by the time the cancer is diagnosed. For these patients, combined modality treatment is necessary. First-line chemotherapy with a platinum-paclitaxel combination given every 3 weeks for 6 cycles has yielded response rates of greater than 80%. However, the median progression-free survival has been only 18 months in these patients and, in most with advanced cancer, the disease eventually relapses and the patient dies. Studies evaluating various cytotoxic agents in recurrent ovarian cancer have generally shown response rates of 10% to 28%.2 This has prompted the search for novel strategies for treatment of ovarian cancer.

Microarray technology has led to a better understanding of the underlying biology of carcinogenesis of ovarian cancer.

Microarray-Based Gene Expression Studies in Ovarian Cancer

Hye Sook Chon, MD, and Johnathan M. Lancaster, MD, PhD

Background: DNA microarray technology is a powerful genomic tool that has the potential to elucidate the relationship between clinical features of cancers and their underlying biological alterations.

Methods: We performed a systemic search in PubMed and Medline databases for recently published articles. The search terms used included “genome-wide,” “microarrays,” “ovarian cancer,” “prognosis,” “gene expression profiling,” “molecular marker,” and “molecular biomarker.”

Results: Genome-wide expression profiling using DNA microarray technology has enhanced our understanding of the genes that influence ovarian cancer development, histopathologic subtype, progression, response to therapy, and overall survival.

Conclusions: Gene expression profiling has demonstrated its utility in ovarian cancer research. It is hoped that with technologic, statistical, and bioinformatic advances, the reliability and reproducibility of this technique will increase, spawing clinical applications that may enhance our understanding of the disease and our ability to care for patients in the future.

Introduction

Despite recent improvements in treatment, ovarian cancer remains the No. 1 cause of death among gynecologic cancers in the United States. In 2010, approximately 21,880 new cases of ovarian cancer were anticipated, and approximately 13,850 women died of the disease.1 In more than 90% of patients with localized disease, surgery alone is curative. However, in most patients, the tumor has disseminated beyond the ovaries...
a time. Measuring the expression of thousands of genes at the same time using microarrays has answered many questions that were impossible to resolve previously. Multigene signatures have been widely adopted in the area of breast cancer treatment. Gene expression assays are now used in daily clinical practice in the care of many patients who are newly diagnosed with breast cancer. The Oncotype DX assay (Genomic Health, Inc, Redwood City, CA) is a 21-gene assay that aims to quantify risk of distant recurrence at 10 years for a subset of women with early-stage breast cancer. The assay includes genes related to cell proliferation (Ki-67, STK15, survivin, cyclin B1, MYBL2), invasion (stromelysin 3, cathepsin L2), HER2 (GRB7, Her2), estrogen (ER, PR, BCL2, SCUBE 2), as well as GSTM1, CD68, BAG1, and several reference genes (beta actin, GAPDH, RPLPO, GUS, and TFRC). MammaPrint (Agendia, Amsterdam, The Netherlands), which is the only breast cancer recurrence assay approved by the

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<td>Gene expression signatures coupled with in vitro drug sensitivity assays to predict sensitivity to various common cytotoxic chemotherapeutic drugs</td>
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<td>Jazaeri et al</td>
<td>45 cDNA</td>
<td>178 genes identified to represent transcripts differently expressed between postchemotherapy tumors and all primary tumors irrespective of intrinsic chemosensitivity</td>
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LMP = low malignant potential
US Food and Drug Administration (FDA), comprises 70 genes that aim to stratify patients into either low or high risk of distant recurrence.

In ovarian cancer, gene expression profiles have so far been used to examine differential gene expression patterns between normal and tumor cells as a way to distinguish between histology subtypes. Several studies have sought to identify gene expression signatures that correlate with clinical outcome, to determine which genes affect survival and relapse, and to generate biomarkers that could predict patient response to chemotherapy. In this review, we examine recently published reports, we summarize the developments in gene expression profiling as they relate to clinical application in ovarian cancer and discuss how gene expression profiling can be used as a potential prognostic and therapeutic tool in the treatment of ovarian cancer (Table).

