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Prostate Cancer

The Proteome of Primary Prostate Cancer

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Abstract

Background: Clinical management of the prostate needs improved prognostic tests and treatment strategies. Because proteins are the ultimate effectors of most cellular reactions, are targets for drug actions and constitute potential biomarkers; a quantitative systemic overview of the proteome changes occurring during prostate cancer (PCa) initiation and progression can result in clinically relevant discoveries.
Objectives: To study cellular processes altered in PCa using system-wide quantitative analysis of changes in protein expression in clinical samples and to identify prognostic biomarkers for disease aggressiveness.
Design, setting, and participants: Mass spectrometry was used for genome-scale quantitative proteomic profiling of 28 prostate tumors (Gleason score 6–9) and neighboring nonmalignant tissue in eight cases, obtained from formalin-fixed paraffin-embedded prostatectomy samples. Two independent cohorts of PCa patients (summing 752 cases) managed by expectancy were used for immunohistochemical evaluation of proneuropeptide-Y (pro-NPY) as a prognostic biomarker.
Results and limitations: Over 9000 proteins were identified as expressed in the human prostate. Tumor tissue exhibited elevated expression of proteins involved in multiple anabolic processes including fatty acid and protein synthesis, ribosomal biogenesis and protein secretion but no overt evidence of increased proliferation was observed. Tumors also showed increased levels of mitochondrial proteins, which was associated with elevated oxidative phosphorylation capacity measured in situ. Molecular analysis indicated that some of the proteins overexpressed in tumors, such as carnitine palmitoyltransferase 2 (CPT2, fatty acid transporter), coatomer protein complex, subunit alpha (COPA, vesicle secretion), and mitogen- and stress-activated protein kinase 1 and 2 (MSK1/2, protein kinase) regulate the proliferation of PCa cells. Additionally, pro-NPY was found overexpressed in PCa (5-fold, $p < 0.05$), but largely absent in other solid tumor types. Pro-NPY expression, alone or in combination with the ERG status of

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the tumor, was associated with an increased risk of PCa specific mortality, especially in patients with Gleason score ≤ 7 tumors.
Conclusions: This study represents the first system-wide quantitative analysis of proteome changes associated to localized prostate cancer and as such constitutes a valuable resource for understanding the complex metabolic changes occurring in this disease. We also demonstrated that pro-NPY, a protein that showed differential expression between high and low risk tumors in our proteomic analysis, is also a PCa specific prognostic biomarker associated with increased risk for disease specific death in patients carrying low risk tumors.
Patient summary: The identification of proteins whose expression change in prostate cancer provides novel mechanistic information related to the disease etiology. We hope that future studies will prove the value of this proteome dataset for development of novel therapies and biomarkers.
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1. Introduction

Prostate Cancer (PCa) is the most commonly diagnosed cancer among men in the Western world and a leading cause of cancer death [1]. As a consequence of routine prostate specific antigen (PSA) testing, most patients currently diagnosed with PCa are middle-aged men with small tumors who have a low, but not a negligible risk of disease progression [2]. Most prostatectomy procedures are performed on patients harboring low-risk tumors. To avoid overtreatment and the associated side effects, active surveillance is increasingly used as a management strategy [3]. Thus, better prognostic biomarkers are needed to properly stratify patients with low and intermediate risk PCa for surveillance or active treatment. Moreover, many patients are still diagnosed with lethal tumors that cannot be cured with prostatectomy or radiation therapy [2]. A better understanding of the mechanisms that drive tumor initiation and progression may identify actionable targets to improve treatment of this patient group.

Rapid advances in genomic technologies have transformed our understanding of the dynamic genomic, epigenetic, and transcriptomic remodeling that occurs in primary PCa [4–7]. However, these insights into the genomic landscape of PCa have yet to prove a major impact to the clinical management of PCa patients. Proteins are essential effectors of cellular functions and as such can provide novel biomarkers or viable drug targets; but our knowledge on the PCa proteome remain limited as genomic and transcriptomic alterations are not always associated with changes in protein levels and activity [8–10]. Methodologies for proteome-wide quantitative profiling [11] have the potential to unveil, hitherto, unknown molecular links between genotype and phenotype and facilitate the identification of accurate prognostic biomarkers or actionable targets for improved treatment of PCa.

To identify protein pathways and biological processes altered in malignant versus benign prostate tissue, we implemented a methodology for quantitative proteome analysis of formalin-fixed paraffin-embedded (FFPE) tissue to study a set of radical prostatectomy specimens. This first-in-class analysis resulted in the most comprehensive quantitative analysis of the PCa proteome to date and sheds light into putative cancer regulatory proteins. Furthermore, we provide insights into the transition from low to high grade PCa and identify a novel biomarker, proneuropeptide-Y, (pro-NPY) of PCa disease aggressiveness.

2. Materials and methods

2.1. Experimental design

Whole cell protein extracts were purified from 28 tumor and eight nonmalignant FFPE radical prostatectomy specimens. Protein extracts were mixed in 1:1 ratio with an isotopically labeled standard obtained from four prostate-derived cells lines (Super-SILAC) to improve quantification accuracy [12]. Tryptic peptides were analyzed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (Fig. 1A). Validation of the differentially expressed proteins was carried out in situ using histochemistry/immunohistochemistry and in vitro in cell experiments to evaluate functionality.

2.2. Proteomic analysis

Protein extracts of stable isotope labeling by amino acids in cell culture (SILAC) labeled LNCaP, PC-3, 22rv1, and WPMY-1 cells were mixed in a 27:27:27:19 ratio and used as spike-in standard. Whole protein extracts were purified from FFPE specimens as previously described [13]. Twenty-five micrograms to 40 μg of protein was mixed in equimolar amounts with the isotopicaly labeled protein standard [12] and trypsin digested following the filter-aid sample preparation methodology [13]. Forty micrograms from the resulting tryptic peptides were fractionated with strong anion exchange chromatography into six fractions to reduce sample complexity and maximize depth of proteome coverage [13]. Each fraction was then analyzed with LC-MS/MS on the Q-Exactive mass spectrometer (Thermo, USA). Peptides were separated using a 4-h gradient of water:acetonitrile, on a 30 cm C18 column. MS spectra were acquired in the orbitrap with a resolution of 70,000. MS/MS spectra were acquired in data-dependent mode, after higher energy collisional dissociation fragmentation, at a resolution of 17,500 [14]. The obtained mass spectrometric raw data were analyzed in the MaxQuant environment, version 1.3.7.1 [15] with the integrated Andromeda searching engine and false discovery rate (FDR) cut-off for peptide identification of 0.1 [16] (Fig. 1A). Proteins were identified by searching MS/MS data against the human proteome sequences from UniProt (UniprotKB, 2012). Normalized light (tissue) to heavy (SILAC standard) intensity ratios were averaged for tumor and control samples. Differences of the mean were evaluated by Student t test followed by Benjamini–Hochberg correction for multiple testing. FDR values smaller than 0.1 were considered statistically significant. No correction was applied when tumors were divided by their Gleason scores (GS) and p values smaller than 0.05 were considered statistically significant.

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