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Studying platinum sensitivity and resistance in high-grade serous ovarian cancer: Different models for different questions

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ABSTRACT

High-grade serous ovarian cancer (HGSOC) has the highest mortality rate among all gynecological cancers. Patients are generally diagnosed in an advanced stage with the majority of cases displaying platinum resistant relapses. Recent genomic interrogation of large numbers of HGSOC patient samples indicated high complexity in terms of genetic aberrations, intra- and intertumor heterogeneity and underscored their lack of targetable oncogenic mutations. Sub-classifications of HGSOC based on expression profiles, termed 'differentiated', 'immunoreactive', 'mesenchymal' and 'proliferative', were shown to have prognostic value. In addition, in almost half of all HGSOC patients, a deficiency in homologous recombination (HR) was found that potentially can be targeted using PARP inhibitors. Developing precision medicine requires advanced experimental models. In the current review, we discuss experimental HGSOC models in which resistance to platinum therapy and the use of novel therapeutics can be carefully studied. Panels of better-defined primary cell lines need to be established to capture the full spectrum of HGSOC subtypes. Further refinement of cell lines is obtained with a 3-dimensional culture model mimicking the tumor microenvironment. Alternatively, *ex vivo* ovarian tumor tissue slices are used. For *in vivo* studies, larger panels of ovarian cancer patient-derived xenografts (PDXs) are being established, encompassing all expression subtypes. Ovarian cancer PDXs grossly retain tumor heterogeneity and clinical response to platinum therapy is preserved. PDXs are currently used in drug screens and as avatars for patient response. The role of the immune system in tumor responses can be assessed using humanized PDXs and immunocompetent genetically engineered mouse models. Dynamic tracking of genetic alterations in PDXs as well as patients during treatment and after relapse is feasible by sequencing circulating cell-free tumor DNA and analyzing circulating tumor cells. We discuss how various models and methods can be combined to delineate the molecular mechanisms underlying platinum resistance and to select HGSOC patients other than *BRCA1/2*-mutation carriers that could potentially benefit from the synthetic lethality of PARP inhibitors. This integrated approach is a first step to improve therapy outcomes in specific subgroups of HGSOC patients.

1. Introduction

Due to the late occurrence of symptoms, epithelial ovarian cancer is diagnosed at an advanced stage in the majority of cases. Therefore, ovarian cancer has the highest mortality rate among all gynecological cancers (Siegel et al., 2012). In the 1980s, first-line treatment of ovarian cancer consisted of cisplatin in combination with cyclophosphamide. Soon after, carboplatin was introduced, which was found to exert a more favorable toxicity profile than cisplatin and a comparable survival outcome (du Bois et al., 2003; Ozols et al., 2003). In the 1990s, paclitaxel replaced cyclophosphamide as part of first-line regimen after a significant survival benefit was proven (McGuire et al., 1996). Current standard of care for patients with advanced stage ovarian cancer consists of surgical debulking of tumor mass combined with neo-adjuvant or adjuvant treatment with 3-weekly paclitaxel and carboplatin for 6 cycles (Aebi et al., 2008). To date, the extent of cytoreduction after debulking surgery is considered the most important prognostic factor for survival when combined with platinum-based regimens (Bristow et al., 2002). Despite high initial response rates, the majority of

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advanced stage ovarian cancer patients will relapse. The progression free interval is a direct predictor of sensitivity to second-line platinum therapy for these patients (Bolis et al., 1994; Gore et al., 1990). The majority of relapses are platinum resistant (*i.e.* with a progression free interval of less than 6 months); these relapses exhibit little to no long-lasting responses to other agents. Consequently, survival rates have not largely improved for advanced stage ovarian cancer over the last two decades, with a 5-year survival rate of 19–28% in advanced stage disease (Siegel et al., 2012). Since 2011, the vascular endothelial growth factor (VEGF) targeting drug bevacizumab has been approved as additional first-line therapy for ovarian cancer. Recently, a benefit in overall survival was shown, however, only in patients with poor prognosis (Oza et al., 2015). Other angiogenesis targeting drugs such as tyrosine kinase inhibitors (TKI) targeting the VEGF receptor are currently under investigation (Mahner et al., 2015).

