Genetic profiling in ovarian cancer

C. Marchetti[1], J.A. Rauh-Hain[2], P. Benedetti Panici[1], M. Birrer[2]

Abstract
Epithelial ovarian cancer (EOC) is a heterogeneous disease that consists of a wide variety of histological types with diverse molecular alterations and is the most lethal gynaecological cancer. Despite debulking surgery and platinum- and taxane-based chemotherapy, the prognosis remains poor. Traditional histological and clinical prognostic factors are insufficient to capture the complex cascade of events that drive the heterogeneous clinical behaviour of this disease. Recently, genomic analysis has confirmed this heterogeneity and has been shown to be a powerful tool to identify dysregulated gene expression, aberrantly activated pathways, and to discover unique molecular signatures among the different histological types of EOC. The promising results obtained to date confirm that genetic analysis might be useful in developing an individualized approach to the management patients with advanced EOC. Identification of the gene-expression profile of a patient could allow better understanding of the specific disease pathogenesis, enabling clinicians to predict an individual response to conventional treatments, ultimately allowing for patients to be triaged to more effective therapies specifically targeting the genetic pathway driving the disease. Major efforts are needed to acquire more accurate gene signatures that can predict chemotherapy resistance and to investigate new targeted small-molecule drugs active with favourable toxicity profiles.

Key words: cancer treatment, epithelial ovarian cancer, gene expression profiling, genetic analysis

Introduction

Epithelial ovarian cancer (EOC) is the second most common gynaecological malignancy and the leading cause of death from gynaecological cancer in the Western World [1]. While more than 90% of patients with cancer confined to the ovary will be alive five years after diagnosis, the majority of patients are diagnosed with disseminated disease, and have only a 30% likelihood of survival at five years, despite the use of radical surgery and platinum- and taxane-based chemotherapy [2]. Current clinical characteristics employed as prognostic indicators include age, International Federation of Gynaecologists and Obstetricians (FIGO) stage, comorbidities, histological tumour grade and subtype, and initial results of surgery. However, these factors are insufficient to capture the important individual variations at the time of diagnosis; consequently all women receive similar chemotherapy regimens, although they will not display the same response to treatment and outcome. Considering the highly diverse population and the variable response to currently standardized therapeutic regimens, a better understanding of the genetic and molecular mechanisms underlying ovarian cancer pathogenesis and chemotherapy resistance is needed to allow optimized and individualized patient care with the aim of improving patient outcome. The advances in genomic technologies have permitted comprehensive genetic profiling of EOC. These technologies allow the simultaneous study of a significant number of genes and provide multi-gene signatures that can classify histologic tumour subtypes and that correlate with clinical outcomes. Furthermore, targeted therapies of some of these pathways have been designed or are under active investigation. In this review, we summarize the contributions of gene expression profiling in the management and treatment of EOC and discuss how this technology could become a useful tool in the management of women with this disease.

Heterogeneity of epithelial ovarian cancer: histology and grade

EOC is a heterogeneous disease that consists of a wide variety of histological types with diverse molecular al-

[1]Department of Gynaecological and Obstetrical Sciences and Urological Sciences, Università di Roma "Sapienza", Rome, Italy.
[2]The Gillette Center for Gynecologic Oncology, Massachusetts General Hospital, Boston, Massachusetts, USA.

Correspondence to: Dr. Claudia Marchetti,
Viale del Policlinico 155, 00161 Roma, Italy.
Phone: +39 338 4652744 – Fax: +39 06 49972564;
E-mail: clamarchetti@libero.it

