From bio-molecular and technology innovations to clinical practice: focus on ovarian cancer

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Ovarian cancer (OC) still represents the most lethal of gynecological malignancies with the chance for death in 5 years exceeding the chance for life. In recent years, the development of knowledge in molecular biology of OC coupled with the new technologies offers enormous opportunity to learn about aetiology of OC, and also give us a powerful tool for early diagnosis, prognosis and treatment of this disease. In particular, small cancer specimens from patients have become extremely informative thanks to techniques such as laser capture microdissection (LCM), tissue lysate arrays (TLAs), reverse transcriptase polymerase chain reaction (RT-PCR), and mass spectrometry. All of this coupled with advancements in bioinformatics have allowed the explosion of genomics, transcriptomics and proteomics. This paper focusses on the influence that advancements in the ‘-omics’ bio-technology will reserve in OC diagnosis, prognostic characterization, and treatment.

Key words: ovarian cancer, molecular biology, technology, proteomics

introduction

Over the past two decades the prognosis of ovarian cancer (OC) has improved with the introduction of platinum-based chemotherapy in the 1980s and taxanes in the 1990s and with the progress in surgical approaches. However, OC still represents the most lethal of gynecological malignancies with the chance of death in 5 years exceeding the chance of life [1]. Two main reasons are responsible for this: (i) OC in its early stages is clinically silent, thus 70%–80% of patients are diagnosed with disseminated intraabdominal disease; and (ii) OC exemplifies the main problem in chemotherapy of solid tumors, because, although relatively chemosensitive, relapse ultimately occurs with drug resistant disease. The greatest hope is a way to positively impact the survival of OC patients through the early detection of the disease and optimized treatment. In recent years, the development of knowledge in molecular biology of OC coupled with new technologies offers enormous opportunity to learn about the etiology of OC, and also give us a powerful tool for early diagnosis, prognosis and treatment of this disease [2]. In particular, small cancer specimens from patients have become extremely informative thanks to techniques such as laser capture microdissection (LCM), tissue lysate arrays (TLAs), RT-PCR and mass spectrometry. All of this coupled with advances in bioinformatics have allowed the explosion of genomics, transcriptomics and proteomics.

This paper focus on the influence that advancement in the ‘-omics’ bio-technology will reserve in OC clinical management. The current ‘-omic’ research is fundamentally based on a hierarchical clustering of genes/transcripts/peptides by computerized algorithms in order to discriminate in a training set of patients a signature characterizing a chosen oncogenic phenotype. In this way researchers can identify specific molecular fingerprints to discriminate tumor versus normal/ different cancer subtypes or good versus bad prognosis disease or sensitive versus resistant tumors to specific therapies. Once the signature is determined it needs to be validated in independent populations in order to test the reproducibility of the method.

From a practical point of view proteomics is probably the most interesting strategy. Indeed, proteomic approaches, by permitting the analysis of peptides shed into the blood, allow tumor characterization by a simple and small serum sample, similarly to tumor markers currently used. Moreover, while genomic and transcriptomic changes may not always translate into changes in the functional protein, proteomics document the structural and functional consequences of the genetic alterations and also the post-translational modifications. From a technical point of view, mass spectroscopy is the principal protein identification technique presently utilized in proteomics, with different platforms used to introduce the proteins, e.g. matrix-assisted laser desorption and ionization (MALDI) and surface-enhanced laser desorption and ionization (SELDI) mass spectroscopy [3]. Bioinformatics can subsequently be used to search genomic and proteomic databases to identify the protein of interest or to analyze the output for discriminating peptide patterns.

Hopefully, clinicians may face the ‘-omic’ strategies as possible means to detect, characterize and treat OC. Each of these issues in clinical management of OC is analyzed below.

early diagnosis and screening

The lack of accurate screening tools in OC justify surveillance via CA125 monitoring and ultrasound scans only in subjects with
a high risk for OC such as BRCA1/BRCA2 mutation carriers, who can benefit from early diagnosis and possibly prophylactic surgery. Indeed, low prevalence of OC in the general population coupled with its dismal prognosis, demand sensitive, specific and possibly low cost biomarkers for early detection.

More than 30 serum markers have been evaluated alone or in combination with CA125 by different investigators. Among these the kallikreins, a serin-protease family including the prostate-specific antigen, seem to be the most promising. However, most of papers published on the possible role of different kallikreins in OC are derived from the experience of a single group and none of these has gained access to clinical practice [4].

On the other hand recent advances in technologies for identifying proteins in complex mixtures have stimulated a new interest in biomarker discovery research. Indeed, tumor microenvironment can shed abnormal peptides, which can be detected in the serum through a non-invasive blood test. Two approaches are possible: pattern recognition and protein identification.