A major advance in achieving upfront selection of ovarian cancer patients is the radical change in classification of ovarian cancer, which is no longer conceived as a single entity. It is now widely accepted that histological subtypes of ovarian cancer, *i.e.* high-grade serous (HGSOC), low-grade serous, endometrioid, mucinous and clear cell carcinoma, are formed through distinct routes of tumorigenesis, sub-divided into low-grade and high-grade groups (Landen et al., 2008). Low-grade, slow growing tumors encompass histological subtypes such as low-grade serous, endometrioid, mucinous and some clear cell carcinomas. They are known to harbor mutations in *KRAS*, *BRAF*, *PTEN*, *CTNNB1* and *TGFBR2* (Cancer Genome Atlas Research Network, 2011; Shih Ie and Kurman, 2004). The presence of *KRAS* and *BRAF* mutations solely in low-grade serous carcinomas and borderline tumors, but not in HGSOC, consistently implies that these tumors develop through independent pathways (Singer et al., 2003). The tumor suppressor gene *ARID1A* is mutated in 46% and 30% of clear cell and endometrioid ovarian cancers, respectively (Wiegand et al., 2010). ARID1A is a component of the large ATP-dependent chromatin remodelling complex SWI/SNF, required for epigenetic transcriptional activation of genes that are normally repressed by chromatin modifications. Interestingly, a synthetic lethal interaction of *ARID1A* inactivation with the inhibition of histone methyltransferase EZH2 was found, opening up new therapeutic possibilities for these ovarian cancer subtypes (Bitler et al., 2015). In contrast, no *ARID1A* mutations were found in HGSOC, indicating a different pathogenesis. HGSOC is characterized by an almost ubiquitous presence of *TP53* mutations (Cancer Genome Atlas Research Network, 2011). In 20% of HGSOC cases, a mutation in *BRCA1* or *BRCA2* is found, predisposing these women to hereditary breast and ovarian cancer. BRCA1 and BRCA2 are both important players in homologous recombination (HR). The nucleotide excision repair (NER) system and HR are the most common pathways for repairing platinum-induced DNA adducts. In addition to *BRCA1/2* mutations, aberration of the HR pathway can also occur *via* *BRCA1* promoter hypermethylation (Baldwin et al., 2000; Catteau et al., 1999; Chan et al., 2002; Esteller et al., 2000). Recently, DNA samples from a large group of HGSOC patients were characterized using whole-genome sequencing. In half of the tumors, germline or somatic mutations in genes associated with the HR pathway or methylation of the *BRCA1* promoter were observed (Patch et al., 2015). In good agreement with these observations, a significant degree of copy number aberrations is present, suggesting disruption of DNA repair pathways in an early stage of tumor development (Berns and Bowtell, 2012; Cancer Genome Atlas Research Network, 2011). In line with the ensuing genomic instability, a high degree of intra-tumor heterogeneity with remarkable genomic alterations was observed using derived samples of primary tumor and metastatic sites (Bashashati et al., 2013; Hoogstraat et al., 2014). Remarkably, HGSOC harbored relatively few other inactivating mutations in *NF1*, *RB1*, *PTEN* and *RAD51B* (Cancer Genome Atlas Research Network, 2011; Patch et al., 2015).

2. Response to platinum-based chemotherapy and resistance mechanisms

The histological subtypes are known for their different sensitivities to platinum-based chemotherapy. The majority of HGSOC patients responds well to first-line chemotherapy, whereas clear cell, mucinous and low-grade serous tumors are known to be more chemoresistant (Itamochi et al., 2008; Pisano et al., 2005). HGSOC patients harboring *BRCA1* or *BRCA2* mutations are known to be sensitive towards platinum-based chemotherapy, resulting in a significantly improved response rate and longer survival (Zhong et al., 2015). A further sub-classification was made for HGSOC based on gene expression profiles (Cancer Genome Atlas Research Network, 2011; Tothill et al., 2008). These expression subtypes (termed 'differentiated', 'immunoreactive', 'mesenchymal' and 'proliferative') were shown to have prognostic value. Among these subtypes, patients with the mesenchymal signature have the poorest outcome. However, this classification is not exclusive and multiple signatures can be found within a single tumor (Verhaak et al., 2013).

Resistance to platinum-based chemotherapy is currently believed to be a Darwinian evolutionary process, driven by selective pressure of chemotherapy (Gerlinger and Swanton, 2010). It has been postulated that at the time of ovarian cancer presentation, minor subpopulations of intrinsically resistant cancer cells already pre-exist (Cooke and Brenton, 2011). Because of the high level of genomic instability in HGSOC, the development of intrinsically resistant sub-clones during the numerous cell divisions before clinical presentation seems a plausible hypothesis. This hypothesis is supported by the observation that some patients are minimally responsive to treatment. Residual disease after surgery is the strongest prognostic factor for survival despite a partial or complete clinical response to first-line platinum-based chemotherapy (Bristow et al., 2002). This also supports the notion of intrinsically resistant sub-clones being present at the time of presentation. Alternatively, one cannot exclude the possibility that acquired drug resistance emerges during treatment as a result of high genomic instability in HGSOC. Assuming that drug resistance mutations occur in a stochastic manner, increased residual tumor mass implies a higher tumor cell load, which increases the chances of acquired drug resistant subclones.

Platinum-based chemotherapy induces toxic damage to dividing cells including cancer cells by the formation of inter- and intra-strand DNA adducts that block DNA replication. The molecular mechanisms underlying resistance to platinum-based chemotherapy have been extensively studied. Several mechanisms were identified including reduced intracellular cisplatin accumulation due to alterations in transmembrane transport, activation of cell growth-promoting and DNA damage repair pathways, aberrant DNA methylation, enhanced epithelial-to-mesenchymal transition and reduced endocytosis of cisplatin (Balch et al., 2004; Galluzzi et al., 2012, 2014; Shen et al., 2012). However, this has not resulted in any translational relevance for the clinical setting. Several resistance mechanisms have been identified by whole-genome sequencing of tumor samples obtained from a large set of HGSOC patients with resistant, refractory or relapsed disease (Patch et al., 2015). Although no platinum-induced driver mutations were found in relapsed tumors, reversion of *BRCA1/2* mutations and loss of *BRCA1* promoter methylation was observed. However, within one patient, multiple exclusive reversion events were found at different sites, again emphasizing the spatial and temporal heterogeneity of HGSOC (Schwarz et al., 2015). Furthermore, an autopsy case displayed a shift in subtype, with the primary tumor being an