CANCER BREAKING NEWS 2014;2(1):30-36

terations. The most common type of EOC is serous, followed by endometrioid, mucinous, and clear-cell, which represent 50–60%, 25%, 4%, and 4% of all ovarian tumours, respectively [3]. Of note, ovarian clear-cell carcinomas are considered rare in the Western World, but are significantly more common in Japan, accounting for 30% of EOC [4]. Serous cancers are thought to arise from the fallopian tube. Mucinous tumours are cystic tumours with mucin-secreting epithelial cells approximating either endocervical or colonic epithelium. Endometrioid and clear-cell carcinomas are thought to originate in part from endometriosis. Of note, mucinous and clear-cell tumours present frequently at an early stage; however, when these tumours present with distant metastasis, patients have a lower rate of response to platinum and taxane-based chemotherapy. In contrast, the more prevalent high-grade serous (HGSOC) and endometrioid types typically present at an advanced-stage, and are more chemosensitive (>70% response rate), although prognosis remains poor. A different subgroup of ovarian neoplasm diseases, that have a favourable prognosis even in advanced-stage disease, are the 'low malignant potential' (LMP or borderline) tumours. These are characterized by the presence of nuclear atypia and micropapillary morphology without invasion of the ovarian stroma. Of interest, serous low-grade (grade 1) ovarian tumours (LGSOC) are more similar to LMP tumours than HGSOC, and are typically less aggressive and slow-growing, with an 85% 5-year survival [5].

Significant efforts over the last decade in genomic analysis of EOC have identified global gene expression profiles and signalling pathways, which have helped to define the molecular features of each of the major subtypes (Table 1). In particular, distinct genomic abnormities (gene amplification, deletion and mutation) have been shown among the various subtypes of ovarian cancer. For example, there is a high prevalence of TP53 mutations among women with HGSOC (>90% frequency), as well as a high prevalence of mutations involving *BRCA1* and *BRCA2* [6]. In contrast, mucinous adenocarcinoma and LGSOC are characterized by aberrant Ras/MAPK signalling due to the prevalence of activating *KRAS* and *BRAF* mutations [7-9]. Mutations of *CTNNB1*/β-catenin and *PTEN*, resulting in hyperactivation of *Wnt* and *PI3K*/Akt signalling, are common in endometrioid tumours, but are rare in the other three major histotypes [10, 11]. Mutations of *PIK3CA* and corresponding *PI3K*/Akt hyperactivation are most frequently observed in clear-cell carcinomas [12-14]. Approximately 50% of clear-cell carcinoma cases also present loss-of-function mutation of the *ARID1A* gene which functions as a tumour suppressor [15-17].

The identification of distinct genetic and molecular patterns among different histologic subtypes has allowed

Table 1. Features of the five major histotypes of ovarian carcinoma

Histotype	Incidence	Presentation	Precursors	Molecular/Genetic abnormalities	Response to chemotherapy	Prognosis
Clear-cell carcinoma	4% (24% in Japanese population)	Initial stages	Endometriosis	*ARID1A*, *PIK3CA*, *PTEN*, microsatellite instability	Low	Intermediate
Endometrioid carcinoma	20–25%	Often confined to pelvis	Endometriosis	*PTEN*, *PIK3CA*, *CTNNB1*	Good	Intermediate
Mucinous carcinoma	4%	Often confined to ovary Histopathological similarity to gastric carcinomas (intestinal type)	Cystadenoma	*KRAS/HER-2*	Low	Favourable
Low-grade serous carcinoma	5–10%	Moderately diffused in the abdominal cavity/ advanced stage	Serous borderline tumour	*BRAF/KRAS* Frequency of *BRCA 1/2* mutations presumed low; chromosomally stable	Intermediate	Intermediate
High-grade serous carcinoma	60%	Present at older age than other histotypes and higher stage (ascites common)	Serous tubal intraepithelial carcinoma (STIC)	Germline and somatic *BRCA 1/2* mutations; *TP53*; chromosomally unstable	High	Poor

the development of targeted therapies of specific pathways, in order to improve treatment efficacy and patient outcome. In clear-cell ovarian cancer, several novel therapeutic approaches have been proposed. A gene expression signature of clear-cell ovarian cancer has recently been derived from clear-cell ovarian cancer cell lines, and involves genes associated with oxidative stress, glyconeogenesis, angiogenesis and cytokine activation [18]. This has led to the development of clinical trials to evaluate the use of anti-angiogenic agents and mammalian target of rapamycin (mTOR) inhibitors in patients with this histotype.

For LGSOC characterized by a poor response to conventional platinum- and taxane-based chemotherapy, targeted therapy against the hyperactivated Ras/Raf/MEK/Erk pathway has been proposed as a novel approach to this disease. A phase II clinical trial (GOG-239) evaluating selumetinib (AZD6244), an oral non-ATP competitive small molecule inhibitor of MEK1/2 in patients with recurrent ovarian LGSC has recently been completed [19]. In this study, selumetinib showed substantial activity in recurrent LGSCs (objective response rate [ORR] 15%, with another 65% of patients presenting with stable disease [SD]), and a substantial improvement in progression-free interval (PFI) was observed (29 weeks *vs* 11 months), with acceptable toxicity [19]. Clinical trials are currently investigating the role of MAPK pathway inhibitors in patients with LMP or LGSOC.

