NEW TECHNOLOGIES FOR BIOMARKER ANALYSIS OF PROSTATE CANCER PROGRESSION: LASER CAPTURE MICRODISSECTION AND TISSUE PROTEOMICS

CLOUD P. PAWELETZ, LANCE A. LIOTTA, AND EMANUEL F. PETRICOIN III

ABSTRACT

The widespread use of serum markers during cancer screenings has led to the belief that there may be tumor markers yet to be discovered that offer better specificity and sensitivity than prostate-specific antigen (PSA). Proteomics, the analysis and characterization of global protein modifications, will add to our understanding of gene function and aid in biomarker and/or therapeutic target discovery. In the past, most proteomic studies were either performed using tumor cell lines or homogenized bulk tissue. Unfortunately, these approaches may not accurately reflect molecular events that take place in the actual ductal epithelium that change as a consequence of the malignant process. This report describes alternative proteomic-based approaches aimed at the identification of protein markers in the actual premalignant and frankly malignant epithelium.


Prostate cancer is the most common noncutaneous malignancy diagnosed and the second most common cause of cancer-related death for men in the US. Despite the widespread use of prostatic-specific antigen (PSA) screenings, derivations thereof (percent-free PSA), and novel molecular marker models, such as the prostatic specific membrane antigen, these tests do not offer optimal specificity and sensitivity required for sophisticated clinical decision making.1–4 It becomes increasingly apparent that the search for a single specific tumor marker for prostate cancer may have a low probability of success,5 particularly when the model systems consist of cell lines or animal tumor models. Our lab has undertaken an alternative approach aimed at the identification of molecular markers in the actual tissue cells of the pathologic prostate specimen. We seek molecules that track with the early pathologic progression of the prostatic cancer and are correlated with aggressive behavior or response to treatment.

The development of rational approaches to the diagnosis and treatment of prostate cancer may start with a basic understanding of the molecular mechanisms that underlie tumor progression. In this regard, discovery-oriented studies such as the human genome project are cataloging a tremendous number of new genes. However, as the human genome project nears its expected publication date of February 2001, it has become obvious that gene sequence alone cannot predict functional consequences that are ultimately reflected in the actual protein contents within the cell.6,7 Normal communications between these proteins trigger signal transduction pathways that determine whether a cell remains quiescent, proliferates, differentiates, commits programmed cell death, adapts to a differentiated state, or migrates. It is envisioned that information regarding proteins governing these processes will likely reveal new drug targets, markers for early detection, or vaccine candidates if the protein is surface expressed.

Furthermore, although studies at the mRNA (messenger RNA) level are capable of rapidly assessing the expression profiles of a large number of transcripts, this kind of analysis measures only the relative abundance of an mRNA encoding a protein and not the actual protein abundance in the disease lesion. It has recently been shown that there is not...
necessarily any direct correlation between mRNA expression and protein levels in vivo.8 Emerging proteomic technologies allow for scanning of cellular proteins in simple, short, reproducible, and quantitative chemical assays.9,10 Discerning the mechanism whereby normal cells transform into premalignant cells, then into tumor cells, and finally into metastatic dissemination can best be understood if the analysis is performed in the actual tissue itself. This is a particularly challenging problem in the study of prostate cancer because relevant cell populations (ie, normal, prostatic intraepithelial neoplasia, frankly invasive) only constitute a small fraction of the whole cellular repertoire, thereby effectively limiting traditional proteomic investigations, such as two-dimensional electrophoresis (2D-PAGE) of homogenized prostate bulk tissue and/or cell lines, such as LNCaP cell line. Results obtained in this fashion need to be analyzed with caution, as it is not clear whether gene or protein expression changes seen in prostate tumor progression are causal, a result of the malignancy itself, or are contributed by interpatient variability.

To address these problems we utilize laser capture microdissection for the procurement of pure cell populations.11 Laser capture microdissection allows for selective procurement of cells with a precision of 3 to 5 μm under direct microscopic visualization. Briefly, a stained slide is placed under a microscope, and a specific adherence cap, housing an ethylene vinyl acetate (EVA) film, is placed onto the tissue. Subsequently, the user moves the slide until the area of interest is in the center of the view field of the microscope. When the cells of interest are located, an infra red laser is fired, which melts the film in the area of the target. The EVA film expands into the void of the stained tissue and solidifies within 200 msec as it rapidly cools.

