Evolution of platinum resistance in high-grade serous ovarian cancer

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High-grade serous ovarian cancers account for most ovarian-cancer mortality. Although this disease initially responds well to platinum-based chemotherapy, relapse and progression to chemotherapy resistance are frequently seen. Time to relapse after first-line therapy is a predictor of response to secondary platinum treatment: more than 12 months is associated with high chance of a secondary response, whereas relapses within 6 months generally indicate platinum resistance. In this Personal View we discuss whether patterns of response, relapse, and the development of drug resistance in high-grade serous ovarian cancers are related to distinct underlying molecular and cellular biological characteristics. In particular, we propose that rapid relapse with platinum-resistant disease is due to minor subpopulations of intrinsically resistant cancer cells at presentation.

Introduction
Understanding of the molecular basis of ovarian cancer has changed rapidly over the past 3 years. The recognition that different histological subtypes are independent disease entities has been accompanied by substantial progress in identifying the cell of origin, precursor lesions, and defining driver mutations for each subtype. Most cases of ovarian cancer are high-grade serous cancers, which account for most ovarian-cancer mortality. High-grade serous cancers are initially extremely sensitive to platinum-based chemotherapy, with response rates to chemotherapy and surgery being as high as 85%. Paradoxically, though, outcomes are poor, with 5-year survival being less than 30% and relapses typically being characterised by the progressive development of drug resistance.

Despite more than 10 years of array-based expression and genomic profiling, no robust signature of drug response has been identified for ovarian cancers. In this Personal View, we compare clinical and genomic data for high-grade serous ovarian cancers with those for haematological cancers, in which drug resistance may be explained by genetic heterogeneity within cancers and evolutionary change.

High-grade serous ovarian cancer has several unique clinical features. The name ovarian cancer suggests that this disease arises from a putative precursor lesion in the normal ovarian surface epithelium. Data indicate, however, that precursor and early invasive lesions can arise in the fimbriae of the fallopian tubes. The fimbriae provide a large epithelial surface area for malignant transformation. The high frequency of apparent primary ovarian masses can be explained by local spread to the ovarian surface. Early metastasis by shedding is then anatomically and mechanistically favourable. Distant metastasis in the abdomen is due to distribution of cancer cells by ascites fluid. This process means that high-grade serous ovarian cancer cells do not have to undergo extensive vascular invasion for metastasis, unlike in other common epithelial cancers, and most patients have stage III–IV disease at diagnosis.

High-grade serous ovarian cancer is also unique in that TP53 mutations are present in almost 100% of cancers, along with extensive rearrangement of the genome. TP53 mutation is an early driver event, which is illustrated by the presence of foci containing TP53 mutations or aberrant accumulations of P53 as precursor lesions in fallopian tubes containing tubular intraepithelial carcinoma. Additionally, mutations can be seen in regions of tubular intraepithelial carcinoma contiguous to foci, which is consistent with disease progression.

In contrast to other common epithelial cancers, high-grade serous ovarian cancers are initially hypersensitive to platinum chemotherapy. Platinum-refractory patients, who have progressive disease during initial treatment, constitute only about 14% of cases (SCOTROC1 data, Paul J, personal communication). Of patients with initial response, up to 75% relapse and are classified according to likelihood of response to re-treatment with platinum-based agents (figure 1). Those who relapse within 6 months of completing initial treatment have response rates to secondary treatment of less than 10% and, therefore, are classified as being platinum resistant. Patients who relapse more than 12 months later are termed platinum sensitive, and more than 60% respond to platinum-based chemotherapy. Patients who relapse between 6 and 12 months are classified as being partially platinum sensitive, as 27% of these patients will respond to a second use of platinum (figure 1).

