Clinical Biochemistry 46 (2013) 524–538

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

The role of proteomics in prostate cancer research: Biomarker discovery and validation

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ARTICLE INFO

Available online 22 December 2012

Keywords:
Prostate cancer
Biomarkers
Proteomics
Tissues
Body fluids
Diagnosis
Prognosis
Treatment

ABSTRACT

Purpose: Prostate Cancer (PCa) represents the second most frequent type of tumor in men worldwide. Incidence increases with patient age and represents the most important risk factor. PCa is mostly characterized by indolence, however in a small percentage of cases (3%) the disease progresses to a metastatic state. To date, the most important issue concerning PCa research is the difficulty in distinguishing indolent from aggressive disease. This problem frequently results in low-grade PCa patient overtreatment and, in parallel; an effective treatment for distant and aggressive disease is not yet available.

Result: Proteomics represents a promising approach for the discovery of new biomarkers able to improve the management of PCa patients. Markers more specific and sensitive than PSA are needed for PCa diagnosis, prognosis and response to treatment. Moreover, proteomics could represent an important tool to identify new molecular targets for PCa tailored therapy. Several possible PCa biomarkers sources, each with advantages and limitations, are under investigation, including tissues, urine, serum, plasma and prostatic fluids. Innovative high-throughput proteomic platforms are now identifying and quantifying new specific and sensitive biomarkers for PCa detection, stratification and treatment. Nevertheless, many putative biomarkers are still far from being applied in clinical practice.

Conclusions: This review aims to discuss the recent advances in PCa proteomics, emphasizing biomarker discovery and their application to clinical utility for diagnosis and patient stratification.

Introduction

Prostate cancer (PCa) is the second most common cancer worldwide and the sixth highest cause of death in men [1]. PCa incidence varies more than 25 times worldwide with the highest rate in developed countries, mostly due to a more frequent practice of biopsies and prostate specific antigen (PSA) testing rather than to a real difference in cancer incidence [2]. Moreover, it is also known that both genetic and environmental factors might affect the risk of PCa onset. A familial history of PCa [3,4], a diet rich in fats [5,6], the presence of BRCA1, BRCA2 and HPC1 mutations [7], or low testosterone levels in serum [8] are reported to predispose to PCa.

The rate of men getting PCa or dying from PCa varies also by race and ethnicity. The highest risk rate is reported for African American men even if the causes of this increase are not yet well understood. Some authors reported that it might be partially due to genetic influences [9], but it also likely attributed to clinical and socio-economical reasons [10,11]. In spite of these influences, age remains the main risk factor for PCa. PCa rarely occurs before 50 years of age yet 30% of men between 55 and 64 are reported to have PCa [12]. Just 8% of PCa will become clinically apparent and most of the cases with localized disease will reach the 5-year survival point [13]. On the contrary, in the case of aggressive disease with distant metastasis in liver, lung, brain and especially bone, the survival rate falls to 30% [1].

The current FDA guidelines for PCa diagnosis are based on Prostate Specific Antigen (PSA) detection in blood together with digital rectal exploration (DRE) for men over 50 years of age. This screening modality helps in detecting PCa in patients without any apparent symptoms and has resulted in both PCa-mortality decrease and -incidence increase. Unfortunately, high levels of blood PSA (>4 ng/ml) are not necessarily due to the presences of PCa [14], but can also result from infection, inflammation (prostatitis) or benign hyperplasia of the prostate (BHP). A major issue with PSA testing is the number of PCa false negatives that arises from the current screening methodology: 15% of PCa patients have a PSA level<4 ng/ml and are negative for a digital rectal exam yet are found to have PCa with a Gleason score of 7 or higher [14]. For these reasons the role of PSA measurement in PCa diagnosis has become controversial and some researchers suggest that it should be considered more as a prostate volume marker rather than a marker for malignancy presence. Moreover, while recent studies have revealed that PSA testing reduced the PCa death-rate by 20%, the fact is that most detected PCa are indolent cancers and overdiagnosis is a huge concern.

Besides the necessity to diagnose PCa as early as possible, the disparity between incidence rates and death-rates highlights the importance to distinguish between aggressive and indolent cancers with the aim to stratify and adequately treat patients with aggressive

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0009-9120/$ – see front matter
http://dx.doi.org/10.1016/j.clinbiochem.2012.12.012

disease. Currently, PCa treatment varies a lot dependent on disease stage, grade and patient age. While PCa stage is evaluated through the classical TNM system, which provides important information concerning disease localization, PCa grade is defined by a morphologic analysis that estimates the tissue differentiation. Low Gleason scores define differentiated cancers that are less likely to spread. For low grade PCa the treatment choice depends on the age of patients. For elderly men (>70 years old) with low Gleason score, watchful-waiting is the standard-of-care approach, together with a periodic PSA test, DRE and, if necessary, more biopsies. For younger patients with low Gleason score, prostatectomy is the usual treatment and generally is curative, even though it has some co-morbid disadvantages like incontinence and impotence. The standard treatment for aggressive disease involves radical prostatectomy, hormonal therapy, radiation therapy, ultrasound treatment (HIFU), chemotherapy or cryosurgery. Surgery and pharmacological castration result in tumor regression in 75% of the cases, however there are a number of side effects (osteoporosis, cognitive decline, cardiovascular morbidity, obesity, insulin resistance, fatigue, sexual dysfunctions) that should be considered before therapy administration. Despite the existence of specific treatment guidelines for each PCa grade, so far, no specific markers are useful to distinguish aggressive disease from indolent forms, regardless of Gleason scoring. Unfortunately, even if histology and Gleason scoring have proven to be effective enough to predict the outcome and guide effective treatment for the majority of <6 and >7 score PCas, histologically-oriented descriptions do not provide clinically useful information for Gleason 6 and 7 PCas, for whom the clinical course is still unpredictable. Moreover, this kind of screening is not 100% specific and sensitive for identifying aggressive disease in the <6 category and indolent disease in the >7 category. Understanding the molecular mechanisms that underlie PCa variety is imperative to design new effective tailored therapy. Another issue in PCa treatment is the acquisition of androgen independency after 12–15 months from the start of ablation therapy. Ablation therapy has been used as a front-line treatment for aggressive PCa since the 1940 [15]. The development of resistance to androgen-independent PCa (AIPC) might arise by clonal selection of hypermutated cells due to ablation therapy, which determines tumor progression and a fatal outcome. Avoiding the development of AIPC is another challenge for PCa treatment and with this aim it is essential to identify when the tumor is likely to become hormone-refractory and to design new specific therapy. The major challenge in this regard, is to find new biomarkers able to answer to important clinical questions like: does the patient have a cancer or a benign disease? Is a biopsy required? Is the cancer indolent or aggressive? Which kind of treatment is most indicated?