Gene expression profiling and patient prognosis

Several studies have been conducted to identify gene expression signatures associated with survival of patients with EOC [20-23]. However, prognostic signatures remain difficult to assess, and these efforts have not generated reproducible and clinically relevant prognostic models. Spentzos et al. [23], by using oligonucleotide microarrays, identified, in a training set of samples from 34 patients, a prognostic 115-gene signature (Ovarian Cancer Prognostic Profile [OCPP]). When applied to a validation set of another 34 samples, the OCPP distinguished patients with a favourable overall survival and was found to be an independent prognostic factor when tested in a multivariate analysis with other known risk factors such as age, tumour stage, tumour grade, and debulking status. The profile consists of 70 genes overexpressed in the unfavourable outcome group and 45 genes underexpressed in the favourable group. In the favourable prognosis group oestrogen-pathway-related genes (such as oestrogen receptor binding site associated antigen 9) were overexpressed; conversely, in the unfavourable prognosis group, genes encoding for angiogenesis-related cytokines, receptor tyrosine kinases, mesenchymal markers (e.g. fibronectin, fibromodulin and vimentin), and proinvasive enzymes (i.e. plasminogen activator inhibitor type 1) were overexpressed. Similarly, Berchuck et al. [20] analyzed 54 patients with advanced-stage EOC and tried to identify a gene expression model that could distinguish short-term (<3 years) and long-term (>7 years) ovarian cancer survivors. The resulting gene expression profile was confirmed to be prognostic when tested in an independent set of 68 patients with EOC, previously reported by Spentzos et al. T-cell differentiation protein, which has been shown to be associated with chemoresistance, anti-apoptotic heat shock protein and lysophospholipase II were considered to be markers of short-term survival. Of note, Berchuck et al. found similarities in genetic profile between early-stage ovarian cancers and a subset of advanced-stage tumours with favourable outcome; accordingly patients with unfavourable advanced EOC showed genetic profiles that were distinct from those of patients with early-stage ovarian cancer, supporting the hypothesis that advanced EOC is a biologically heterogeneous EOC subtype, distinct from early-stage disease [20].

Recently, a systematic validation of all gene expression-based prognostic models for late-stage, high-grade serous ovarian cancer has been published [24]. Fourteen prognostic models were identified that were compared and ranked by validation in 10 published datasets comprising 1,251 primarily HGSOC patients. Twelve published models had 95% confidence intervals (CI) of the C-index (interpretable as the probability that a patient predicted to be at lower risk than another patient will survive longer than that patient: its expected value is 0.5 for random predictions and 1 for a perfect risk model) that did not include the null value of 0.5. Four top-ranked models achieved overall validation C-indices of 0.56 to 0.60 and shared negative correlation with expression of immune response pathways. This database has been used to generate a gene expression signature predictive of survival and debulking status in late-stage ovarian cancer [25]. The study involved meta-analytic techniques using integrated data from 13 publicly available datasets including 1,525 patients. The identified survival gene signature stratified patients into high- and low-risk groups exhibiting significant or near-significant differences in overall survival in each of six training data sets and seven validation data sets, including patients with early-stage disease. Use of the gene signature stratified patients with late-stage disease into high- and low-risk groups with median overall survival of 29.6 *versus* 60.1 months (haz-

ard ratio [HR]=2.19; 95% CI=1.84–2.61). Importantly, HR represented an increase of 0.36 (p=0.04) over the HR for low *versus* high risk derived by The Cancer Genome Atlas (TCGA) consortium gene signature (HR=1.83; 95% CI=1.54–2.17). The original TCGA signature and an updated TCGA signature had similar performance in distinguishing low and high risk (HR difference=0.00; 95% CI=−0.33 to 0.34) [24-26]. Pathway analysis of the newly derived signature showed enrichment of TGF-β and PDGF signalling in poor prognosis patients. Nonetheless, the authors of the study concluded that the survival signature, although significantly improved over other published signatures, does not meet standard requirements in order to effectively alter clinical management of patients with advanced-stage EOC.

