian carcinomas, the penetrance differs in these two populations, with a lifetime risk of 36%–60% in *BRCA1* carriers and 16%–27% in *BRCA2* [48]. In addition, the development of OC may be ten years earlier in *BRCA1* carriers than in *BRCA2* carriers [49] Mutations in other members of the HRR pathway, as *BRIP1*, *RAD1C* and *RAD1D* have estimated lifetime risk of developing OC of 5.8%, 5.2% and 12%, respectively [50]. Genetic variations in other DNA repair genes such as *PALB2*, *RAD51*, *RAD50*, *BARD1* and *CHK2* have been also detected, although in a lower proportion of cases [51]. The term 'BRCAness' is currently used to describe tumours with deficiencies in homologous recombinant repair (HRD). The HRD may occur, in both, hereditary and sporadic OC with mutations in *BRCA1* and *BRCA2* genes, as well as in those tumours in which epigenetic silencing of *BRCA* expression is detected. BRCAness tumours show a favourable treatment response and better clinical outcome [13]. As mentioned previously, the standard of care for OC treatment depends on the histological subtype and clinical factors. Then, the platinum-based chemotherapy is currently recommended as primary systemic therapy for most patients with epithelial OC. Furthermore, the addition of targeted therapies has considerably improved the patient outcome [52]. Thus, one of the most relevant advances in the OC treatment have been the development of specific poly (ADP-ribose) polymerase inhibitors (PARPi) [53]. Importantly, PARPi treatment in OC is significantly associated with longer progression-free survival, especially in patients with BRCA-mutated tumours [54]. In fact, this targeted treatment has also modified the molecular diagnosis of OC which now includes the mutational analysis of *BRCA1/2* [55]. The PARPs are a family of enzymes involved in excision repair, a key pathway which repairs DNA single-strands breaks (SSBs) [56]. The inhibition of this pathway by specific PARPi leads the persistence of spontaneous SSBs and consequently the cell collapses [57]. It has been reported the synthetic lethality induction of PARPi in *BRCA* deficient tumour context reinforcing the important role of *BRCA1/2* status analysis in OC (Fig. 4). Furthermore, it has been recently reported that the PARPi treatment in patients with advanced OC who had a response to platinum-based chemotherapy shows a significant longer progression-free survival than those patients who received placebo [54]. This relevant study has shown that specific PARPi inhibitor could be a clinical benefit not only in patients with *BRCA1/2* mutations but also inpatients with OC with homologous recombination deficiency [58].

3.   Omics and OC: TCGA

During the last decades, the 'omics' revolution has shed light on the molecular characterisation and classification of

multiple tumour types, including OC [59]. In this regard, TCGA Research Network published in 2011 an integrated genomic analysis of HGSOCs including 489 untreated stage II-IV tumours and its corresponding normal DNA [15]. Analyses of mRNA expression, microRNA expression, promoter methylation and DNA copy number alterations were performed in the totality of the patients, while whole-exome sequencing (WES) was carried out only in 316 of them [15].

WES analysis confirmed *TP53* as the most frequently mutated gene in HGSOCs (96% of the samples), whereas lower prevalence, but statistically recurrent somatic mutations, was found in eight further genes including *BRCA1* and *BRCA2* (22% of the cases, including germline and somatic mutations) and *RB1*, *NF1*, *FAT3*, *CSMD3*, *GABRA6* and *CDK12* (2–6%).

By contrast, 113 significant focal somatic copy number alterations were identified, supporting the relevance of chromosomal instability in this type of tumour. Focal amplifications in *CCNE1*, *MYC* and *MECOM* (detected in more than 20% of tumours) and focal deletions of *PTEN*, *RB1* and *NF1* (observed at least in 2% of the tumours) were found. In addition, many other studies have been also performed as Macintyre et al. [60] who analysed 117 HGSOC samples and identified seven copy number signatures which were found correlated to mutation data and overall survival [60]. Copy number (CN) 1 signature was associated with breakage-fusion-bridge and inferior survival. CN2 was enriched in patients with mutations in *CDK12* and also presented poor outcome. Samples with mutations in *BRCA1/2* were enriched in CN3 group which was also characterised by break events and copy number changes from diploid to single copy. Similarly, CN7 also presented breaks across all chromosomes but the copy number changes were from a tetraploid state and no relation was found with *BRCA1/2* mutations. These last two CN signatures presented a good outcome that agrees with previous results that correlated *BRCA1/2* mutations with better prognosis [60]. High copy number characterised CN4 that presented aberrant PI3K/AKT signalling. In the same line, CN6 showed whole copy number changes as well as a great number of changes for small segments and this signature was associated with mutations in genes encoding proteins related with cell cycle checkpoints. Finally, signature 5 was related with chromothriptic-like events [60].