**Basic Principles and Techniques in DNA Microarray Data Analysis and Interpretation**

DNA microarray is a multiplex technology and consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing a specific DNA sequence (known as probes). This can be a short section of a gene or other DNA element that is used to hybridize a cDNA sample. Hybridization is usually detected and quantified by detection of a fluorophore-, silver-, or chemiluminescence-labeled target to determine relative abundance of nucleic acid sequences in the target (Figure). The process of measuring gene expression or activity is called expression analysis or expression profiling.

Multiple array platforms vary depending on the kind of probes utilized (e.g., short-oligonucleotide, long-oligonucleotide, cDNA), the production method (e.g., in situ polymerization, spotting, microbeads), or the labeling method. Different types of arrays can address different biological assays. Spotted arrays are suitable for applications that require small to medium numbers of probes, such as focused genotyping, bacterial diagnostics, and gene expression analysis. In situ synthesized arrays and random bead arrays are suitable for applications that require medium to large numbers of probes, such as genome-wide screens for single nucleotide polymorphisms.

In oligonucleotide microarrays, the probes are short sequences designed to match parts of the sequence of known or predicted open reading frames. Oligonucleotide arrays are produced by printing short oligonucleotide sequences designed to represent a single gene or family of gene splice variants by synthesizing this sequence directly onto the array surface instead of depositing intact sequences. Oligonucleotide arrays can be synthesized in situ or ex situ and attached to a derivative substrate. Affymetrix (Affymetrix Inc, Santa Clara, CA) and Agilent (Agilent Technologies Inc, Palo Alto, CA) are commercial platforms that rely on in situ synthesis of probes. Sequences may be longer (60-mer probes such as the Agilent design) or shorter (25-mer probes produced by Affymetrix) depending on the desired purpose. Longer probes are more specific to individual target genes, while shorter probes may be spotted in higher density across the array and are cheaper to manufacture. One technique used to produce oligonucleotide arrays is photolithographic synthesis (Agilent and Affymetrix) on a silica substrate, in which light and light-sensitive masking agents are used to build a sequence, one nucleotide at a time, across the entire array.

The alternative bead array is a collection of microscopic polystyrene beads, each with a specific probe and a ratio of two or more dyes. Beads are randomly assembled in the well. Advantages of this approach are the dense packing that can be achieved and the ability to have multiple copies of each sequence-specific bead in an array. Randomness and redundancy increase the precision and robustness of measurements.

Another recent method is the cDNA-mediated annealing, selection, extension and ligation (DASL) assay (Illunima Inc, San Diego, CA), which is capable of transcriptional profiling in formalin-fixed, paraffin-embedded samples instead of frozen tissue sections. This assay displays high specificity and sensitivity in interrogating partially degraded targets in hepatocellular cancer and ovarian cancer.

There are several challenges in the analysis of microarray data and in the comparison of the results between different investigations and techniques used due to the lack of standardization in platform fabrication, assay protocol, and analysis methods. Various projects are underway to facilitate the exchange and analysis of data. The Minimum Information About a Microarray Experiment (MIAME) is one such project. This checklist assists in defining the level of detail and interpreting the microarray data that are derived from independent verifica-
Molecular Classification/Subtype of Ovarian Cancer

Molecular classification of tumors based on their gene expression profiles can significantly refine diagnosis and management for cancer patients. A generic approach to cancer classification based on gene expression monitoring by DNA microarrays has been reported in human acute leukemia.18 As a result of this report, acute myeloid leukemia can be distinguished from acute lymphoblastic leukemia without previous knowledge of these classes, leading to a general strategy for discovering and predicting cancer classes for other types of cancer, independent of previous biological knowledge.

Giordano et al19 generated gene expression profiles from 154 primary adenocarcinomas of the lung, colon, and ovary using oligonucleotide arrays. They used two statistical methods (principal component analysis and cross-validated prediction based on differentially expressed genes) that resulted in the classification of 152 of 154 of the adenocarcinomas in an organ-specific manner and identified genes expressed in a putative tissue-specific manner for each tumor type. These results suggest a strong discrimination of these tumors based solely on gene expression profiles. Furthermore, the remaining two tumors were of other origin, as shown by further investigation with immunohistochemical profiling. Subsequently, this group analyzed 113 patients with epithelial ovarian cancer (EOC) and demonstrated that mucinous and clear cell EOCs could be readily distinguished from serous tumors on the basis of gene expression profile, whereas endometrioid EOCs exhibited significant gene expression overlap with other histologic subtypes.20 Zorn et al21 reported gene expression profiling of 75 cancers (endometrioid, serous, and clear cell) of the ovary and endometrium, and five renal clear cell carcinomas using cDNA array. Comparisons across endometrial and ovarian cancers and serous and endometrioid tumors showed expression patterns reflecting their origin. However, clear cell tumors showed remarkably similar expression patterns regardless of their origin. This suggests that to identify better therapies for clear cell cancer, future trials may need to enroll based primarily on the presence of clear cell histology rather than on the anatomic site of origin of the tumor. In this regard, sunitinib, an oral multitargeted tyrosine kinase inhibitor of the vascular endothelial growth factor receptor and platelet-derived growth factor receptor, is now a standard first-line therapy for metastatic clear cell renal cancer22 and therefore may also represent a biologically rational option for recurrent or refractory clear cell type of ovarian cancer.23

