Proteomics in cancer

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Mass spectrometric approaches for cancer diagnosis

Serum protein profiling by mass spectrometry (MS) is a promising direction for clinical and scientific advancement in diagnosis and treatment. This technology has been reported by many investigators [1–11] to achieve higher ovarian, breast and prostate cancer diagnostic sensitivity and specificity. Newer results show sensitivity and specificity approaching 100% in some cases, compared with conventional and other investigational cancer biomarkers [12]. The predecessor to MS for discovering disease-associated proteins, two-dimensional polyacrylamide gel electrophoresis, is laborious, requires large quantities of protein and is not easily converted into a diagnostic test. The development of an MS and bioinformatics coupled approach has largely overcome many of these limitations, proving to be a fast, potentially cost-efficient, minimally invasive, highly sensitive and accurate diagnostic tool.

Surface-enhanced laser desorption and ionization (SELDI) or matrix-assisted laser desorption and ionization (MALDI) with time-of-flight (TOF) MS detection, coupled with artificial intelligence-based informatics algorithms, have become a powerful tool with which to identify biomarker disease profiles [3, 13–15]. Mass spectrometry has been used successfully to detect several disease-associated proteins in samples of <10 μl from cell lysates, seminal plasma, urine, cerebrospinal fluid, nipple aspirate fluid and serum. The SELDI technology procedure involves application of samples to protein capture chips composed of chemically modified surfaces, causing the proteins to be selectively adsorbed to the surface. These protein chips have multiple spots containing varied surfaces, including hydrophobic, ion exchange, metal affinity binding surfaces or normal phase chromatographic surface. The use of multiple capture chip matrices can provide different views of the proteome. The sample protein-bound chips are introduced into the MS unit and separation results through ionization of the proteins with laser energy (Figure 1). Detection is in direct proportion to the size and net electrical charge of the protein (m/z). The mass spectrometry output is shown as a chromatographic pattern wherein the peak amplitude is represented on the y-axis at a given mass/charge assignment (m/z or x-axis). The resolution of the mass spectrometry unit directly reflects the sensitivity of this technique; the datastream can contain 15,000–350,000 data points in the region below 20,000 Da/charge ratio, depending on the type of machine and its mass accuracy [14]. Different investigative groups have found that different proteomic chip surfaces may be optimal for their disease diagnosis [3, 14, 16–20]. Electrospray and MALDI are also under investigation.

Higher-order analytical bioinformatics approaches are used to define optimal discriminatory signature proteomic patterns to distinguish cancer versus non-cancer patients. The signature pattern developed from the initial genetic algorithm bioinformatics program used identified a set of five key features, m/z species, which as an event in N-space fully segregated ovarian cancer from non-cancer within a supervised teaching set of defined populations [3]. This was then applied to a blinded series of samples from which its true discriminative ability was evaluated. Mass spectra from the blinded unknown samples were classified by likeness to the pattern created by the key features in the discriminative signature. This technology and similar other high order bioinformatics has achieved signature patterns by scientific investigators that are 99–100% sensitive and 99–100% specific, proving the concept to be promising as a new biomarker tool [3]. Eventually, with extensive validation by independent research groups, mass spectra proteomic pattern analysis could be applied to medical screening clinics as a diagnostic test.

Limitations of mass spectrometry

Many scientists have utilized MS as a disease biomarker discovery platform, although there are limitations with this technology [21]. The discriminatory signature patterns that distinguish peaks between cancer and non-cancer or other entities are different among scientific groups and report differing sensitivity and specificity [3, 14, 16–20, 22, 23]. This may be due to the extent of the information cache used, e.g. up to 500,000 points (0–20,000 Da), and for that there may be more than a single reliable sensitive and specific diagnostic pattern. MS resolution capability varies between older and newer machines, proteomic chips containing different chromatographic surfaces and the different bioinformatics analysis programs that reveal other discriminatory peaks. Advancement with this technology will occur with higher resolution instruments, positive identification of signature patterns by independent research groups and identification of the molecules detected through MS that are the cancer diagnostic markers. It remains paramount that each profile put forward and each biomarker system undergoes rigorous blinded assessment using reliable validated standard operating procedures and cross-platform validation.