Gene profiling and surgery

Optimal cytoreductive surgery is the most important prognostic factor in patients with EOC, numerous studies have demonstrated that patients who are optimally cytoreduced, enjoy prolonged progression-free and overall survival compared with those who are suboptimally debulked [27]. Several authors have hypothesized that optimal debulking is not directly related to the technique or efforts of the surgeon but that resectability of disease may reflect the underlying tumour biology [25, 28]. A recent study, looking at genes differentially expressed between patients with optimally *versus* suboptimally debulked advanced-stage EOC, showed that among the 120 differentially expressed genes, *RARB*, a gene coding for the retinoid acid receptor-beta, and *MAP2K4*, a metastasis-suppressor gene coding for a mitogen-activating protein kinase, were upregulated in optimally debulked tumours, perhaps conferring a low ability to local and distant extension [28]. From these 120 genes, a 32-gene model was derived that predicted the debulking status in 72.7% of advanced-stage cases in cross-validation, and 60% of five additional early-stage cases in an independent validation [28]. These findings suggest that molecular differences exist between patients with optimally and suboptimally debulking procedures. This predictive model, could allow a more rational selection for an initial debulking surgery or for less extensive surgery and neoadjuvant chemotherapy. More recently, Reister et al. [25] characterized a gene expression signature, which identifies advanced-stage serous tumours that could not be optimally debulked to ≤1 cm of residual tumour. It was shown that suboptimal debulking is correlated with the upregulation of genes that support tumour dissemination, decreasing the likelihood of total surgical removal and optimal cytoreduction (Figure 1). From an initial group of identified genes in a debulking signature, six were validated by immunohistochemistry and quantitative reverse-transcription polymerase chain reaction (PCR) in two independent cohorts of 78 and 179 patients. POSTN, CXCL14, FAP, NUAK1, PTCH1 and TGFBR2 were validated by quantitative reverse-transcription PCR (all $p<0.05$) as independent predictors of debulking status in one cohort, with a model using all genes classifying 76.9% of all samples correctly (area under the curve [AUC]=0.76; 95% CI=0.66–0.87). Protein expression of three of the associated proteins, POSTN, CXCL14, and pSmad2/3 (a surrogate marker of TGF-β pathway activation) was validated by immunohistochemistry as an independent predictor of debulking status in the other cohort. The sum of immunohistochemistry intensities for these three proteins provided an index that classified 92.8% of samples correctly in high- and low-risk groups for suboptimal debulking (AUC=0.89; 95% CI=0.84–0.93) [25]. This means that the debulking signature, identified in this study, will have clinical utility if the 93% accuracy of the immunohistochemistry tool observed in the 179-patient validation cohort will be confirmed in a prospective validation. This is the strongest evidence to date for the existence of a biologic basis and a predictive gene signature for debulking ability of ovarian tumours.

Gene profiling and chemotherapy

Multiple studies have been conducted in an attempt to define and identify a genetic profile corresponding to chemotherapy response [29-32]. Since Selvanayagam et al. in 2004 [31] published their first small but significant experience, many other attempts have been made, but results are still not conclusive. In particular, in 2005, Spentzos et al. [32] identified a 93-gene signature known as the Chemotherapy Response Profile (CRP) predictive of pathological complete response to chemotherapy by profiling 24 tumours from patients who had undergone second-look surgery. Gene families with potential relevance to chemosensitivity were represented including genes that regulate apoptosis (*BAX*, *Ephrin B2*, *Ephrin B3*), cell cycle control and DNA repair (*RB1*, *RASSF1*). When applied to a separate validation set of 36 patients who had not undergone second-look surgery, the CRP distinguished between patients with unfavourable *versus* those with favourable survival outlook. Their prognostic 115-gene profile for overall survival, described above [23], was determined from the same data but, interestingly, there was no overlap between the gene signatures identified by the CRP and those in the previously described OCPP, although both demonstrated similar prognostic value. Finally, the combination of the predictors yield-