By using SELDI-mass spectrometry, Petricoin et al. [5] dramatically described a pattern of peptides, able to detect all the 50 tested patients with OC, while falsely identifying just three healthy patients as suffering from the disease from a total of 66 controls. From the same dataset, a 100% sensitivity and specificity was later claimed by Conrads et al. by using a high-resolution proteomic profiling [6]. Three other biomarker protein panels were also identified by an independent group [7]. With these promising results, commercial laboratories planned to quickly translate this approach into a routine diagnostic test with the brand name ‘OvaCheck’, but the US Food and Drug Administration delayed these plans. Indeed, reanalysis of the data, posted on the web first by Sorace and Zhan [8] and later by Baggerly et al. [9], raised serious questions about the reliability and reproducibility of this technology. Indeed, both critics suggested that peptides' patterns discriminating cancer versus unaffected patients looked more like experimental artifacts than real biological differences. Undoubtedly, better designed studies and more evidence from different and independent populations and laboratories are necessary to eliminate differences in peptide patterns due to chance or possible bias [10]. The controversy not only into whether serum proteomics may diagnose OC but also into the validation and reproducibility of the results in different ‘omics’ fields has been extensively discussed in the last years [9–12], warning the dangers of moving these immature technologies to the clinic.

More interestingly, a similar method was recently used to identify potential biomarkers in OC. The proteins identified (i.e. apolipoprotein A1, truncated transthyretin, and an inter-alfa-trypsin inhibitor heavy chain H4 cleavage fragment) were then tested in an independent population. The three biomarkers combination with CA125 demonstrated improved sensitivity over CA125 (74% versus 65%) when specificity was fixed at 97%. This kind of study, in which the proteins comprised in the signature panel are purified and individual assays are developed to analyze the peptides, obviously avoids problems of chance and limits possible bias [12].

However, large scale prospective and blinded studies are required to determine the robustness of these early findings and to form the basis for its application in prospective screening trials [12].

According to the early detection of recurrent OC, rising values of serum CA125 correlate with disease progression in approximately 90% of cases. However, debate still exists according to the timing of treatment in patients without clinical evident disease. In this situation new imaging technologies, such as positron emission tomography (PET), evaluating active and proliferating OC cells could be helpful [15].

**prognostic characterization**

Since patients with similar clinico-pathologic factors such as FIGO (International Federation of Gynecology and Obstetrics) stage, histotype and residual tumor after surgery, often present disparate clinical outcome, it is conceivable that the assessment of molecular markers more strictly related to individual tumor cell and intrinsic biologic aggressiveness could help to identify high-risk patients and facilitate management of this disease. Different biochemical markers involved in OC progression such as p53, HER2/neu, epidermal growth factor receptor, kallikreins and COX-2 have been reported as possible prognostic factors: none of these has been definitively validated [2]. More interestingly, new profiling techniques, such as DNA and protein microarrays, have enabled high-throughput screening of tumors. Spentzos et al. found a putative gene panel that is able to characterize high-risk versus low-risk patients, independently from other clinico-pahological characteristics [16]. More recently, by analysing gene expression signatures that reflect the activation status of several oncogenic pathways, the co-deregulation pattern of β-catenin and Src (both elevated) has been found to identify OC patients with very poor survival [17]. Moreover, the patterns identified by ‘-omic’ approaches could be useful in order to identify specific genes/proteins to be investigated as serum or tissue biomarkers with techniques more easily performed in clinical practice such as enzyme ligand immunossay (ELISA), immunohistochemistry (IHC) or fluorescent in situ hybridisation (FISH).

**design of new drugs and tailored treatment**

Up till now, investigators have attempted to find combinations, dosages and schedules of drugs that would cure the ‘average patient’. However, it is really difficult to expect that OC survival rate will improve by treating all patients uniformly according to standard guidelines. Targeted therapy aims at personalizing the cure for each patient, tailoring peculiar biological characteristics of the tumor. In addition to the key signals elucidated in the last years with the classical hypothesis-driven approaches, new ones are identified with genomic strategies: for example, Bild et al. [17] identified specific gene expression signatures that reflect the activation status of several oncogenic pathways, such as those on which cancer cells depend to proliferate, invade, metastasize and prevent apoptosis. These signal cascades could be targeted by specific drugs designed and/or screened against specific cancer-related molecules. Moreover, these genomic-based predictions of pathway deregulation in cancer cell lines are also shown to
predict the sensitivity to therapeutic agents that target components of the pathway, thus potentially guiding targeted drugs [17].

A novel and more direct approach to identify therapeutic targets is by using protein microarray biochips to systematically analyze large numbers of proteins. This approach has been used, for example, to identify new possible mechanisms involved in resistance to cisplatin in ovarian cancer cell lines [18, 19]. Moreover, this technology, by using a specific detector (i.e. an antibody) placed on a solid matrix, permits the protein in a biological sample to be quantified and characterizes its selective binding with antibodies, drugs and other proteins. In particular, protein microarrays can be utilized to analyze multiple steps in a specific signaling pathway to identify the alterations to be targeted or to monitor the activity of the therapeutics on that particular pathway [20, 21]. This issue is particular important in the early development of non-cytotoxic drugs, because these agents need the measure of the target effect instead of toxicity end points to define the recommended doses. Moreover, protein profiling could provide new tools to molecular imaging. This in vivo imaging technique, targeting specific molecular pathway involved in cancer biology, in animal models have shown promising results, in particular as an early marker of response [22].

conclusions

The next step will be to integrate the skills of molecular biologists, statisticians, informatics and clinicians to better understand and simplify the bulky evidence of data given by ‘-omic’ approaches. Only by this way, promises about an in vivo imaging technique, targeting specific molecular pathway involved in cancer biology, in animal models have shown promising results, in particular as an early marker of response [22].

references