Promising Directions for the Diagnosis and Management of Gynecological Cancers

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ABSTRACT

Diagnosis and management of cancer requires tools with both high sensitivity and specificity. The minimally invasive cervical smear has demonstrated how a test, even one with low specificity, can change the public health profile of a cancer from a late stage deadly disease to early diagnosis with rare tumor-related deaths. The benefit of such a test is best demonstrated by the low frequency of cervix cancer and its good outcome in countries where this test is readily available and used with appropriate secondary follow up. Early and specific symptoms, and identification and prevention for high risk groups has had similar impact for endometrial cancer. Neither a robust test, nor reliable or specific early symptoms are available for ovarian cancer, making clinical and scientific advances in this area a critical world-wide need. Current approaches testing one protein or gene marker at a time will not address this crisis expeditiously. New sensitive, specific, accurate, and reliable technologies that can be implemented using high throughput mechanisms are needed at as low a cost as possible. Ideally, these technologies should be focused on readily available patient resources, such as blood or urine, or as in the case of cervix cancer, minimally invasive informative approaches such as cervical smears. Techniques that allow data mining from a large input database overcome the slow advances of one protein–one gene investigation, and further address the multi-faceted carcinogenesis process occurring even in germ line mutation-associated malignancy. Proteomics, the study of the cellular proteins and their activation states, has led the progress in biomarker development for ovarian and other cancers and is being applied to management assessment. Amenable to high throughput, internet interface, and representative of the proteome spectrum, proteomic technology is the newest and most promising direction for translational developments in gynecologic cancers.

NEED FOR BIOMARKERS IN GYNECOLOGIC MALIGNANCIES

Clinical conundrums currently facing our discipline are the detection of ovarian cancer at a stage of development where intervention has a high likelihood of cure or long term remission, as well as the identification of the subset of early stage gynecologic cancer patients who are at high risk of recurrence and death from cancer. Early detection of ovarian cancer has been plagued by the lack of clinically specific symptoms, poor specificity of the physical examination, and a minimally invasive highly specific screening test. Like most other solid tumors when identified early, ovarian cancer is amenable to surgical extirpation and, with additional chemotherapy where indicated, can have up to a 95% five and ten year survival [1]. Unfortunately, only 15–20% of patients are diagnosed with stage I ovarian cancer and of those, many are not anticipated to have a malignancy prior to surgery and may not receive optimal surgical evaluation [2].

A biomarker that allows early detection in the absence of a change in surgical or medical treatment may cause a population stage shift and lead time outcome bias as was initially seen with mammography and adjuvant therapy in breast cancer [3]. However, longer term follow up of the impact of mammography and adjuvant chemotherapy has now been shown to cause a real benefit and increase in overall longevity. Thus, a shift of stage at diagnosis in ovarian cancer should have a marked impact on public health. A shift to early detection would lend increasing importance to a second goal for biomarker development for early diagnosis. We need a reliable mechanism to identify those patients with early stage limited disease who will relapse and die of disease. Again, as with other solid tumors including breast cancer, there remains approximately 15–25% of early stage patients who will succumb to disease [1]. Do these patients have a unique molecular subtype of cancer not discernable with current technology? Newer data with cDNA microarray analysis is uncovering gene transcription signatures that suggest a new molecular subtyping may be useful in ovarian and other cancers [4,5]. We need to determine how to identify this patient subgroup and then to tailor therapy accordingly. This approach would limit the number of early stage patients subjected to chemotherapy for whom it may not have been necessary. Identification of biomarker diagnostics for early detection and for discrimination of those cancers likely to recur will allow individualization and optimization of therapy for women with gynecologic malignancies [6].