A protein cancer biomarker is a protein measured in body fluids or in tissues that could reflect the presence of cancer and indicate its aggressiveness, staging and response to therapy. Protein cancer biomarkers can be divided in several categories [16]:

a) Diagnostic screening biomarkers: proteins that are used to detect cancer in an individual. A diagnostic biomarker is required to have high sensitivity and specificity.
b) Prognostic biomarkers, which are used to predict the course of the disease, including recurrence and aggressiveness. They are useful once the disease status has been established to make the more appropriate therapeutic choice.
c) Stratification biomarkers: proteins that predict response to a specific therapy, permitting a stratification of patients in responders/non-responders. A stratification biomarker can be identified by molecular profiling analysis of tissues, which could uncover specific analytes that correlated with response to therapy. Biomarkers that predict response to therapy do not need to be cancer specific to be useful [16].

The discovery of new biomarkers in blood, urine or tissue can help to develop more sensitive and specific PCa diagnostic and prognostic tests that will permit the early detection and treatment of patients with aggressive disease and, concomitantly, avoid overtreatment for low risk cases. Proteomics, together with the innovative high-throughput technologies, might be a highly promising way to identify new biomarkers for both detection and tailoring therapy. The recent advances in proteomics are producing powerful platforms that are able not just to detect proteins but also to quantify them in many different body fluids (urine, blood, seminal fluid) and in tissue (Fig. 1).

This review will discuss the most recent advancements of proteomics in the PCa field, with special emphasis on new approaches for biomarker discovery and characterization and their possible future clinical application in diagnosis and patient stratification (Table 1).

Tissue biomarkers

The key to a more effective diagnosis, prognosis, prediction and therapeutic management of PCa could lie in direct analysis of cancer tissue. Through the analysis of the tissue itself it might be possible to clarify the mechanisms at the basis of the transformation of a prostate normal cell to a tumor cell and that, subsequently, permit the progression to a metastatic state. The Human Genome Project in 2001 catalogued the human genome and the recent advances in deep-sequencing has enabled the field to analyze the fine structure of the genome, however simple cataloging genomic mutations and derangements and the transcriptional archive is not enough to elucidate complex biochemical processes like migration, proliferation, differentiation, apoptosis or quiescence. In fact, these processes are regulated by and between complex networks of proteins transducing biological signals through the cells into the nucleus, and the proteomic architecture is likewise orchestrated by post-translational epigenetic modifications such as phosphorylation. Indeed, this phosphorylation/activation status is not directly correlated to transcription level. For these reasons and also because proteins are the functional units of these signaling pathways, proteomic technologies have become a powerful tool to reveal new drug targets, markers for early diagnosis or vaccine candidates.

An important factor to be considered for proteomic analysis is that the tumor tissue is usually not constituted by a group of homogeneous cells. On the contrary, tumor tissue is comprised of many different subpopulations (e.g. fibroblasts, nerve cells, endothelial cells, infiltrating lymphocytes, epithelial cells, etc) that cross-talk with each other and collaborate for sustaining tumor growth and proliferation. To clarify which are the pathways responsible for PCa onset and progression it is important to disassemble this complex tissue ecosystem. The difficulty in isolating pure cell subpopulations from heterogeneous tissue was an issue for inaugural proteomic analysis that has been overcome by the introduction of techniques like cell sorting and laser capture microdissection (LCM) [17]. Cell sorting permits the isolation of cells based on surface CD markers using a cytofluorimeter. The isolated cells can be directly analyzed or subcultured. LCM, instead, is a method that permits the isolation of homogeneous cell types from a tissue based on their histomorphology after a specific staining and under microscopic visualization. Proteins extracted from selected cells can be analyzed using techniques like forward/reverse phase microarrays or mass spectrometry (MS). The most widely used mass spectrometry applications are 2DE-MS, MALDI-MS and SELDI-MS, all permitting a qualitative analysis of the proteome [18]. Just more recently, i-TRAQ was introduced giving the possibility to quantitatively analyze the proteome variations [19].

Tissue diagnostic and prognostic markers

One of the most successfully used tissue protein biomarker for PCa prognosis and prediction is of course the Androgen Receptor (AR). Androgens are the key regulators of PCa growth by both proliferation stimulation and apoptosis inhibition. Testosterone—a steroid hormone secreted by Leydig cells in testis and synthesized starting from cholesterol through a process known as androgenesis—is the main circulating