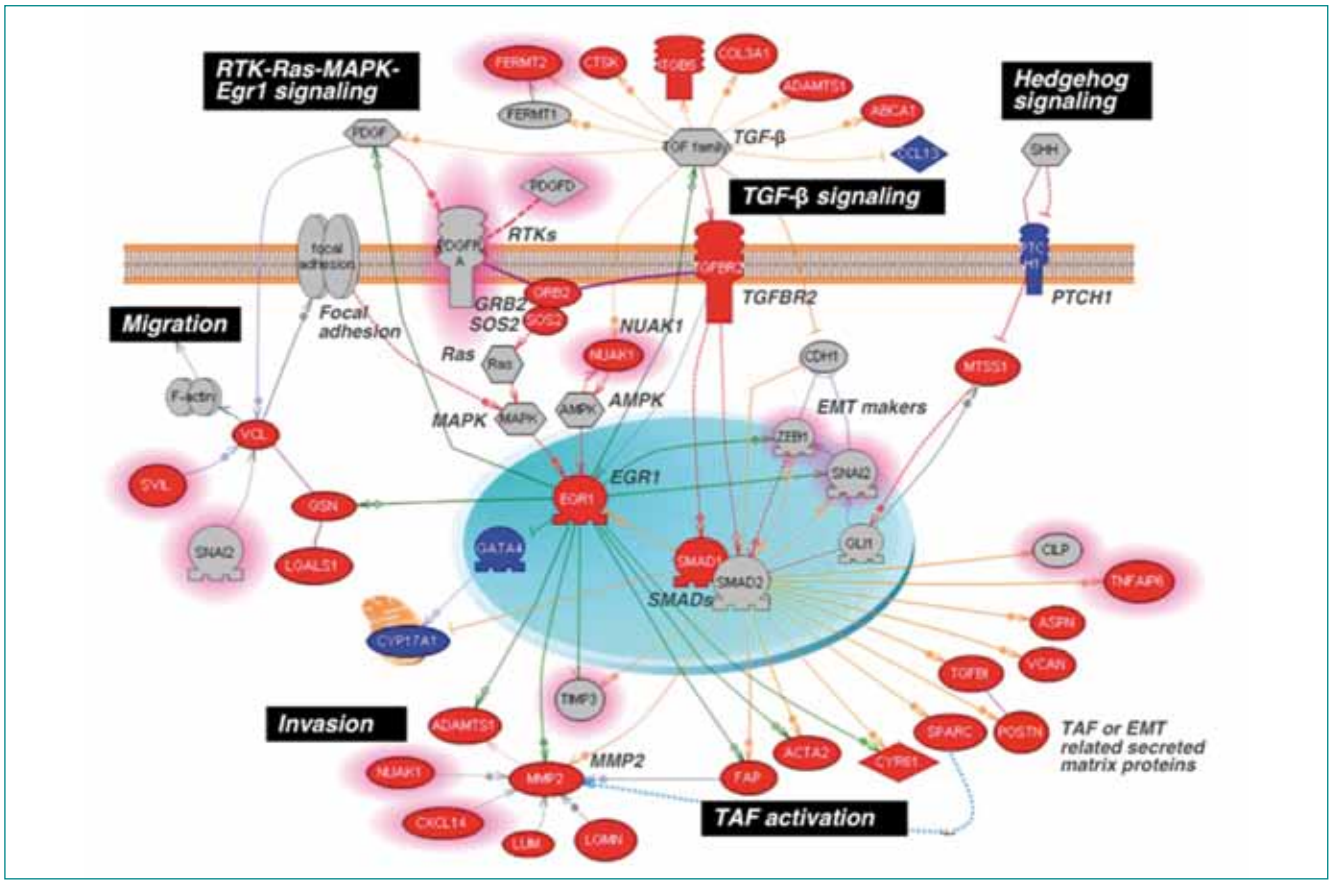

Fig. 1. Pathway analysis of the debulking signature [25]: using the Pathway Studio 7.1 (Ariadne Genomics) software and a novel signature of 200 debulking-associated genes, several pathways statistically significantly associated with suboptimal debulking surgery have been identified. RED: genes overexpressed in tumours that were subsequently suboptimally debulked; BLUE: genes overexpressed in tumours with optimal cytoreduction; PINK: genes with predictive power toward poor prognosis based on the meta-analysis.