PROTEOMICS: DEFINITIONS AND TECHNIQUES

The proteome is the array of expressed proteins. It varies

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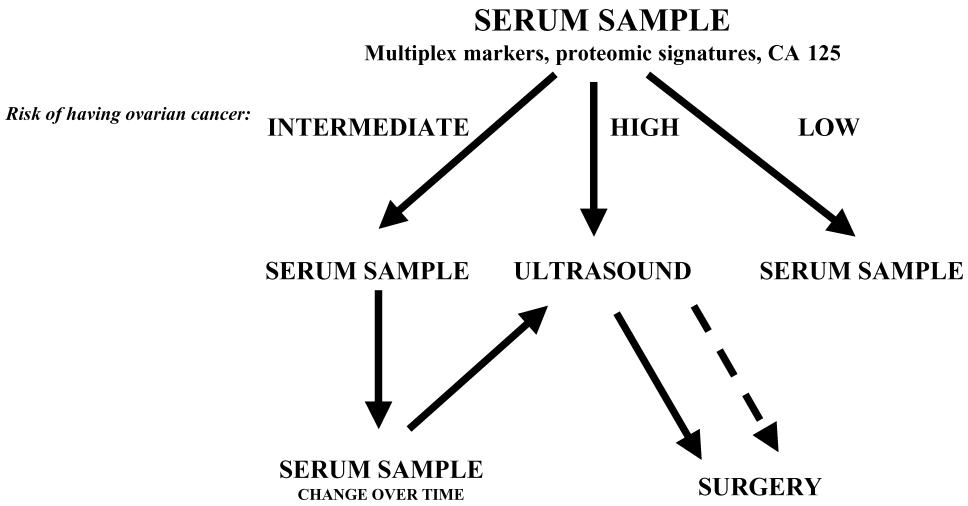

Fig. 1. Application of serum marker screen/ultrasound approach to ovarian cancer screening. Single biomarkers, such as CA125, multiplex biomarkers, or signature comprising information of hundreds to hundreds of thousands of input points can be used as a primary screen in the diagnostic paradigm for ovarian cancer.

temporally and spatially within and between organs. The complexity of this dynamic component of the body is further expanded through co- and post-translational protein modifications, activation status, and breakdown products. This array of variation is important. As is demonstrated in the immune system, a very small peptide epitope may stimulate an active immune response. The human genome contains only 30–35,000 genes; signal amplification occurs at the level of gene transcription in promoter regulation, copy number, alternative splicing and transcript stability. Further amplification occurs with protein translation and modification coupled with protein stability. The proteome, predicted to be between 1.5 and 100 million different units, is therefore the most broad and actively changing representation of an organ's function at any given time. The serum proteome, proteins and protein products in circulating blood, is thus the most comprehensive general source of information in the body that can contain information regarding organ-confined events, and host responses. New technologies are under development for optimal mining of this data reserve and to apply it for patient benefit [7].

Mass spectroscopy is a technique long used to characterize and identify chemicals and proteins in the laboratory and the clinic. Its sensitivity has increased with more complex machinery such that mass spectroscopy tools now can discriminate hundreds of thousands of signals. Mass spectroscopy can "see" changes in the snapshot of the proteome at a given time. The complexity of the signal can be pared down by coupling with chip-based protein capture systems to create a first level sample fractionation. Solid phase bait chips can narrow the selection to hydrophobic interactive proteins or those with selected properties, such as aliphatic or minimally charged proteins. The spectroscopy pattern from a given sample will differ with the bait used for selection and therefore can provide opportunities to analyze the potential dataspace from multiple views. The resultant datastreams must then be mined for their hidden values and properly applied for patient benefit.

Bioinformatics has advanced dramatically in the past decade and is now an important adjunct tool in clinical diagnostics. Multiple higher order analytic programs have been developed with which to mine the large databases developed using mass spectroscopy proteomic tools and those emanating from genomic and transcriptomic array analyses [8,9]. Decision trees have been developed, tested, and shown to offer discriminating capacity. These directions of mass spectroscopy coupled with complex bioinformatics analysis are being harnessed and applied for clinical biomarker development [10].

PROTEOMIC BIOMARKERS OF DIAGNOSIS

The search for biomarkers of ovarian cancer diagnosis and specifically early diagnosis has been ongoing for decades [11–16]. CA 125, the work horse of ovarian cancer detection, has an unacceptably high false positive rate leading to invasive assessment and a high false negative rate, especially in early stage disease and low volume disease leading to inappropriate reassurance [17].