Promoter methylation TCGA analysis showed 168 genes silenced by epigenetic events in HGSOCs comparing with normal controls, including *BRCA1* in more than 10% of the cases, as previously reported [47]. This study additionally revealed four epigenetic subtypes which differed in the diagnosis-age and in the frequency of the BRCA inactivation but were not as stable as gene expression clustering subtypes [22]. Furthermore, other studies performed on HGSOC have shown a subset of 543 hypermethylated genes whose expression was significantly reduced [61]. In general, this global hypomethylation of OC is associated with higher stages, grades and mortality. Different studies have observed that the methylation profiles depend on the histotype, and then the hypomethylation is more frequent in HGSOC than in EOC or OCCC [62].

From the transcriptomic point of view, four HGSOC subgroups have been defined, which include 'immunoreactive',
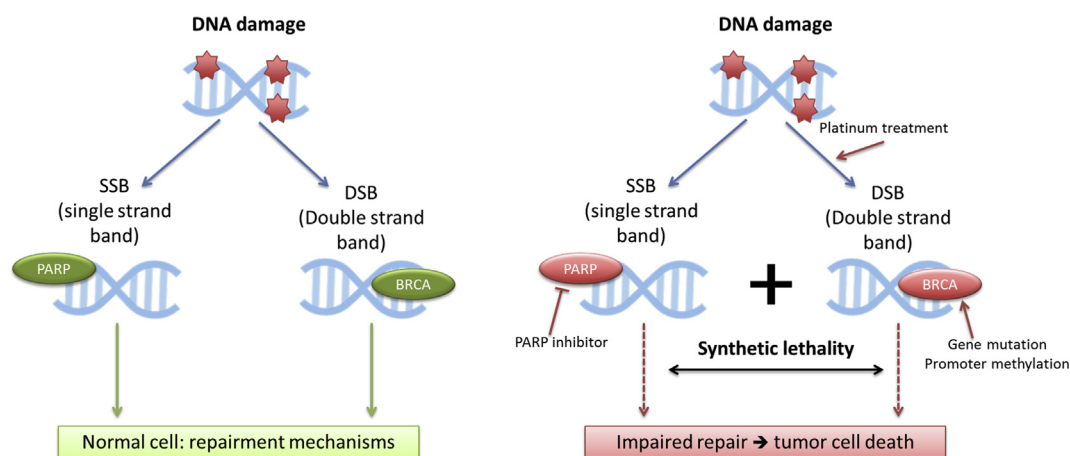
Fig. 4 – DNA damage influence in chemotherapy treatment. Different mechanisms are implicated in DNA damage repair: Poly(ADP-ribose) polymerases (PARPs) are implicated in single-strand band damage repair, while BRCA1 and BRCA2 genes are involved in double-strand band damage repair. Normal cells with DNA repair mechanisms intact can solve the damage and continue with cell division and replication. However, tumour cells with defective BRCA genes are impaired to repair double-strand band damage caused, for example, by platinum-containing chemotherapy, and consequently being highly sensitive to this type of treatment. In addition, cancer cells with BRCA defects show an increased PARP dependence, so PARP inhibitor treatment generates a synthetic lethality giving rise to an impaired DNA damage repair and tumour cell death.