Bonome et al7 reported gene expression profiles among low malignant potential (LMP) and high-grade serous ovarian carcinomas using oligonucleotide microarray. Results revealed enhanced expression of genes linked to cell proliferation, chromosomal instability, and epigenetic silencing in high-grade cancers, whereas LMP tumors displayed activated p53 signaling. A close association between LMP and low-grade lesions was observed. Prominent expression of TP53, CDKN1A, and other p53-modulated genes in the LMP tumors suggests that this signaling pathway may play an important role in the distinct phenotypes associated with this lesion. Furthermore, a return of TP53 and CDKN1A to levels expressed in ovarian surface epithelium may precede progression of these low proliferative cancers to more aggressive low-grade tumors. Targeting deregulated genes that are repressed in high-grade cancers for therapeutic intervention may attenuate the progression of the disease. These findings are supporting by the explanations of serous carcinogenesis in ovarian cancer, in which LMP is the precursor of low-grade serous carcinomas, whereas high-grade serous carcinoma is a genetically distinct entity that does not simply represent a transition from a low-grade to a high-grade phenotype.24

Anglesio et al25 described LMP tumors as associated with KRAS and BRAF mutations in 18% and 48% of cases, respectively. Interrogation of expression profiles in serous LMP tumors suggested overall redundancy of RAS-MAPK pathway mutations and a distinct mechanism of oncogenesis compared with high-grade ovarian carcinomas. In addition, ErbB2 mutations (6%), but not epidermal growth factor receptor (EGFR), are prevalent among serous LMP tumors. As such, therapies that target this pathway may have utility in treating recurrent LMP tumors, particularly in younger patients who desire fertility.

Tothill et al26 identified molecular subtypes of ovarian cancer by gene expression profiling with linkage to clinical and pathologic features from 285 serous and endometrioid tumors of the ovary, peritoneum, and fallopian tube using oligonucleotide array followed by K-means clustering. Optimal clustering of array data identified six molecular subtypes. Two subtypes represented predominantly serous LMP and low-grade endometrioid subtypes, whereas the remaining four subtypes represented higher-grade and advanced-stage cancers of serous and endometrioid morphology. A subtype of high-grade serous cancers reflected a mesenchymal cell type, characterized by overexpression of N-cadherin and P-cadherin and low expression of differentiation markers, including CA125 and MUC1. Each subtype displayed distinct levels and patterns of immune cell infiltration. The identification of molecular subsets provides a context for additional genomic studies to understand the biology of the subtypes and could be utilized to triage patients with ovarian cancer to an appropriate treatment plan.
Early Detection of Ovarian Cancer

Despite recent advances in the understanding of the pathogenesis of ovarian cancer, it remains the most lethal gynecologic malignancy in developed countries. EOC is diagnosed at advanced stages in most patients, resulting in low overall cure rates. This is partially due to the absence of specific signs and symptoms and the lack of effective screening programs. Therefore, improving our understanding of the biology of early-stage EOC to rationally design experimental approaches and clinical studies to identify and evaluate biomarkers associated with early-stage disease is of critical importance.

Mok et al. used cDNA arrays to identify overexpressed genes for secretory proteins as potential serum markers in ovarian cancer cells compared with normal human ovarian surface epithelial cell lines. When tested in an independent set of 64 patients with ovarian cancer and 137 control subjects, the mean level of serum prostasin was significantly higher in patients with ovarian cancer. In patients in whom levels of CA125 and prostasin were available, the combination markers gave a sensitivity of 92% and a specificity of 94% for detecting ovarian cancer. Similar microarray studies have generated a substantial number of new markers, but only some of these have been validated in small patient cohorts. These markers include osteopontin, Kallikrein native cell adhesion molecule, 30 kallikrein 10, creatinine kinase B, and insulin-like growth factor 2.

