ELSEVIER

Urologic Oncology: Seminars and Original Investigations 24 (2006) 231–236

UROLOGIC ONCOLOGY

Seminar article

Proteomics for the identification of new prostate cancer biomarkers

David K. Ornstein, M.D.[a,*], Darren R. Tyson, Ph.D.[b]

[a] *Department of Urology, The University of California, Irvine, Orange, CA 92868, USA*
[b] *Department of Urology and Physiology & Biophysics, The University of California, Irvine, Orange, CA 92868, USA*

Abstract

Molecular profiling studies of human prostate cancer provide great opportunities to identify new prostate cancer biomarkers to improve prostate cancer detection and treatment. Proteomics has distinct advantages over genomic and ribonucleic acid expression studies because it is the proteins that are ultimately responsible for the malignant phenotype. The goal of traditional proteomic studies is to identify disease-specific biomarkers. Two-dimensional (2-D) gel electrophoresis (polyacrylamide gel electrophoresis; PAGE) coupled with mass spectrometry is the most widely used experimental strategy and, to date, has yielded several potentially relevant prostate cancer biomarkers. A promising prostate cancer biomarker identified by 2-D PAGE and mass spectrometry is annexin I. Studies have already confirmed that annexin I is underexpressed in a majority of early stage prostate cancers. Other nongel based proteomic technologies that may have improved sensitivity as compared to 2-D PAGE have recently been developed. An example of this is the ProteomeLab PF 2-D (Beckman Coulter, Inc., Fullerton, CA).

The goal of most proteomic studies is to identify biomarkers that can be measured by enzyme-linked immunosorbent assay or immunohistochemistry. Improvements in proteomic technology may be changing this paradigm because there are now efforts to develop proteomic technologies directly into clinical diagnostic tests. An example of this technology is surface-enhanced laser desorption ionization time-of-flight mass spectrometry. Using this technology combined with a pattern recognition based bioinformatics tool, discriminatory spectrum proteomic profiles were generated that could help discriminate men with prostate cancer from those with benign prostates. If several technologic hurdles can be overcome, it is possible that methodology will improve the specificity and sensitivity of prostate cancer detection.

Keywords: Proteomics; Prostate cancer detection; Annexin I

Introduction

Although the introduction of serum prostate-specific antigen (PSA) measurements into clinical practice has revolutionized the care of patients with prostate cancer, there are well-recognized limitations of PSA, and there is a critical need to identify additional prostate cancer biomarkers to assist in early detection and prognosis. Advances in biotechnology have made high-throughput molecular analysis of human tissue a reality, and the opportunity to identify novel prostate cancer biomarkers is greater than ever. To date, most molecular profiling studies of prostate cancer have focused on messenger ribonucleic acid (RNA) transcript analysis. However, there are distinct advantages of proteomic studies because proteins are ultimately responsible for the disease phenotype. Proteomics can identify alterations in post-translational modifications, cellular trafficking, and even total expression levels that may not be detected by RNA-based expression studies.

Furthermore, because most Food and Drug Administration approved diagnostic tests are protein based, it should be possible to use findings from proteomic studies to develop clinically useful tests. Traditionally, proteomic studies have been used for biomarker discovery, and the clinical test is typically an enzyme-linked immunosorbent assay (ELISA)-based assay. Because technologic advances have increased the throughput potential of protein analysis, it is possible that some of these analytic instruments will become usable for proteomic-based clinical diagnostics. In the past, most proteomic studies have used the tumor tissue itself as the source for biomarker discovery but, with advances in proteomic technologies and the realization that the molecular milieu of even localized cancer is reflected in circulating fluids, serum has become an increasingly desirable source

* Corresponding author. Tel: +1-714-456-5179; fax: +1-714-456-5062.
E-mail address: dornstei@uci.edu (D.K. Ornstein).

