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Proteomic Characterization of Prostate Cancer to Distinguish Nonmetastasizing and Metastasizing Primary Tumors and Lymph Node Metastases

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Abstract

Patients with metastatic prostate cancer (PCa) have a poorer prognosis than patients with organ-confined tumors. We strove to uncover the proteome signature of primary PCa and associated lymph node metastases (LNMs) in order to identify proteins that may indicate or potentially promote metastases formation. We performed a proteomic comparative profiling of PCa tissue from radical prostatectomy (RPE) of patients without nodal metastases or relapse at the time of surgical resection ($n = 5$) to PCa tissue from RPE of patients who suffered from nodal relapse ($n = 5$). For the latter group, we also included patient-matched tissue of the nodal metastases. All samples were formalin fixed and paraffin embedded. We identified and quantified more than 1200 proteins by liquid chromatography tandem mass spectrometry with subsequent label-free quantification. An increase of ribosomal or proteasomal proteins in LNM (compared to corresponding PCa) became apparent, while extracellular matrix components rather decreased. Immunohistochemistry (IHC) corroborated accumulation of poly-(ADP-ribose)-polymerase 1 and N-myc-downstream-regulated-gene 3, alpha/beta hydrolase domain-containing protein 11, and protein phosphatase slingshot homolog 3 in LNM. These findings strengthen the present interest in examining PARP inhibitors for the treatment of aggressive PCa. IHC also corroborated increased abundance of retinol dehydrogenase 11 in metastasized primary PCa compared to organ-confined PCa. Generally, metastasizing primary tumors were characterized by an enrichment of proteins involved in cellular lipid metabolic processes with concomitant decrease of cell adhesion proteins. This study highlights the usefulness of a combined proteomic-IHC approach to explore novel aspects in tumor biology. Our initial results open novel opportunities for follow-up studies.

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Abbreviations: PCa, prostate cancer; LNM, lymph node metastasis; TU, primary tumor; RPE, radical prostatectomy; LND, lymph node dissection; FFPE, formalin-fixed, paraffin-embedded; MS, mass-spectrometry; IHC, immunohistochemistry; FC, fold change.

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Introduction

The American Cancer Society estimates 160,000 new cases of prostate cancer (PCa) in 2017 and expects 26,000 patients to die from PCa [1]. PCa prognosis is often favorable, especially when the disease is organ-confined as 5-year survival rates of over 95% show. The total removal of the prostate, called radical prostatectomy (RPE), if necessary in combination with the radical removal of pelvic lymph nodes, offers curative treatment. Apart from surgery, external beam radiation or the implantation of radioactive metal seeds into the prostate, called brachytherapy, is a further treatment possibility [2]. However, PCa returns in 15% to 30% of all cases as local relapse or in terms of metastases [3]. Especially in metastatic state, the survival rates drop to around 28% [4]. PCa metastasizes predominantly via the lymphatic drainage ways to local lymph nodes in the small pelvis, e.g., to those alongside the iliacal vessels, in the fossa obturatoria, or presacral. In advanced stages or in case of relapse, PCa often affects also aortal or caval lymph nodes [5], which are classified as distant metastases.

The nodal relapse of PCa, meaning PCa-positive lymph nodes after primary therapy, can be treated with metastases-directed therapy, such as salvage lymph node dissection (salvage-LND) or targeted radiation therapy [2,5,6].

For PCa risk and prognosis assessment, several well-established prognostic schemes exist, e.g., the Gleason Grading/ISUP grade groups [7], TNM classification, surgical margin status, or the height of prostate-specific antigen (PSA) value at primary diagnosis. However, these tools cannot predict the biological course and the metastatic potential of primary PCa as precisely and reliable as desired.

In cancer research, mass spectrometry (MS)–based proteomic approaches are predestined methods for the initial identification of potential new prognostic, diagnostic, or therapeutic markers, hence opening novel opportunities for follow-up studies. The ability to perform proteomic researches on formalin-fixed, paraffin-embedded (FFPE) tissue [8–10] provided the possibility for retrospective proteomic investigations on sample cohorts with a long-term follow up.