Initial response to platinum
Platinum induces cross-linking within and between DNA strands and subsequent single-strand and double-strand breaks. The double-strand breaks require repair by the error-prone non-homologous end-joining pathway (G phase) or conservative repair through error-free homologous recombination (G1 and S phases). Cells deficient in either BRCA1 or BRCA2 cannot induce homologous recombination and, therefore, are highly sensitive to apoptosis triggered by platinum-induced DNA damage. Consistent with this feature is that patients with high-grade serous ovarian cancer due to hereditary BRCA1 or BRCA2 mutation respond extremely well to platinum treatment and have substantially longer survival than do non-carriers.
Less than 20% of high-grade serous ovarian cancers are hereditary,15 yet initial platinum response rates are around 85%, which suggests that homologous recombination dysfunction could also occur in sporadic cases. The early loss of P53 function seen in sporadic cancers could create a permissive environment for loss of BRCA1 or BRCA2 function (or other phenotypes of DNA-repair deficiency) that would otherwise lead to apoptosis owing to checkpoint activation.16 Many sporadic high-grade serous cancers show inactivation of BRCA1 or BRCA2 through methylation, mutation, or alteration of other regulators in the BRCA2 pathway.17–20 Only 7–9% of sporadic ovarian cancers have BRCA1 mutations leading to inactivation of BRCA1,17,20 and 4% have BRCA2 mutations.20 In a further 8–13%, however, BRCA1 silencing via promoter methylation is seen17,20 and 13% lose BRCA2 expression, although only rarely because of promoter methylation.16 BRCA2 can also be disrupted through silencing of its upstream regulator, FANCE, by promoter methylation (13–21%) and amplification of C11orf30 (also known as EMSY; 17%).19,22,23 The Cancer Genome Atlas project has refined these estimates and shown that 51% of high-grade serous ovarian cancers have compromised homologous recombination-based repair.24 Acquired platinum resistance mechanisms, therefore, need to overcome hypersensitivity conferred by loss of homologous recombination.

The use of in-vitro drug treatment to induce platinum resistance in cell lines has identified decreased import, increased export, and trapping of cisplatin in intracellular complexes as mechanisms of resistance.25 Most of these mechanisms, however, are not clinically important in high-grade serous ovarian cancers. On the basis of the hypothesis that functional homologous recombination is required for DNA repair and, therefore, survival after exposure to platinum, restoration of BRCA1 and BRCA2 function was identified as a resistance mechanism in patients who have hereditary BRCA1 or BRCA2 mutations and relapse with platinum-resistant disease.26–28 Secondary mutations can restore wild-type aminoacid sequences and in-frame deletion of the original mutation can restore at least partial protein function.26–28 In-vitro knockdown of BRCA2 in cells that have acquired platinum resistance by secondary BRCA2 mutation almost completely restores platinum sensitivity and completely restores sensitivity to PARP inhibition, which indirectly affects the same homologous recombination repair pathway.29 In a mouse mammary tumour model, where Brca1 had been irreversibly removed in mammary epithelial cells by cre-mediated deletion and, therefore, could not undergo a reversion mutation, platinum resistance did not develop even after repeated exposure. Resistance to alternative drugs with different mechanisms of action could, however, be reliably induced.30 These findings suggest that hypersensitivity to platinum treatment from loss of Brca1 function in this model was dominant over any potential acquired resistance mechanism.

The evolution of resistance

The paradigms we suggest for the evolution of resistance traits in cancer have parallels with classic experiments in which resistance to antibiotics was evolved in bacterial populations. Resistance resulted from the outgrowth of rare, highly resistant mutants that were present at low frequency before antibiotic exposure.31 In wild-type bacterial cultures, variation in antibiotic sensitivity follows a normal distribution, which encompasses a tenfold range of antibiotic concentrations.31 In a sufficiently large culture, however, spontaneously resistant mutants that can survive doses of antibiotic several hundred times greater than the normal inhibitory concentration exist at low frequencies. When selective pressure from antibiotic is applied, the outgrowth of resistant subclones is inevitable because of their relatively high fitness in the new environment. Acquired drug resistance in bacteria can result, therefore, from one genetic event that yields a large phenotypic effect, and is present owing to normal genetic variation generated by the error rate intrinsic to cell division.

Clinical evidence supports selection for drug resistance in cancers. Point mutations conferring resistance to imatinib have been reported to be present in small numbers of cells at presentation in some cases of acute lymphocytic leukaemia and chronic myeloid leukaemia.32–34 Cells with these mutations dominate at relapse.32–34 Furthermore, in acute lymphocytic leukaemia, retrospective quantification of resistance mutations in archived samples collected at presentation has shown that the time to relapse decreases with increasing concentration of resistant subclones at presentation.32 Mutations in EGFR and in-frame deletions within EGFR exon 19 are activating driver events found in up to 13% of non-small-cell lung cancers. Addition to the EGFR oncogene makes these cancers sensitive to tyrosine kinase inhibitors, including gefitinib and erlotinib.35–37 In patients with EGFR mutant carcinoma, 38% also have low-frequency Thr790Met mutations in EGFR before treatment, which reverse sensitivity and are significantly associated with decreased progression-free survival.38 In
these examples, genetic heterogeneity within cancers facilitates therapy-driven selection for an intrinsically resistant cancer subclone and inevitably results in relapse and drug resistance. Whether resistance mutations have any selective advantage in the absence of therapy, or whether they are merely low-frequency events until the application of chemotherapy is unclear.