1078-1439/06/$ – see front matter
doi:10.1016/j.urolonc.2005.11.035

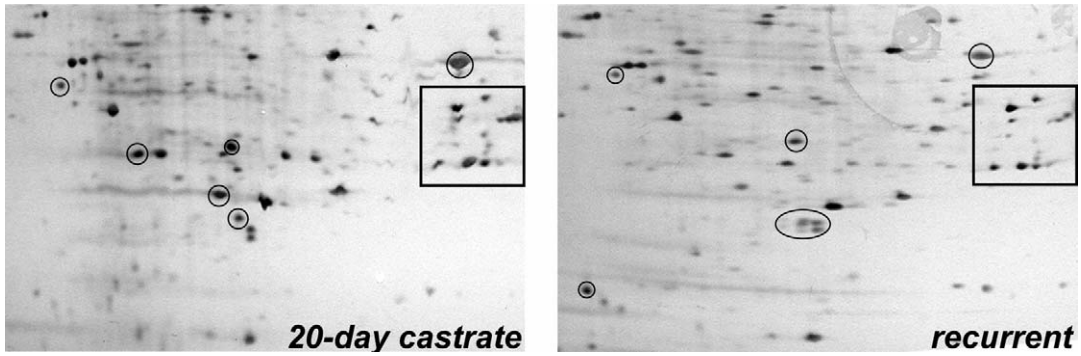

Fig. 1. 2-D PAGE separation of insoluble fractions of CWR22 xenografts. Xenografts were subjected to an ultraturrax homogenizer in Tris buffer, and insoluble proteins were pelleted by ultracentrifugation at 450,000 × *g* for 10 minutes. 2-D PAGE then separated insoluble proteins, and gels were silver stained. Differentially migrating spots are circled. Spot patterns with very high similarity between the samples are indicated with boxes.

for biomarker discovery. To date, several prostate cancer biomarkers have been discovered, and there are currently intense efforts to define the clinical use of these biomarkers. There is great promise that proteomics will improve how urologists diagnose and treat prostate cancer in the not too distant future.

Discovery based proteomics. The use of 2-dimensional gel electrophoresis to identify markers associated with early stage prostate cancer

Traditional proteomic studies have relied on 2-dimensional (D)-polyacrylamide gel electrophoresis (PAGE) to compare protein expression patterns from different tissues or cell lines. The first dimension separates proteins by pH (isoelectric focusing) and the second dimension by molecular mass (sodium dodecyl sulfate PAGE). Although, 2-D PAGE has been available for several decades, improvements in this technology have dramatically improved sensitivity, spot resolution, and reproducibility. The use of fluorescent-based dyes, such as SYPRO Red (Cambrex Corp., East Rutherford, NJ), has highly improved the dynamic range and sensitivity of protein detection, while the development of immobilized pH gradients and image analysis software has dramatically improved the reproducibility.

The major limitation of 2-D PAGE is that it is labor-intensive and does not resolve highly basic proteins or those smaller than 10 kDa. Because most clinically useful biomarkers are high-abundant large proteins, 2-D PAGE is still an ideal technology for cancer biomarker discovery studies. Although 2-D PAGE coupled with mass spectrometry has been used to identify protein changes associated with a variety of human cancers, until recently, it has had limited applications regarding the study of early stage prostate cancer. The primary reason for this limitation is the heterogeneous and infiltrative quality of prostate cancer that makes it difficult to isolate a pure population of malignant prostatic epithelium. To overcome this investigative hurdle, different microdissection techniques have been developed for procuring pure populations of cells from human tissue sections.

Laser capture microdissection is a relatively new technique that allows researchers to visualize a tissue section via light microscopy and procure the desired cells by activating a 7.5–30-micron diameter infrared laser beam to "weld" the tissue to a plastic cap. Intact deoxyribonucleic acid, RNA, and protein can then be extracted from the "welded" tissue and analyzed using conventional methods [1,2]. Specific to prostate cancer studies, laser capture microdissection has been used to procure pure populations of patient-matched benign and malignant prostatic epithelium. Protein expression has been compared using 2-D PAGE and differentially expressed proteins identified by mass spectrometry. This approach has identified several potentially useful biomarkers of early stage prostate cancer, and the most promising ones from this research laboratory are annexins I and II [3,4].

More detailed studies of annexin protein expression in prostate cancer have shown that the expression of both annexins I and II is commonly reduced in high-grade prostatic epithelial neoplasia and invasive prostate cancer [5,6]. Because annexins I and II are strongly expressed in benign lesions, including atrophic cells and basal cell hyperplasia (Fig. 1), annexin I/II immunohistochemistry may be a useful adjunct to help resolve diagnostic dilemmas that occur during the interpretation of needle biopsies. Protein expression of annexins I and II is further reduced in androgen-independent, as compared to androgen-dependent prostate cancer [6]. Studies are ongoing to determine if expression levels of annexins I, II, and VII correlate with Gleason grade and clinical aggressiveness.

The use of 2-D gel electrophoresis to identify markers associated with progression to androgen independence

Further studies using 2-D PAGE and mass spectrometry to identify additional predictive prostate cancer biomarkers are underway. The CWR22 xenograft is an excellent model to study prostate cancer progression because this tumor responds to castration by regressing, but, analogous to human prostate cancer, the CWR22