Chien et al. reported 285 differential gene expressions from formalin-fixed paraffin-embedded samples of five high-grade stage I serous carcinomas and five stage I borderline tumors of the ovary using the Illumina Whole-Genome DASL Assay (Illumina Inc, San Diego, CA) corresponding to 24,000 genes. FOLR3 (folate receptor gamma), survivin, MCM3 (minichromosome maintenance genes), E2Fs, and VTCN1 were overexpressed, and SYNE1, AKAP14, KNDC1, and DLEC1 were underexpressed in serous carcinoma. These differential gene expression levels in stage I serous carcinoma indicate alterations in pathways associated with cell cycle regulation, cell cycle-related cytoskeletal signaling, transcription-related chromatin modification, and kallikrein-related inflammatory signaling, which may be important in ovarian pathogenesis and biomarker development.

Berchuck et al. using a microarray-based model, recently reported that early-stage invasive and borderline serous ovarian cancers have gene expression profiles predictive of favorable outcome. Serous ovarian cancers detected at an early stage generally have a favorable underlying biology similar to that shown in long-term survivors with advanced-stage disease. Along with the findings that LMP is the precursor of low-grade serous carcinomas, whereas high-grade serous carcinoma is a genetically distinct entity that does not simply represent a transition from a low-grade to a high-grade phenotype in ovarian cancer, this study suggested that to develop a successful screening method for ovarian cancer, approaches that are able to detect most late-stage virulent ovarian cancers at an early stage should be made to improve survival.

Microarray Analysis as a Prognostic Tool

Several studies have examined the role of gene expression profiles identified with microarray analysis in regard to clinical outcome of patients with ovarian cancer. Spentzos et al. identified a 115-gene signature referred to as the Ovarian Cancer Prognostic Profile (OCPP) using oligonucleotide microarrays. Sample tissues from 68 patients were randomly split into training (n = 34) and validation sets (n = 34). When a 115-gene signature from the training set was applied to the validation set, a strong survival discrimination was observed on the basis of the prognostic profile, with the median overall survival of 30 months in the unfavorable group and not yet reached in the favorable group, at a median follow-up of 47 months (log rank P = .004). The signature maintained independent prognostic value in multivariate analysis with other known prognostic factors such as age, stage, grade, and debulking status. Genes encoding for angiogenesis-related cytokines, receptor tyrosine kinases, mesenchymal markers including fibrinectin, and proinvasive enzymes were overexpressed in the unfavorable prognosis group. On the other hand, Hartmann et al. evaluated specimens from 79 patients with EOC using cDNA microarrays to develop profiles that could distinguish patients who have a high risk for early (< 21 months) recurrence after initial platinum-paclitaxel chemotherapy. A 14-gene predictive model was developed with 51 samples (training set), which were subsequently validated with 28 independent samples. This model correctly predicted the outcome of 24 of the 28 samples (86% accuracy) with 95% positive predictive value for early relapse. Elevated mRNA levels of protein tyrosine phosphatase receptor, pre-RNA processing factor 31 homolog, hexamethylene bisacetamide-inducible RhoGFE and pleckstrin domain protein 1, putative nuclear factor κB activator, zinc finger protein 200, and protein kinase C-γ were observed in the early recurrence group. Lancaster et al. initially analyzed specimens from 31 patients with advanced-stage EOC using oligonucleotides and identified 43 gene profiles prognostic of survival < 2 years vs > 7 years using hierarchical clustering. This study was subsequently extended by the same group; Berchuck et al. developed an expression model to identify patterns of genes to distinguish short-term (< 3 years) and long-term (> 7 years) ovarian cancer survivors. The study included patients with serous ovarian cancers, 30 short-term survivors who lived < 3 years, 24 long-term survivors who lived > 7 years, and 11 patients with early-stage disease. The expression model developed for advanced-stage disease classified all 11 early-stage ovarian cancers as long-term
survivors. Genes that discriminated between long-term and short-term survivors included MAL gene (T-lymphocyte maturation-associated protein), heat shock protein 27, and lysophospholipase II. The identified gene expression profiles for survival were confirmed to have a prognostic value in an independent set of tumors that were processed and analyzed by Spentzos et al. using a different microarray platform. Berchuck et al. recently validated a microarray-based (linear discriminant) model for long- vs short-term survival using samples from 101 patients with serous ovarian cancer (42 with advanced stage, 39 with early stage, 20 with borderline). The model correctly predicted 81% (34 of 42) of the advanced-stage cancers. Correct predictions were obtained in 82% (27 of 33) of short-term survivors and in 78% (7 of 9) of long-term survivors. All but one of the 39 early-stage invasive cancers were predicted to be long-term survivors, and 15 of 20 (75%) of those with borderline tumors were predicted to be long-term survivors. Tothill et al. also reported that a poor prognosis subtype was defined by a reactive stroma gene expression signature, correlating with extensive desmoplasia. This subtype overlaps with the 115 genes in the report from Spentzos et al. This study demonstrated that class prediction identified similar subtypes in an independent ovarian dataset with similar prognostic trends.