Many studies on the proteome of PCa, e.g., from Aiello et al. [11], Davalieva et al. [12], or Iglesias-Gato et al. [13], compare malignant to benign tissue. We considered it especially interesting to investigate whether the proteome composition of malignant tissue differs from other malignant tissue. Therefore, we compare tissue of primary PCa from RPE of relapse-free patients (n = 5) to tissue of primary PCa from RPE with nodal relapse in the course of median 32 months (n = 5). Additionally, patient-matched primary tumor tissue was compared to the tissue of recurrent lymph node metastases (LNMs) (n = 5). Our focus laid on patients with LNM relapse only, as there is increasing evidence for superior overall survival rates compared to patients with metastases confined to other organs [14,15].

On FFPE tissue of the above-described sample cohorts, we conducted a retrospective MS-based proteomic analysis with label-free quantification with twofold purpose: a) We strove to investigate whether metastasized primary PCa tissue, nonmetastasized primary PCa tissue, and tissue of corresponding nodal metastases exhibit measurable differences in their proteomic profile. b) We aimed to identify individual proteins, which are differentially expressed in metastasized versus nonmetastasized primary PCa tissue and furthermore with significant divergent abundance between metastasized primary PCa tissue of RPEs and corresponding tumor tissue of nodal relapse. We hypothesize that these proteins could on the one hand be predictors of LNM formation and/or indicate its promotion or suppression. Potential candidates identified in this initial study might also serve as starting point for biomarker research on larger and randomized sample cohorts.

Materials and Methods

Ethics Statement

The study was approved by the Ethics Committee of the University Medical Center Freiburg (no. 562/15). Before study inclusion, all patient data were pseudonymized. Patients gave written informed consent for the use of their tissues for research purposes.

Patient Cohort

This study includes 10 male patients with primary diagnosis of PCa between 2003 and 2011. All patients underwent RPE. Five patients (nos. 1-5) had LNM, either already at the time of RPE or LND or they developed biochemical recurrence over time with local (rpN1) or distant (rpM1 (LYM)) nodal relapse exclusively. In case of relapse, they underwent a salvage-LND with therapeutic approach. Before salvage-LND, patients received temporary androgen deprivation therapy with bicalutamide (no. 1), radiation of the prostatic loge (no. 2-4), or no additional therapy (no. 5). The remaining five patients (nos. 6-10) were free of biochemical relapse (postoperative PSA< 0.2 ng/ml). The median relapse-free interval was 10 years, with the latest follow-up examination performed in 2017. Patient characteristics including TNM classification at the time of RPE and salvage-LND, Gleason Score/ISUP grade group, and the localization of the recurrent LNM used for this study are specified in Table 1.

Tissue Collection, Fixation, and Macrodissection

Tissue specimens were harvested at the time of radical prostatectomy with pelvic lymphadenectomy or at salvage-LND. Formalin fixation was started latest within 20 minutes after surgical removal. Tissue fixation in formalin and paraffin embedding was conducted according to routine protocols. After processing, samples were immediately anonymized.

Hematoxylin-eosin (HE)–stained sections of all samples were inspected under a light microscope by experienced pathologists to confirm diagnosis and to mark eligible tumor areas of invasive PCa or LNM (0.5 cm^2-3.0 cm^2) for macrodissection of consecutive sections. These sections were obtained by cutting 10-μm–thick slices from the paraffin block on a microtome, mounted on a glass slide, and dried overnight at 37°C. Deparaffinization in xylene and decreasing concentrations of ethanol was performed as described previously [16] and in an automated manner using the Medite Tissue Stainer COT 20. Tumor areas corresponding to the adjacent HE-stained tumor template were macrodissected of the deparaffinized tissue sections with a scalpel or hollow needle. The tumor tissue was transferred into 1.5-ml microreaction tubes.

Sample Preparation for LC-MS/MS Analysis and Data Acquisition

For further tissue preparation for MS analysis, 100 μl of lysis buffer (0.09 mM HEPES pH 8.0, 0.02 mM DTT, 0.1% Rapigest, in water) was added per mm^3 tissue. Samples were incubated at 95°C, shaking at 750 rpm, for 1 hour. When cooled down to 25°C, pH was adjusted to 7 to 8. Two micrograms of trypsin (sequencing grade, Worthington, Lakewood, NJ)/mm^3 tissue was added to the samples, and they were incubated at 37°C for 18 hours. The samples were

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