To date the most commonly used proteomic tool for the study of disease-associated protein changes is the combination of 2D-PAGE analysis with mass spectrometric identification of deregulated proteins. For the analyses of microdissected 2D-PAGE, approximately 50,000 cells (7000 laser acquisition shots) are required.12 However, 2D-PAGE analysis of laser capture microdissection (LCM) procured cells, although a powerful technique for the discovery of novel proteins in a given disease state, is a laborious, time-intensive process and is not amenable to rapidly assessing changes in protein expression in a clinical setting, thereby effectively limiting the usage of 2D gels for urologists to determine diagnostic and prognostic outcome of prostate cancer on a routine basis.

Alternatively, a possible method to improve on existing screening technologies, which focuses on single proteins and genes such as PSA, “free PSA,” mPSA, p53, or PTEN, would be to utilize specific protein profile phenotypes that correlate with changes in signal transduction pathways.13,14 It is envisioned that if a global survey of a large protein population can rapidly be visualized and correlated with a prognostic or diagnostic stage, limitations of common screening techniques may be overcome.

Surface-enhanced laser desorption ionization (SELDI) spectrometry is a highly sensitive, specific, and high-throughput technology for the study of protein lysates. The foundation of this technology is based on the same principle as in affinity-based mass spectrometry. Proteins of interest are directly applied to a surface utilizing a defined chemical chromatographic characteristic (ie, hydrophobic, hydrophilic, cationic, anionic) or biochemical ligands such as proteins, receptors, antibodies, or DNA oligonucleotides. Consequently, the bound proteins are treated with wash buffers to wash nonspecific binding partners away and analyzed by time-of-flight mass spectrometry to selectively present a protein phenotype that shares only common chemical characteristics. It differs from conventional matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry in that it does not rely on preclearing complex biological mixtures of most extraneous material in the matrix-analyte mixtures by high-performance liquid chromatography or gas chromatography.15 The extension of this technology to construct sensitive, specific, and reproducible protein profiles that span progressions of a variety of malignancies from microdissected samples was validated just recently.14

In a specific example that demonstrates the utility of protein biomarker changes within prostate progression, we microdissected 1500 patient-matched normal, prostatic intraepithelial neoplastic, frankly invasive, and endothelial cells and analyzed them by SELDI-TOF mass spectrometry. A schematic of the process and the results are shown in Figures 1 and 2, respectively. Not only was a specific and reproducible protein phenotype obtained for each individual cell type, but the spectra for microdissected premalignant cells also represented a unique subset that was a mix of both the normal and tumor protein fingerprints. Precisely, the ratio that is concomitantly changing between protein identities A and B seems to be disease associated between normal and tumor microdissected cells as ongoing studies in our laboratory are currently showing. Furthermore, this kind of analysis would be amendable in a clinical setting because the whole analysis from the microdissection until the readout takes only 10 minutes.
SUMMARY

Studies demonstrating statistically useful prognostic and diagnostic values from microdissected tissue samples are warranted and currently ongoing in our laboratory. The need to correlate diagnostic value of serum and biopsy samples is here realized and actively being pursued in a large study set. Nonetheless, even though still in its infancy, we believe that the combination of novel technologies, such as SELDI and LCM, surface plasma resonance screening, and others will provide a substantial basis for the improvement and the development of multiparametric, efficient, and cost-effective clinical screenings in prostate cancer.16,17

FIGURE 1. Schematic overview of longitudinal cancer progression investigation by surface-enhanced laser desorption ionization (SELDI) spectrometry. Defined patient-matched cell populations are microdissected, lysed, and immobilized onto the SELDI chip. After ionization and subsequent desorption from the surface, a time-of-flight protein profile from the entire cellular repertoire is observed. MW = molecular weight.
REFERENCES


FIGURE 2. Representative protein phenotypical time-of-flight mass map of microdissected patient-matched normal, prostatic intraepithelial neoplasia, and invasive prostate carcinoma cell populations. In total, 1500 patient-matched normal, prostatic intraepithelial neoplasia, and invasive carcinoma cell populations were microdissected and analyzed by surface-enhanced laser desorption ionization spectrometry. Two protein identities (A and B) seem to change concomitantly with prostatic disease progression in a variety of individually analyzed cases.