'differentiated', 'proliferative' and 'mesenchymal' subtypes, although no significant differences in survival rate or an enrichment of mutation signatures was found among them [15]. Different works have used *in silico* dataset combinations for finding the best classification, but all of them obtained similar TCGA subtypes with the exception of Tan et al. [63] who using a large cohort of OC samples identified five molecular subtypes (Epi-A, Epi-B, Mes, Stem-A and Stem-B) with distinct clinicopathological features and overall survival rates. However, some similarities were related with TCGA groups. The Stem-A was similar to proliferative and Mes to mesenchymal both related with poor outcomes, and Epi-A was represented by tumours with low malignant potential. Moreover, miRNA expression analysis identified three subtypes that partially overlapped to the mRNA results. In this case, one of the clusters showed a significantly longer survival time [15]. Interestingly, a systems biology study revealed five main altered signalling pathways in HGSOCs, comprising RB (67% of cases altered), PI3K/RAS (45% of cases altered), NOTCH (22% of cases altered), HRR (51% of cases altered) and FOXM1 signalling (84% cases altered) [15].

Recently, a systemic framework for HGSOC subtyping on the basis of multi-omics data from the TCGA study has been published [64]. In this work, a total of nine subtypes based on RNA sequencing data were found, being associated with the activation and/or suppression of four biological processes (immunoactivity, hormone metabolic, mesenchymal development and MAPK signalling pathway). In addition, these subtypes overlapped with other subtypes obtained across different omics platforms, suggesting that multi-omics can be used to describe the OC profile (Fig. 5).

Nonetheless, it is worth to mention that TCGA analysed samples that included untreated primary tumours [15]. Given that most HGSOCs recur owing to platinum resistance, a recent publication based on WES analysis compared primary refractory, resistant, sensitive and matched acquired resistant tumours to further investigate in this sense [61]. Inactivation of the tumour suppressor genes *RB1*, *NF1*, *RAD51B* and *PTEN* by gene breakage was shown to contribute to acquire chemotherapy resistance, while *CCNE1* amplification was associated with primary platinum resistance. Other events implicated in platinum resistance were germline *BRCA1/2* mutation reversion, loss of *BRCA1* promoter methylation and overexpression of the drug efflux pump *MDR1*.

The next generation sequencing (NGS) molecular techniques have allowed researchers to obtain a more accurate OC molecular subtypes and their correlation with the clinic. Nevertheless, more efforts should be addressed to obtain good biomarkers with clinical applicability with the objective of improving the lives of patients with OC.

3.1. Applications of NGS in cancer: use in the clinic

The widespread characterisation of cancer genomes has increased the number of clinically relevant biomarkers for cancer risk assessment, diagnosis and treatment, including the tailoring of therapeutic strategies based on actionable molecular alterations and resistance mechanisms [65]. However, large-scale genome-sequencing studies are still unaffordable not only from the economical point of view but also because of the limitations to apply its results into the clinic. It is important to note that few of described mutations have been functionally validated, and the prediction of its consequence continues being a real challenge nowadays.

Currently, the implementation of NGS technologies into the clinic is mainly based on targeted sequencing of specific
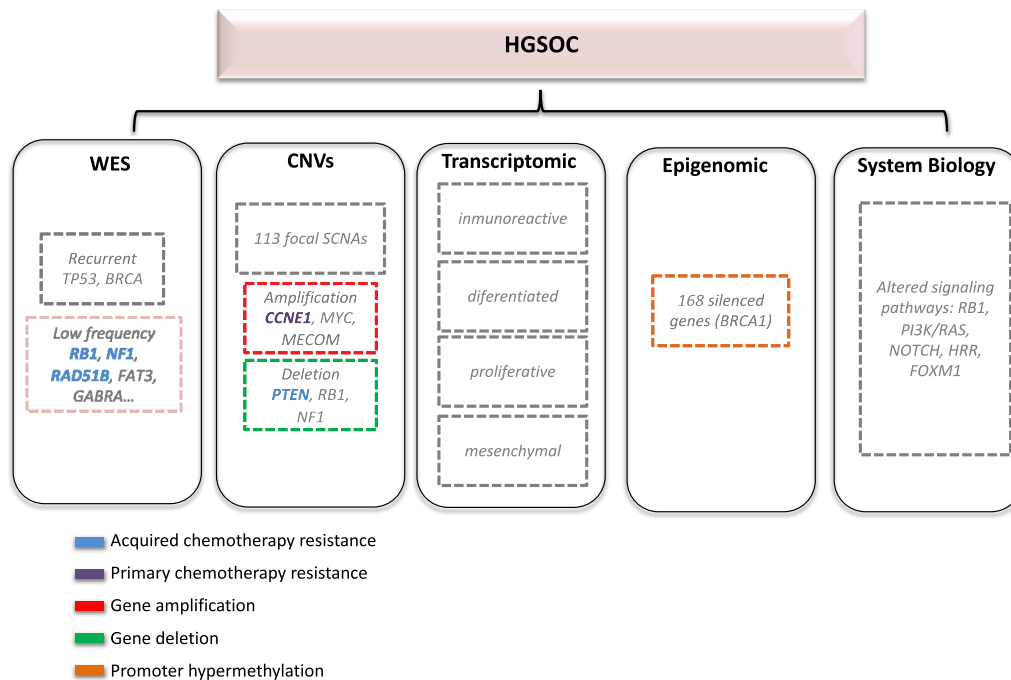
Fig. 5 — Summary of the TGCA data obtained of genomic, transcriptomic, epigenomic and system biology studies in high-grade serous ovarian carcinoma. The genes involved in a specific clinical feature are indicated in bold in the legend. Blue: genes associated with acquired chemotherapy resistance, purple: genes associated to primary chemotherapy resistance, red: gene amplification, green: gene deletion, orange: makes reference to the hypermethylation status of indicated genes. HGSOC, high-grade serous ovarian carcinoma; WES, whole-exome sequencing; CNVs, copy number variations; TCGA, The Cancer Genome Atlas.