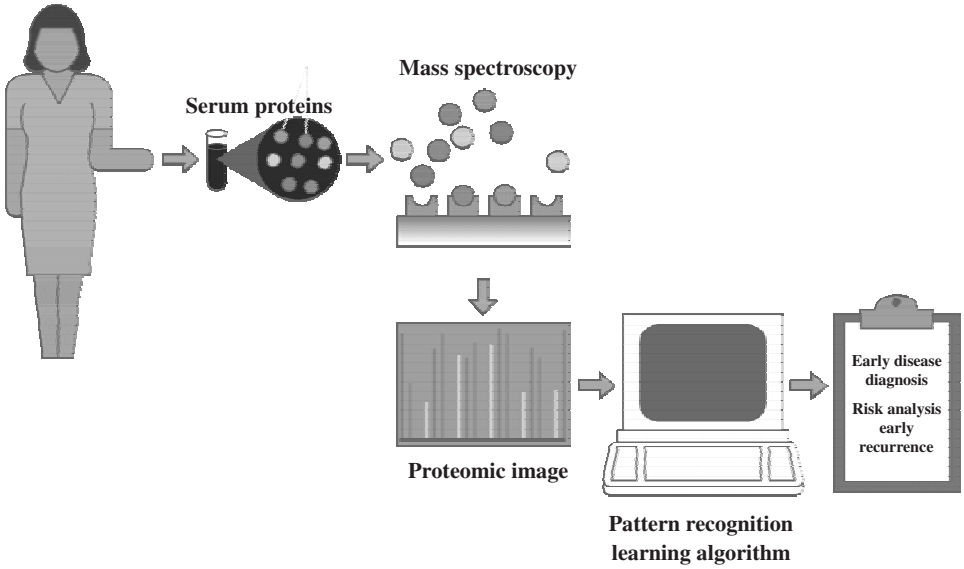

Fig. 2. Paradigm for application of serum protein pattern diagnostics. Proteomic signature biomarker analysis, following validation in prospective trials, can be developed for high throughput. Blood samples can be subjected to mass spectroscopy in a central reference laboratory, datastreams sent to bioinformatics algorithms, and results received in a confidential webcentric platform.

However, recent data in the longitudinal series of studies of Jacobs and coworkers and Skates has shown that the rate of change of CA 125 may increase its predictive value [18,19]. A small but real increase in detection of early stage cancer has been projected. However, CA 125 alone or in combination with color Doppler ultrasound is unlikely to be sufficient for the detection of early stage ovarian cancer [20,21].

Numerous investigators are working on biomarker discovery in ovarian cancer using cell lines, tumor samples, and patient serum samples. Applied techniques include candidate marker validation [22–24], and global unbiased searches using cDNA microarray screening [25,26], gene expression patterns [5,27,28], protein screening [29,30], and proteomic pattern analysis [10,31,32]. The lack of a precursor lesion or known pre-cancer syndrome combined with the relative rarity of ovarian cancer has made this challenge more difficult. As approximately 1 in 2500 postmenopausal women will develop ovarian cancer in their lifetime, coupled with 70% or more of patients presenting with advanced stage disease, the detection of early stage disease is a “needle in a haystack” search [18]. Since there is no true consensus as to the precursor cell or entity, and only recently a transgenic mouse model [33], researchers are hampered by lack of models from which to identify individual biomarker candidates.

Application of advanced bioinformatics to genomic or proteomic datastreams from patient materials has led to progress in discerning gene and protein signatures of ovarian cancer [10,25,34]. The serum proteome has the greatest potential to provide information from organ-confined early stage disease [10,35–37]. Serum suffuses the tumor and the tumor microenvironment and may also be acted upon by the tumor microenvironment. Thus, changes in protein patterns in sera can reflect the status of distant organs. Several groups have analyzed serum samples using mass spectroscopy coupled with different bioinformatics techniques. All have reported the ability to identify some pattern or collection of proteins that have predictive potential in the experimental setting. Our group led the way using surface enhanced laser desorption and ionization with a genetic cluster bioinformatics algorithm to show that there is a discriminative pattern of mass spectroscopy features discerned from plasma samples that can differentiate between unaffected women and those with ovarian cancer. The algorithm was trained using serum samples from women with all stages of ovarian cancer. It was able to recognize all stage I cancers in a separate cohort of blinded multi-stage samples [10]. This addresses the first goal, to develop a biomarker that can identify early stage disease. The findings have been extended using different selection chip matrices and a more sensitive tandem mass spectroscopy system capable of detecting several hundred thousand features in the low molecular weight protein/peptide range (Conrads et al, manuscript submitted). Data are reproducible with samples from different institutions and identify signature patterns with several features that are identified