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Patient-tailored therapy

Pharmacogenomics is the utilization of a patient’s molecular profile for prediction of response or toxicity to therapy. A plethora of new potential targets for cancer therapy are now being uncovered with the recent sequencing of the entire genome and the availability of huge bioinformatics databases providing new views into the complexity of oncogenesis. The next step towards developing more effective molecular targeted therapies is determining the functions of genes and gene products as they function in the patient. Recovering resources with which to complete transcriptional profiling from patient samples and applying the global gene expression data to patient care and drug development has proven to be problematic. Aside from issues related to tissue quality, there is the breadth of information and the inability to capture it all at one time. Furthermore, transcriptional studies do not address post-transcriptional modifications and cellular signal transduction pathways. In cellular signal transduction pathways, location of the signal may also affect the result of the signal. Proteomics is an emerging field that examines the final protein product of gene expression, and can be applied to yield different answers to functional and pharmacological questions. This approach has proven to be a promising direction for the identification of prognostic and diagnostic markers, as well as a method for proof of principle for putative therapeutic targets.

Protein microarray technologies provide new means of monitoring chemotherapeutic treatment [24]. Pharmacological targets may be hit more selectively if key signal pathways involved are known and how their activity is regulated can be assessed. A new proteomics technology, reverse-phase protein arrays (or tissue lysate arrays), has been developed to allow the study of the dynamic proteome of human cancer. Laser capture microdissection allows for molecular analysis of pure cell populations from biopsy material and, when coupled with reverse phase protein arrays, allows protein circuitry mapping of pathways of interest. These may include pro-survival, mitogenic, apoptotic, growth regulation and other pathways involved in tumor progression and chemotherapeutic cytotoxicity (Figure 2) [25]. Integration of proteomics into clinical trials could lead to individualized patient care.

Combinatorial therapy provides a mechanism through which one can simultaneously target different mechanisms of action to cause greater metabolic disruption [26]. The optimal use of this concept requires knowledge of the mechanism(s) of action of the agents, and the status of the targeted tumor cell and stromal protein pathways. Clinical drug trials with compounds shown to work synergically in vitro are now in progress. D’Incalci and Jimeno [27] presented phase I/II clinical results exploring the combination of cisplatin with a novel partially characterized drug, trabectedin (ET-743, Yondelis), in ovarian cancer. Trabectedin is known to form monoadducts at the N2 position of guanine in the minor groove of DNA, whereas cisplatin binds at the N7 position of the guanine, in the major groove of DNA [28]. Cisplatin resistance is primarily mediated through enhancement of the DNA repair pathway, the transcription-coupled nucleotide excision repair (TC-NER) pathway [29]. Trabectedin may counteract this cisplatin resistance through targeting the tumor cells containing upregulated TC-NER. Early clinical results with trabectedin in relapsed ovarian cancer are promising. Signal profiling of patients through proteomic monitoring, if incorporated into these clinical trials, may allow assessment of the success and failure of combination treatment in patients.

Our group has initiated a series of clinical trials employing these technologies to understand the activity of several targeted agents. One such study involves the use of imatinib mesylate (Gleevec) in ovarian cancer. Imatinib targets c-kit, platelet-derived growth factor receptor-β and abl kinase. In addition to monitoring the agent’s clinical activity, we have proceeded to collect serial biopsies of index lesions and have begun to profile effects on signaling pathways involved in survival, proliferation and angiogenesis. These profiling studies...
have provided insight into imatinib-associated toxicities, such as marked fluid accumulation observed in ovarian cancer patients while on the drug. Fluid accumulation waxed and waned in these patients with drug exposure, and correlated to induction of proangiogenic cytokines in the blood (Posadas et al., 2004). Additionally, we are in the process of initiating a series of clinical studies to test combinatorial anti-signaling therapy using agents that have been found to have greater than additive efficacy upon signaling events during in vitro testing. These studies are designed to incorporate a series of biological end points in addition to standard clinical end points, in a scientific effort to demonstrate biological proof of principle.