ed better prognostic discrimination than either predictor alone. Helleman et al. [29] profiled a training set of patients (24 samples including 5 non-responders and 19 responders) using microarray and reverse transcription-polymerase chain reaction; this allowed the identification of 69 differentially expressed genes, from which a 9-gene predictor was defined. The predictor was validated in an independent set of 72 patients (9 non-responders and 63 responders) profiled using quantitative real-time PCR with a sensitivity of 89% and a specificity of 59%. Of note, Helleman et al. assessed a clinical response according to World Health Organization criteria and defined as non-responders only patients whose tumours showed progression [29]. This is different to the analysis by Spentzos et al. [32], who assessed response pathologically and considered all patients without pathological complete response as non-responders. This could explain the low number of genes shared by the two profiles. Finally, Jazaeri et al. [30] compared the profiles of 21 pre-treatment chemosensitive tumours (complete response to chemotherapy, progression free interval ≥13 months) with those of 24 pre-treatment chemoresistant tumours (presence of residual disease after chemotherapy or complete response with relapse <6 months after initiation of chemotherapy). A total of 85 genes were differentially expressed between the two groups, with a modest difference between the mean expression levels of the two groups (≤2-fold). These genes represent those potentially involved in intrinsic chemoresistance, including proliferation genes that were overexpressed in sensitive tumours. A predictive model of response to chemotherapy based on the 9 most differentially expressed genes yielded a rate of accurate prediction of 77.8% in leave-one-out cross-validation. Presuming that an important part of chemoresistance is acquired during treatment and that tumours obtained shortly after chemotherapy are enriched in resistant clones, a 7,585-gene microarray was next used to compare the 45 pre-chemotherapy samples with 15 post-chemotherapy samples collected during interval or second-look surgery. Consistent with this hypothesis, fewer and smaller magnitude gene expression differences were found between post-

chemotherapy and primary chemoresistant samples than between post-chemotherapy and primary chemosensitive samples. Proliferation genes were underexpressed in post-chemotherapy samples, supporting the idea that a decreased proliferation state may also be involved in the development of acquired chemoresistance. The lack of significant overlap between the gene list differentiating primary chemosensitive and chemoresistant tumours and that differentiating each of them from post-chemotherapy samples suggests that non-overlapping molecular pathways are likely involved in intrinsic and acquired chemoresistance. Several extra-cellular matrix-related genes were overexpressed in post-chemotherapy samples when compared with primary chemosensitive samples, suggesting that stromal-epithelial interactions or the extra-cellular matrix may be involved in acquired chemoresistance in ovarian cancer.

Genomic analysis of high-grade sserous ovarian cancer

Extensive molecular analysis of women with EOC has provided a more detailed genomic profile [6]. These genomic data include alterations in DNA copy number, methylation of gene promoter regions, gene expression profiles, and mutation patterns. Most of the molecular analyses has focused on high-grade serous ovarian cancer, the most common and lethal ovarian neoplasia. Noted above, the results suggest that HGSOC is very different from other epithelial cancers, presenting with high frequency mutational inactivation of the p53 tumour-suppressor gene along with *BRCA1/2*. These mutations result in a severe abnormality in DNA repair, which causes extensive DNA copy number abnormalities including gene deletion and amplifications, many of which are thought to be important for the development of the tumour [33].