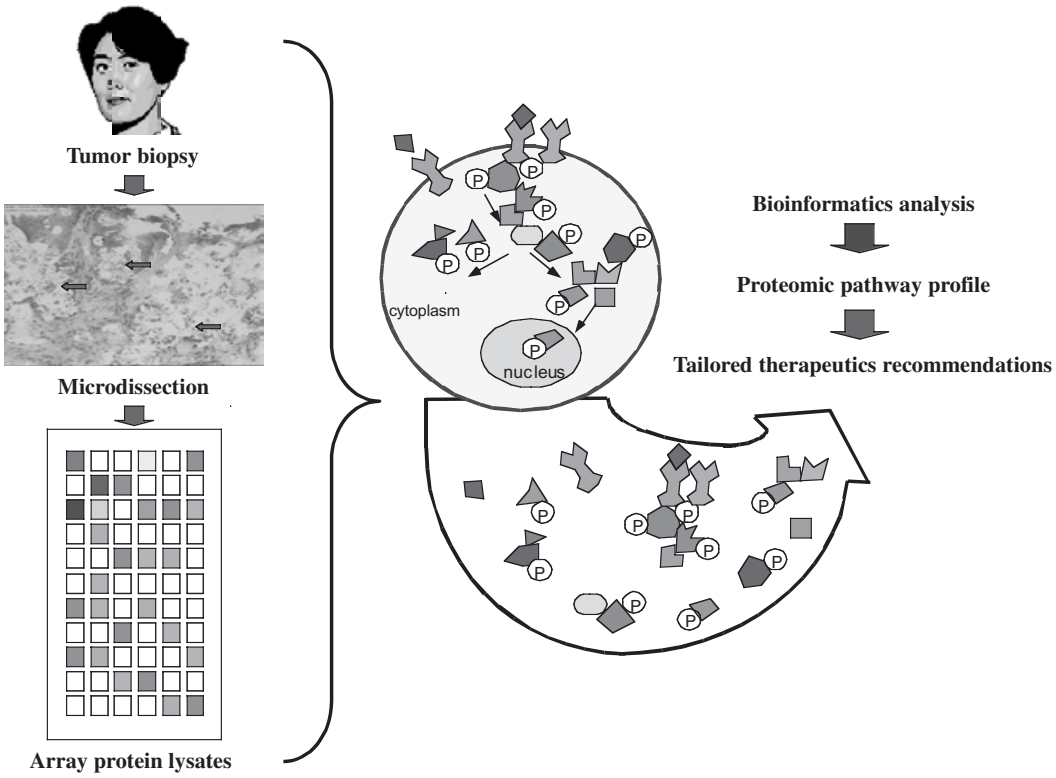

Fig. 3. Application of molecular signature for clinical decision making. Protein expression and signal pathway activation status can be monitored in tumor tissue by minimally invasive biopsy acquisition followed by protein lysate array analysis. Bioinformatic integration of the array data can yield a description of the molecular profile of the cancer driving logical targeted therapeutic combinations individualized to the expressed genetic aberrations in the patient's tumor.

reproducibly (Petricoin and Liotta, preliminary results). Work is underway to isolate the features in order to learn more about the tumor and its local microenvironment.

The field is now charged with demonstrating robustness of the lead biomarker approaches. This includes demonstration that the assay can be translated to the community serum collection standards, that results transcend bias that may occur related to the time of day, meal, medication, or other patient variables, that it is applicable to genetically at-risk as well as sporadic risk cancer patients, and that it can be processed in a high throughput fashion at acceptable cost. The value of these assays as monitors of cancer recurrence need to be evaluated in prospective randomized trials designed to ascertain sensitivity and specificity impact on outcomes and quality of life.

BIOMARKERS REPRESENT HOST AND TUMOR EVENTS

Investigation of the serum proteome or circulating DNA or RNA moieties for biomarker determination is logical. Aside from the ease of obtaining the patient materials, blood products have the highest likelihood of representing changes going on in organ confined disease. However, that does not guarantee that the signature obtained is from the tumor cells themselves. It is more likely that any signature identified reflects the local microenvironment occurring during and after epithelial cell transformation [38]. Changes in the stroma and the local stroma/hyperplasia interface can precede neoplasia. This has been shown in prostate and ovarian cancer, amongst others [39–41]. Thus, incorporation of cohorts of women with preneoplastic lesions, CIN in cervix cancer, UIN and atypical uterine hyperplasia for endometrial cancer, and benign lesions that may have some relevance to ovarian cancer are important in any biomarker development program.