Whether resistance to non-targeted therapies, including platinum chemotherapy, follows the same paradigm as targeted therapies is unclear. However, the restoration of BRCA1 or BRCA2 function as a mechanism of drug resistance is analogous to the development of resistance to targeted therapies, as a single genetic event provides a large phenotypic effect by switching between sensitivity and resistance. Whether these secondary BRCA1 or BRCA2 mutations exist at the time of presentation is unknown. In studies of solid tumours where the resistance event is unknown, however, clones with relapse-specific mutations can be found at low frequencies in samples taken at presentation. In a case of lobular breast cancer, relapse-specific mutation events were retrospectively identified at a frequency of 1–13% in the primary tumour, which occurred 7 years before the relapse clone reached dominance.39 In three cases of high-grade serous ovarian cancer, presentation and resistant relapse did not show a linear genetic relation, which means that the relapse genotype cannot have arisen through direct descent from the dominant presentation clone.40 Similar to the findings for leukaemias, these data are most consistent with relapse resulting from outgrowth of a small cancer cell population that preferentially survived first-line treatment, and support resistance existing in a proportion of cancer cells before the first treatment.

In view of mutation being a stochastic process, the probability of a carcinoma harbouring a resistance mutation depends on the number of cell divisions it has undergone and the mutation rate.41 Far more cell divisions are likely to occur in the years between the onset of cancer and presentation than occur during the relatively short period of treatment. For example, mathematical models of pancreatic cancer evolution suggest at least 15 years are required from cancer initiation to metastasis,42 yet median survival is less than 6 months in patients who have metastatic disease at diagnosis. At autopsy, advanced pancreatic cancers are genetically heterogeneous and up to 52% of mutations are subclonal, including mutations in potential cancer genes such as OVCH1.43 The primary cancers contain multiple genetically divergent clones, which seed independent metastases at different sites.42,43 Thus, extensive genetic heterogeneity precedes metastasis and provides a wide range of genotypes and phenotypes on which therapy can selectively act. Cancer cells with substantial genomic instability and proliferation rates, as seen in high-grade serous ovarian cancer, show high rates of cell death and, therefore, need to undergo a large number of cell divisions to generate sufficient bulk to be clinically detectable, which makes acquisition of resistance likely.44 Thus, the presence of intrinsically resistant cells in a high number of high-grade serous ovarian cancers at presentation would be unsurprising.

**Platinum-resistant high-grade serous ovarian cancer**

Whether platinum-resistant relapse in patients with high-grade serous ovarian cancer arises owing to low-frequency mutations that exist within the tumour bulk at the time of first treatment (figure 2) needs to be explored. Small populations of resistant cells can continue to grow after treatment and will repopulate the tumour bulk, owing to the removal of competing cells. Patients harbouring such cells will, therefore, relapse quickly and be resistant to further treatment. The proportions of patients with advanced disease who are classified as platinum sensitive or platinum resistant is hard to assess because many studies include patients with non-serous cancer and low-stage disease and use combination therapies. Median progression-free survival for patients treated with carboplatin alone or with cisplatin combination therapy (which have equal response rates), however, is 16-1 months from diagnosis.
for patients with serous and non-serous cancers and, therefore, just over half of patients fall into the platinum-resistant or partially sensitive categories (figure 1). Even among patients with platinum-sensitive cancer, response rates are only around 50%. In an early study of 54 patients with ovarian cancer in whom response after first-line platinum-based chemotherapy was partial or complete, only 30% responded to the second use of platinum. These data support the existence of resistance mutations before treatment in many cases of ovarian cancer, since platinum resistance at first relapse occurs more frequently than does a second response. The collection of sequential samples from patients at relapse will be critical in addressing this question, as even with next-generation sequencing the small populations of putative resistant subclones could be below the threshold of detection at presentation.

Platinum-sensitive high-grade serous ovarian cancer

In the absence of any pre-existing resistant cells within the cancer mass, any residual disease or regrowth will be platinum sensitive and, therefore, will enable re-treatment with platinum chemotherapy (figure 2). Whether treatment-sensitive cells that persist despite exposure to platinum comprise a random proportion or a specific subtype of cancer cells is unclear. Without intrinsic resistance mechanisms cancer can persist after chemotherapy for many reasons, including cancer-cell dormancy, the presence of cancer stem cells, or inaccessibility to drugs. The dynamics of chronic myeloid leukaemia after imatinib treatment reflected those in a model where differentiated but not stem cells were depleted after chemotherapy, and emergence of true resistance in the stem-cell compartment was a stochastic process. Genetic heterogeneity, including diversity of selectable traits, such as growth rate, has been found within the leukaemic stem-cell compartment.