New directions

Innovative high-throughput proteomic applications

The Developmental Therapeutics Program of the National Cancer Institute has profiled numerous aspects of 60 human cancer cell lines (NCI-60) for the past 11 years [30]. These 60 cell lines include leukemias, melanomas, and renal, ovarian, colon, breast, prostate, lung and central nervous system cancer cell lines. The cell lines have been characterized pharmacologically by exposure to >100 000 defined chemical compounds (http://dtp.nci.nih.gov). Many laboratories have taken the ‘omic’ approach to characterizing the DNA, mRNA and protein in the NCI-60 cell lines, resulting in abundant public information resources. High-density reverse-phase protein lysate microarrays have been applied for proteomic profiling of this set of cell lines [31]. Each protein array contains >30 000 datapoints, and was probed with 52 antibodies and analyzed by p-scan (available at http://abs.cit.nih.gov/index.html) with a quantitative dose interpolation method developed specifically for this study. The arrays identified promising markers for distinguishing different cancers, for example colon from ovarian adenocarcinomas [32]. Tumor biopsies from patients have been used by the Tissue Array Program consortium at the NCI to produce tissue arrays containing up to 600 human tumors of different types, plus normal human tissues, for scientists to probe with protein markers of interest. These resources can be used to validate markers identified using proteomic and other technologies. Additional information about these arrays can be seen online at http://ccr.cancer.gov/tech_initiatives/tarp/. This high-throughput technology is expected to contribute significantly to our basic biological understanding, and guide in the identification of molecular markers and targets for therapy.

Biological database construction and interpretation

Recent advances in high-throughput proteomic studies have led to intensive statistical analysis, database construction and biological interpretation [33]. Weinstein and Pommier [30] have developed a number of algorithms and computer program packages to assist in the analysis and interpretations, which are publicly available at http://discover.nci.nih.gov. CIM-Miner generates color-coded cluster image maps (Figure 2) to represent high dimensional datasets like gene expression or protein expression profiles. After identification of a key set of interesting genes, GoMiner is able to identify thousands of genes and organizes them in the framework of the Gene Ontology hierarchical tree [34, 35]. Another useful database that is
available, LeadScope/LeadMiner, links molecular markers with drug discovery, comparing gene expression profiles for the NCI-60 or other cell lines used for screening with a set of 27,000 drugs tested against these cell lines. The integration of so many types of information available from multiple sources into universal, publicly available databases is a necessity.

Conclusions

The field of proteomics represents an important medical advancement and may yield important tools in the war against cancer. While this remains a paradigm shift from the genomic approach to medicine, it is important to remember that it is the protein that is the final arbiter of function, the level at which molecular therapeutics are active. The introduction of new technologies such as those described here, along with the development of highly sensitive function-specific antibodies and advanced data mining techniques, are yielding results of significant advances. Elucidation of new biomarkers for cancer and identification of proteomic signatures are leading towards development and validation of diagnostic and prognostic tools. Despite our aggregate advances in oncology, the paradigm of early detection and intervention must remain our primary goal, as prevention is preferable to disease and risk of cure. The new tools necessary to advance this are being invented, validated and improved. Clinical studies are opening with new agents and new assessment tools. Application of these assessment tools to new molecular targeted therapeutics could improve the method of testing and selection of these agents for human subjects. The application of proteomic technologies to clinical studies will allow us to acquire crucial information in the activity and efficacy of these agents and lead to rational combinations to optimize antitumor effect and minimize toxicity.

References