The use of gene expression profiling may eventually permit identification of patients with ovarian cancer appropriate for investigational treatment approaches rather than standard first-line chemotherapy with paclitaxel-platinum chemotherapy, given the fact that there is a low likelihood of achieving prolonged survival with standard therapy.

**Molecular Predictions of Therapeutic Response**

Although optimal debulking surgery and early stage of disease can mean better survival for patients with ovarian cancer, it is impossible to predict who will progress or recur during or after chemotherapy. This prediction is essential since patients who are chemoresistant might benefit from a different treatment rather than first-line chemotherapy with a paclitaxel-platinum combination. The use of molecular analyses may enable important distinctions to be made between cancers that appear similar based on traditional clinical and histopathologic features. The development of such predictive molecular markers may facilitate the identification of subpopulations of patients most likely to respond to existing systemic chemotherapy and, moreover, may identify specific new targets for pharmacologic development. Indeed, several studies have identified a profile predictive of response to chemotherapy.

Spentzos et al. identified a 93-gene signature referred to as the Chemotherapy Response Profile that predicted pathologic complete response using a training set of 24 patients with EOC who had undergone second-look laparoscopy. This profile was subsequently able to distinguish 44 patients with favorable vs unfavorable overall survival in a validation set. Interestingly, their prognostic 115-gene profile for overall survival was determined from the same data but, surprisingly, showed no gene overlap with the 93-gene profile associated with predicting response to chemotherapy.

Helleman et al. identified a 9-gene profile using cDNA microarray and reverse transcription-polymerase chain reaction (RT-PCR) from 24 patients based on their response to platinum-based chemotherapy (5 nonresponders and 19 responders). With this model, they were able to predict platinum resistance in an independent validation set of 72 tumors with a sensitivity of 89% (95% CI, 0.68–1.09) and a specificity of 59% (95% CI, 0.47–0.71; odds ratio = 0.09; P = .026). In their study, response was assessed according to World Health Organization criteria, and nonresponders were defined as patients whose tumors showed progression. Spentzos et al. defined nonresponders as patients whose tumors showed no pathologic complete response, in addition to the difference of aggressiveness of the tumor and debulking status. In another microarray study, genes associated with cisplatin resistance in 14 ovarian cancer cell lines were reported. However, there was no overlap between these genes and the Helleman group gene set, which is probably due to the difference between cell lines and tumors. Dressman et al. developed a gene expression model that predicts response to platinum-based therapy using a training set of 83 advanced-stage serous ovarian cancers and tested on a 36-sample external validation set. Platinum response was predicted accurately in 70 of 83 samples, achieving an overall accuracy rate of 84.3% (specificity 85%; sensitivity 83%). In parallel, expression signatures that define the status of oncogenic signaling pathways, including Src, β-Cat, MYC, E2F3, and RAS, were evaluated in 119 primary ovarian cancer and 12 ovarian cancer cell lines. The Src and E2F3 pathways were frequently found to be activated in patients with platinum-resistant disease. Furthermore, a greater sensitivity was achieved when the Src-specific inhibitor SU6656 was added to cisplatin in ovarian cancer cell lines compared with either agent alone. This study shows the strong potential of translational research of microarray technology to clinical settings in ovarian cancer.