Relapse in patients with platinum-sensitive, high-grade, serous ovarian cancers is caused by regeneration of tumour bulk by residual platinum-sensitive cells. The observation that platinum cannot cure cancer in the Brca1-null mouse tumour model supports this theory, as treatment-sensitive cells persist even in the absence of acquired resistance mechanisms. Cell division during regrowth can lead to the occurrence of resistance mutations. Whether further relapses are sensitive or resistant to platinum, therefore, reflects the stochastic nature of mutation (figure 2).

Refractory high-grade serous ovarian cancer

In around 14% of patients with high-grade serous ovarian cancer, no objectively measureable response to initial platinum therapy can be seen. Several potential explanations exist for drug resistance being a dominant trait in chemotherapy-naive cancer. First, driver mutations could be downstream of the drug target. For instance, lung tumours sensitive and refractory to tyrosine kinase inhibitors have disruption of the EGFR/MAPK signalling pathway, which leads to increased cellular proliferation. In sensitive cases the mutations affecting this pathway are in EGFR, which is the target of the tyrosine kinase inhibitors. In refractory cases, however, the mutations are in KRAS, which is downstream of EGFR in the signalling cascade. Cancer with pathway-activating mutations downstream of the target of the tyrosine kinase inhibitor will not respond. Second, a drug-resistance mutation might have occurred early and become widespread through random drift or hitchhiking. Platinum-refractory disease, however, is seen arguably more frequently than might be anticipated for a chance event, especially in view of the potentially small window of opportunity that might be available for such a mutation to arise in a common ancestor of bulk disease. Third, the processes and pathways that lead to refractory disease might be fundamentally different from those that lead to initially sensitive high-grade serous ovarian cancer (figure 2). Resistance might be a secondary effect of a driver mutation that enables the clone to reach dominance in the absence of treatment, or refractory disease could be due to a subset of high-grade serous ovarian cancers that do not lose homologous recombination proficiency during carcinogenesis. Paradoxically, refractory disease might be defined by the absence of mutations associated with platinum sensitivity, namely BRCA1 and BRCA2 defects, rather than the presence of platinum-resistance mechanisms.

What remains surprising is that despite fundamental clinical differences between disease refractory and sensitive to platinum, no robust biomarkers of poor response exist. A genomics study of refractory disease showed amplification of CCNE1 as a preadaptation that conferred primary platinum resistance. The definition of refractory disease in this study, however, was extended to include patients who relapsed within 6 months of first-line therapy because treatment was primary surgery and adjuvant chemotherapy rather than neoadjuvant chemotherapy. Thus, truly refractory patients were difficult to identify.

In view of the hypothesis that refractory high-grade serous ovarian cancers evolve via distinct molecular pathways compared with initially sensitive cancers, and that the differences affect mechanisms of genomic instability, whether genomic profiles vary between refractory and responsive patients needs to be established. Defects in repair by homologous recombination result in deletions and translocations, whereas overexpression of cyclin-E1 leads to chromosomal instability—the gain and loss of whole chromosomes. In a comparative genetic analysis of 33 refractory or resistant and 52 initially responsive patients, the refractory and resistant group had fewer recurrent copy number gains and losses than did the sensitive group (31 vs 40). No data on whether chromosome instability or aneuploidy was increased in refractory cases, however, were reported.
for this dataset, and more work is needed to assess whether refractory and sensitive disease truly have different patterns of genomic instability.

Conclusions

Advances in sequencing technologies are providing the tools to test whether evolutionary models, integrated with analyses of genetic heterogeneity within cancers, can explain the clinical patterns of response and resistance in high-grade serous ovarian cancer. If they can, measures of genetic diversity could have predictive potential. Genetic heterogeneity presents major challenges to personalisation of treatment, particularly for strategies to prevent loss of platinum sensitivity. Deep sequencing of large series of high-grade serous ovarian cancers is necessary to identify small subpopulations and to increase sensitivity for detection of resistance mutations at presentation. The analysis of subpopulations might be more important than profiling of bulk disease at presentation in the identification of prognostic indicators and new targets to overcome platinum resistance, because at presentation disease is generally platinum sensitive and not a major cause of death. A particular challenge will be the collection of the sequential clinical samples necessary to test these hypotheses. Collection of multiple samples at the time of initial surgery and at each relapse or change in treatment is required to track molecular changes. Studies of platinum-refractory disease are particularly challenging and post-mortem programmes might be needed to make rapid progress, as was the case for pancreatic cancer.

Contributors

SC researched and wrote the paper. JB reviewed and revised the manuscript.

Conflict of interest

We declare that we have no conflicts of interest.

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