gene panels owing to the cost and the complexity of data analysis are significantly lower. A clear example has been indicated in the 2015 National Comprehensive Cancer Network guidelines, which recommends the use of NGS gene panels for patients with hereditary OC without mutations in the high-penetrance genes [66]. The use of this kind of panels allows the simultaneous analysis of multiple genes in several samples with low DNA input and high sensitivity. In addition, these platforms can be applied in the analysis of formalin-fixed paraffin-embedded samples, favouring their utility in the clinical setting. Nevertheless, the selection of suitable genes for panel design, the need of additional validation, together with long-term storage and retrieval of data are still challenging [67].

4. New challenges for NGS: unravelling intra-tumour genetic heterogeneity

Intra-tumour phenotypic heterogeneity has been observed by pathologists since the early days of cancer knowledge, which led to propose the existence of a genetic heterogeneity implicated in the clonal evolution of tumours [68—70]. However, it has not been observed until the last years, with the development of NGS technologies, that intra-tumour heterogeneity (ITH) at a genetic and genomic point of view has been well demonstrated and deeply characterised. Initial sequencing studies comparing subsets of regionally [71,72] and temporally [73] separated areas from the same tumour confirmed the existence of ITH not only in the primary lesions

but also in metastatic regions. These findings highlight the relevance of considering ITH when carrying out genetic and genomic studies, especially when these are aimed to diagnostic procedures or to uncover possible therapeutic strategies [40]. In fact, ITH has been described in numerous solid tumour types [74]. The clinical implication of ITH remains a controversial issue nowadays, being necessary further studies to analyse its real impact on cancer progression, risk of relapse and treatment response and resistance [75]. Nevertheless, recent studies have suggested that ITH could be an independent prognostic factor of disease progression and survival [76,77].

4.1. ITH in OC

OC encompasses several tumour subgroups with distinct clinicopathological and molecular features and prognosis; however, it is treated as a single disease so far [74]. The advances in the NGS and in other 'omics' studies have revealed the intrinsic complexity within the OC subtypes and within an individual patient with OC [78—87]. First, studies based on loss of heterozygosity data by microsatellite and single-nucleotide polymorphism analyses demonstrated widespread ITH in primary ovarian tumours, suggesting a monoclonal origin [78]. This process was also found between metastatic lesions, being clonally related with the primary tumour [79]. These studies proposed a model in which OCs have a common clonal origin, evolving to polyclonal tumours due to genetic divergence. The role derived from ITH in cisplatin resistance was also

described by array comparative genomic hybridisation analysis, showing pre-existing minor resistant clones even before treatment [80].