Mechanisms of DNA repair can be grouped into: 1) single-stranded breaks, which involved the activity of polyadenosine diphosphate-ribose polymerase (PARP) and include base excision repair (BER), nucleotide excision repair and mismatch excision repair; or 2) those following double-strand breaks (DSB), which comprise non-homologous end-joining (NHEJ) and homologous recombination repair (HRR) utilising a complex containing *BRCA1/2*. The defect in DNA repair found in HGSOC is in itself targetable. PARP is a complex enzyme initially identified in 1963 involved in DNA repair [34]. PARP is involved in DNA repair utilizing the BER pathway. DNA damage stimulates the catalytic activity of PARP 1 [35, 36], which by two zinc finger motifs in the DNA-binding domain binds to DNA single-stranded DNA-binding protein (SSB), thus activating the BER machinery to repair the single-stranded break. Inhibition of PARP blocks the BER pathway leading to the generation of DSB. Normal cells can readily repair this DNA damage through the HRR pathway, mediated by the *BRCA1/2* complex. However, cells with deficient HRR, such as BRCA-mutated ovarian cancer cells, without both copies of *BRCA1* or *BRCA2*, have to use alternative pathways for repair such as NHEJ; this is error-prone and eventually leads to cell death. Normal cells (heterozygous for the defect with one functional allele) retain *BRCA1/2* protein expression and maintain the HRR pathway. This difference between tumour and normal cells means that PARP inhibitors kill tumour cells selectively compared with the effects in normal cells and this has led to the concept of 'synthetic lethality', which describes the situation whereby one pathway is mutated in the cancer cell and another pathway can be inhibited [37, 38]. Drugs that inhibit PARP have been developed and this targeted approach has demonstrated remarkable success in high-grade serous ovarian cancers; phase I and II trials have demonstrated a high response rate in patients with germline-mutated disease [39-45]. Interestingly, it is now well established that 10–15% of women with ovarian cancer has germ-line *BRCA1* or *BRCA2* mutations [46, 47]. In addition, data from TGCA [6] indicate that up to 50% of women with high-grade EOC could have functional loss of proteins involved in the HRC pathways of DNA repair and behave like *BRCA1/2* mutant cancers, even in the absence of germ line *BRCA* mutations. This is the phenomenon called 'BRCAness' and the identification of the BRCA-like EOC population could define a larger population of patients who might potentially benefit from PARP inhibition, due to genetic instability.

Conclusions

EOC is a heterogeneous disease that consists of a wide variety of histological types with diverse molecular alterations. Recent genomic analysis has confirmed this heterogeneity. Furthermore the promising results obtained to date confirmed that genetic analysis might be used in the near future to develop an individualised approach to the management of patients with advanced EOC. Identification of the gene-expression profile of a patient could allow a better understanding of the pathogenesis of disease, enabling clinicians to predict response to conventional treatments. This may ultimately allow for patients to be triaged to more effective therapies as well as receiving chemotherapy agents that specifically target the driving genetic pathway. Major efforts should

be directed towards the acquisition of more accurate gene signatures that can predict chemotherapy resistance and, in the meantime, new small-molecule drugs active against specific pathways, with favourable toxicity profile should be investigated.