In an attempt to properly guide the use of available therapeutics to achieve the most favorable outcome, Potti et al. developed gene expression signatures using microarray data coupled with in vitro drug sensitivity assays to predict sensitivity to various common cytotoxic chemotherapeutic drugs (docetaxel, paclitaxel, 5-fluorouracil, topotecan, doxorubicin, etoposide, and cyclophosphamide) including ovarian cancer cell lines and primary advanced serous EOCs. These gene signa-
tures were also validated with response data from an independent set of other cell line studies. Assessment of data from studies that linked gene expression with clinical response showed that these signatures predicted drug response in clinical samples with an accuracy of 81%. With evaluation of signatures that reflect the activation of several oncogenic pathways, regression analysis showed a relationship between phosphatidylinositol 3-OH kinase pathway deregulation and docetaxel resistance, indicating a potential relevance of future use of a phosphatidylinositol 3-OH kinase inhibitor in this docetaxel-resistant subgroup.

On the other hand, Jazaeri et al\(^4\) attempted to evaluate whether distinct gene expression profiles are associated with intrinsic and/or acquired chemoresistance in EOCs, which potentially can be used as molecular mediators of chemoresistance. From 21 primary chemosensitive tumors and 24 primary chemoresistant tumors using cDNA microarrays, 178 genes were identified that represented transcripts differentially expressed between primary chemosensitive vs post-chemotherapy tumors and primary chemoresistant vs postchemotherapy tumors. Higher expression levels of CTSD, PCNA, and KI-67 in chemosensitive tumors were postchemotherapy tumors. Higher expression levels of CTSD, PCNA, and KI-67 in chemosensitive tumors were observed. The gene expression profile of postchemotherapy tumors compared with the profile of primary tumors revealed statistically significant overrepresentation of genes encoding extracellular matrix-related proteins including DCN, COL6A3, and SPARC.

Although microarray technology has had a profound impact on gene expression research in ovarian cancer over the past decade, reports with contradictory results, obtained using different microarray platforms to analyze identical RNA samples, have led to concerns about the reliability and reproducibility of this technology. One of the reasons that may explain the limited overlap between prognostic profiles in ovarian cancer microarray studies might be sample size. Small sample sizes can lead to a discriminatory pattern by chance. Therefore, additional large, independent studies are warranted to find reliable, reproducible prognostic values in ovarian cancer.

Conclusions

Despite recent improvements in treatment, ovarian cancer remains the No. 1 killer among gynecologic cancers in the United States. First-line chemotherapy with a paclitaxel-platinum combination has yielded response rates of greater than 80%. However, the disease eventually relapses and most patients with advanced cancer die. Studies evaluating various cytotoxic agents in recurrent ovarian cancer have generally shown response rates of 10% to 28%, in addition to accompanying progressive increases in the number of drug-resistant tumors. Therefore, novel strategies for treating ovarian cancer are needed.

Over the past decade, microarray technology has facilitated studies that have improved the understandings of the underlying biology in carcinogenesis of ovarian cancer. Gene expression profiles have been used to identify the differential gene expression patterns in normal and tumor tissues, distinguishing between histology subtypes. Of particular importance to patients with this disease, several studies have sought to identify gene expression signatures that correlate with clinical outcome, to identify genes that determine survival and relapse, and to generate predictive biomarkers of response to chemotherapy as well as molecular subtypes. Data from these studies have deepened and widened our understanding of the biology of ovarian cancer despite some challenges. Several challenges exist in the analysis of microarray data and in the comparison of the results between different investigations and techniques used due to the lack of standardization in platform fabrication, assay protocol, and analysis methods. MIAME and MAQC have now been implemented to improve the exchange and analysis of these data.

Studies on the role of microarray analysis to identify gene expression profiles associated with prognostic values and prognostic and predictive molecular markers will help identify patient groups who could benefit more from individualized treatment rather than the current standard first-line chemotherapy with a paclitaxel-platinum combination. In addition, identification of biomarkers associated with early detection of disease and molecular subsets will also improve overall survival for patients with ovarian cancer, as the early signs of this disease are often undetectable.

It is clear that gene expression profiling has a clinical application in the area of ovarian cancer treatment and could provide useful tools as novel prognostic and therapeutic options for patients with this disease.

References