Further analyses using more sensitive techniques, including WES and targeted massive parallel sequencing, have led to a deeper understanding of ITH in OC with a single nucleotide resolution. Most studies agreed on the presence of extensive genomic and transcriptomic ITH in OCs, showing different degrees of heterogeneity depending on the patient [81—85]. These studies also subscribed the presence of sub-clones in the untreated primary tumour that would give rise to recurrent disease, although the possibility of metastasis spread has been also proposed [83]. Interestingly, the quantification of ITH may have a predictive value, showing a decreased progression-free survival and overall survival for those patients with extensive ITH [83]. Nonetheless, the major differences regarding samples from the same patients were found between distant metastases and ovarian tumours [86]. One way to elucidate the ITH consists in the use of single-cell RNA sequencing technique that allows the examination of a unique tumour cell to gain insights into the tumour biology [88].

This ITH represents another degree of complexity and has even been blamed for the failure of treatment. The tumour heterogeneity between patients and in tumours reinforces the need of a more personalised treatment not only in OC but also in other cancer types. In this sense, the analysis of ascites appear to be a way to overcome ITH because most of somatic mutations, SCNAs and methylation patterns are represented in ascitic cells [84]. Ascites is accessible and often therapeutically removed from patients and supposes a valuable source of tumour material, and consequently, information on the molecular perturbations of the tumour. Thus, it could isolate and analyse malignant ascites for identifying potential therapeutic targets, as well as prognostic and predictive biomarkers [89].

4.2. Non-invasive biopsies: liquid biopsies and others

There has been considerable interest in the use of non-invasive liquid biopsies to monitor and diagnose early relapse in patients with OC [90]. Up to now, although the study of CA-125 and HE4 levels in blood has received FDA approval as predictive biomarkers and for the detection of recurrent disease after the completion of first-line treatment, several studies show their low sensitivity in early stages of the disease [91]. Currently, the determination of other circulating tumour-derived material known as liquid biopsy has emerged strongly in the cancer diagnosis. This involves the analysis of circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), and circulating cell-free mRNA/microRNAs and circulating extracellular vesicles [92]. In OC, recent studies in CTCs have demonstrated a significant association between the presence of CTCs and worse survival [93]. Regarding ctDNA, detections of different genetic and epigenetic alterations were correlated with diagnosis, prognosis and response to treatment. Methylation status of COL23A1, C2CD4D and WNT6 in ctDNA has been described as predictors of response to platinum-based neoadjuvant chemotherapy [94]. In addition, the

detection of TP53 mutations in plasma ctDNA could suggest earlier response to chemotherapy [95]. In this line, BRCA1/2 reversions detected in patient's tumour are also observed in paired ctDNA [96]. Instead, extracellular vesicles such as tumour exosomes which may contain mRNA, miRNA and proteins carry information of the original tumour cells and have been considered as useful diagnosis biomarkers. Different exosomal protein biomarkers have been identified in OC but their role in progression, occurrence and treatment response is still unclear [90]. Despite the fact that it has been proposed as a solution in other types of cancer [97,98], preliminary analyses with this source of genetic material in OC allowed the detection of mutations but with high variations in sensitivity among patients [81]. Another important limitation in liquid biopsies in OC nowadays is the requirement of validation of the potential identified biomarkers.

As aforementioned, beyond to the limitations of the liquid biopsies in the typification of ITH or more specifically in the identification of a potential biomarker in OC, ascites provides an opportunity to understand the drug resistance and mechanisms of tumour progression [89]. Ascites represents a rich source of tumour cells and the local microenvironment of ovarian tumours and it can be used to study various biological aspects of the underlying tumour. Previous evidence showed that malignant ascites can stimulate the growth and invasion of OC cells [4,99]. Thus, understanding the composition of ascites may advance in understanding the mechanisms triggering malignant ascites and develop some novel therapies of OC.

5.   Conclusion remarks

OC is the fourth leading cause of death in women. Despite the high efforts to obtain accurate classification and treatment for them, patients with OC face many challenges such as chemoresistance, high ITH, therapy resistance among others. The last NGS studies in OC have revealed new molecular subgroups associated to specific molecular features, although it is still necessary to clarify their clinical impact. They have also shown the high rate of intra-tumour molecular heterogeneity in OCs. Advances in the NGS analyses of alternative biopsies such as ascetic liquid could be considered a new tool for the study of molecular complexity of OCs and open new avenues into more effective strategies to combat the disease.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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