References

1. Seidman JD, Yemelyanova A, Cosin JA, et al. Survival rates for international federation of gynecology and obstetrics stage III ovarian carcinoma by cell type: a study of 262 unselected patients with uniform pathologic review. Int J Gynecol Cancer. 2012;22:367-71.
2. Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006;95(Suppl 1):S161-92.
3. Feeley KM, Wells M. Precursor lesions of ovarian epithelial malignancy. Histopathology. 2001;38:87-95.
4. Oncology Committee of Japan Society of Obstetrics and Gynecology. Annual report of the patients with ovarian cancers. Acta Obstet Gynaecol Jpn. 2010;62:827-910.
5. Lachance JA, Shutter J, Atkins KA, et al. Utilization of a uniform grading system for interpreting serous ovarian cancer. Am J Obstet Gynecol. 2008;199:189 e1-6.
6. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-15.
7. Enomoto T, Weghorst CM, Inoue M, et al. K-ras activation occurs frequently in mucinous adenocarcinomas and rarely in other common epithelial tumors of the human ovary. Am J Pathol. 1991;139:777-85.
8. Gemignani ML, Schlaerth AC, Bogomolniy F, et al. Role of KRAS and BRAF gene mutations in mucinous ovarian carcinoma. Gynecol Oncol. 2003;90:378-81.
9. Singer G, Oldt R, 3rd, Cohen Y, et al. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. 2003;95:484-6.
10. Obata K, Morland SJ, Watson RH, et al. Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. Cancer Res. 1998;58:2095-7.
11. Wright K, Wilson P, Morland S, et al. Beta-catenin mutation and expression analysis in ovarian cancer: exon 3 mutations and nuclear translocation in 16% of endometrioid tumours. Int J Cancer. 1999;82:625-9.
12. Anglesio MS, Carey MS, Kobel M, et al. Clear cell carcinoma of the ovary: a report from the first Ovarian Clear Cell Symposium, June 24th, 2010. Gynecol Oncol. 2011;121:407-15.
13. Campbell IG, Russell SE, Choong DY, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res. 2004;64:7678-81.
14. Kuo KT, Mao TL, Jones S, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol. 2009;174:1597-601.
15. Jones S, Wang TL, Shih Ie M, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010;330:228-31.
16. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. Oncogene. 2009;28:1653-68.
17. Wiegand KC, Shah SP, Al-Agha OM, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010;363:1532-43.
18. Stany MP, Vathipadiekal V, Ozbun L, et al. Identification of novel therapeutic targets in microdissected clear cell ovarian cancers. PLoS One. 2011;6:e21121.
19. Farley J, Brady WE, Vathipadiekal V, et al. Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase 2 study. Lancet Oncol. 2013;14:134-40.
20. Berchuck A, Iversen ES, Lancaster JM, et al. Patterns of gene expression that characterize long-term survival in advanced stage serous ovarian cancers. Clin Cancer Res. 2005;11:3686-96.
21. Hartmann LC, Lu KH, Linette GP, et al. Gene expression profiles predict early relapse in ovarian cancer after platinum-paclitaxel chemotherapy. Clin Cancer Res. 2005;11:2149-55.
22. Lancaster JM, Dressman HK, Whitaker RS, et al. Gene expression patterns that characterize advanced stage serous ovarian cancers. J Soc Gynecol Investig. 2004;11:51-9.
23. Spentzos D, Levine DA, Ramoni MF, et al. Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol. 2004;22:4700-10.
24. Waldron L, Haibe-Kains B, Culhane AC, et al. Comparative meta-analysis of prognostic gene signatures for late-stage ovarian cancer. J Natl Cancer Inst. 2014;106.
25. Riester M, Wei W, Waldron L, et al. Risk prediction for late-stage ovarian cancer by meta-analysis of 1525 patient samples. J Natl Cancer Inst. 2014;106.
26. Verhaak RG, Tamayo P, Yang JY, et al. Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest. 2013;123:517-25.
27. Bristow RE, Tomacruz RS, Armstrong DK, et al. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol. 2002;20:1248-59.
28. Berchuck A, Iversen ES, Lancaster JM, et al. Prediction of optimal versus suboptimal cytoreduction of advanced-stage serous ovarian cancer with the use of microarrays. Am J Obstet Gynecol. 2004;190:910-25.
29. Helleman J, Jansen MP, Span PN, et al. Molecular profiling of platinum resistant ovarian cancer. Int J Cancer. 2006;118:1963-71.
30. Jazaeri AA, Awtrey CS, Chandramouli GV, et al. Gene expression profiles associated with response to chemotherapy in epithelial ovarian cancers. Clin Cancer Res. 2005;11:6300-10.

31. Selvanayagam ZE, Cheung TH, Wei N, et al. Prediction of chemotherapeutic response in ovarian cancer with DNA microarray expression profiling. Cancer Genet Cytogenet. 2004;154:63-6.
32. Spentzos D, Levine DA, Kolia S, et al. Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol. 2005;23:7911-8.
33. Wei W, Dizon D, Vathipadiekal V, Birrer MJ. Ovarian cancer: genomic analysis. Ann Oncol. 2013 Dec;24(Suppl 10):x7-15.
34. Chambon P, Weill JD, Mandel P. Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun. 1963;11:39-43.
35. de Murcia JM, Niedergang C, Trucco C, et al. Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci U S A. 1997;94:7303-7.
36. Szabo C, Zingarelli B, O'Connor M, Salzman AL. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci U S A. 1996;93:1753-8.
37. Ashworth A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol. 2008;26:3785-90.
38. Mendes-Pereira AM, Martin SA, Brough R, et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med. 2009;1:315-22.
39. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet. 2010;376:245-51.
40. Fong PC, Yap TA, Boss DS, et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol. 2010;28:2512-9.
41. Gelmon KA, Tischkowitz M, Mackay H, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol. 2011;12:852-61.
42. Kaye SB, Lubinski J, Matulonis U, et al. Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer. J Clin Oncol. 2012;30:372-9.
43. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366:1382-92.
44. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15:852-61.
45. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germ-line *BRCA1/2* mutation: An open-label phase II study. J Clin Oncol. 2013 31:abstr 11024.
46. Pal T, Permuth-Wey J, Betts JA, et al. *BRCA1* and *BRCA2* mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104:2807-16.
47. Zhang S, Royer R, Li S, et al. Frequencies of *BRCA1* and *BRCA2* mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol Oncol. 2011;121: 353-7.