TOOLS FOR MONITORING MOLECULAR THERAPEUTIC INTERVENTIONS

Early diagnosis is an imperative in ovarian cancer and

a mechanism to discriminate those early stage disease patients destined to have recurrent and fatal disease is needed for all gynecologic cancers. In concert with the latter is the need for surrogate markers of response. CA125 is helpful in monitoring patients for whom it has been elevated [11,42]. However, CA125 may not increase with serial tumor recurrences or its magnitude of change may decrease such that it ceases to be a reliable marker. The increased use of molecularly targeted therapeutics and the further dissection of the mechanisms of action of cytostatic agents is providing new likely surrogate endpoint targets for response monitoring. Proteomic technologies have been developed and/or adapted for surrogate marker analysis, and if validated in prospective trials, may advance our predictive abilities. The concept of proteomic pattern signatures for disease recurrence prediction has been applied to clinical trial. It is not yet known if a general signature pattern of recurrence can be developed or if patterns validated for screening will serve a dual duty in recurrence detection.

Evaluation of genomic, genetic, and proteomic disease- or treatment-specific events can be tested in relationship to treatment outcome. The finding that c-kit mutation positive gastrointestinal stromal tumor patients are more likely to have a response to STI-571 (Gleevec) therapy is an example of this approach [43,44]. This is being tested for epidermal growth factor receptor inhibitors in ovarian and other cancers. Currently, these events must be evaluated in the tumor tissue directly. Since that requires an invasive procedure, it is important to develop techniques to glean the most information from this limited clinical sample. We and others have created protein microarrays allowing micro-format analysis of selected and specific proteins [7,45,46]. This approach is amenable to high throughput analysis and serial analysis in a quantitative fashion. Further, it is amenable to microdissection to interrogate tumor and stroma separately, if needed. Repetitive printing of the tumor cell or stromal cell lysate array maximizes internal controls and potential to replicate results. Arrays are subjected to immunostaining, much like immunohistochemistry, and then applied to a reader. Quantitative results are gleaned and can be analyzed against treatment outcome or other clinical variables of interest [7]. A criticism of the technique, also a criticism of the tissue array, is that only a microcosm of the tumor is being sampled. A true representation of the tumor may not be present if there is marked heterogeneity.

Many investigators have recognized that immediately proximate signaling events, such as the presence of the target receptor or of its activated state, may not be the best biomarkers for comparison of molecular surrogate marker results to patient outcome. Downstream events regulated by the molecular target and other interactive pathways, converging on a final cell survival or invasion pathway, may be more reliable surrogates of response. For example, regulation of the activation status of Akt reproducibly correlates with patient response to therapy in a small subset of patients receiving Herceptin treatment (Liotta, preliminary results). Thus, the proteomic tools under development have the potential to provide high throughput focused molecular dissection of the cancer and its response to therapy. These approaches may rapidly become commonly used, robust methods of clinical patient assessment.

SUMMARY

The science of cancer biology, cancer detection, prevention and treatment has advanced at a lightening pace. New technology has been married to clinical practice to identify and test new approaches to cancer detection and for optimization of patient care. The excitement of these advances must, as always, be tempered by cautious review of the supporting data, appropriateness of use, and careful interpretation of results. Application of genomics, genetics, and proteomics to clinical diagnosis, prognostication, treatment, and triage is in our immediate future. Clinical trials of new tools need to be done with adequate design and power to allow us to adopt effective new technologies as quickly as feasible, while discarding inadequate alternatives. Recognition of the importance of the translational scientist in the clinical trials and clinical treatment venues is necessary. We cannot make important advances quickly or safely without developing teams for patient recruitment, trial execution, careful data analysis, and dissemination of new tests. Use of these new technologies for early diagnosis of gynecologic cancers, identification of high risk or low risk patient subsets for triage to appropriate treatment, and new mechanisms for reliable surrogate markers of response will advance medical and surgical gynecologic oncology into the new century and provide the best clinical and scientific care for our patients. This will permit attainment of the goal of individualized molecular medicine and with ensuring improvement in outcome.

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