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Prostate Cancer

Low-Molecular-Weight Protein Tyrosine Phosphatase Predicts Prostate Cancer Outcome by Increasing the Metastatic Potential

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Abstract

Background: Low-risk patients suffering from prostate cancer (PCa) are currently placed under active surveillance rather than undergoing radical prostatectomy. However, clear parameters for selecting the right patient for each strategy are not available, and new biomarkers and treatment modalities are needed. Low-molecular-weight protein tyrosine phosphatase (LMWPTP) could present such a target.
Objective: To correlate expression levels of LMWPTP in primary PCa to clinical outcome, and determine the role of LMWPTP in prostate tumor cell biology.
Design, setting, and participants: Acid phosphatase 1, soluble (*ACP1*) expression was analyzed on microarray data sets, which were subsequently used in Ingenuity Pathway Analysis. Immunohistochemistry was performed on a tissue microarray containing material of 481 PCa patients whose clinicopathologic data were recorded. PCa cell line models were used to investigate the role of LMWPTP in cell proliferation, migration, adhesion, and anoikis resistance.
Outcome measurements and statistical analysis: The association between LMWPTP expression and clinical and pathologic outcomes was calculated using chi-square correlations and multivariable Cox regression analysis. Functional consequences of LMWPTP overexpression or downregulation were determined using migration and adhesion assays, confocal microscopy, Western blotting, and proliferation assays.
Results and limitations: LMWPTP expression was significantly increased in human PCa and correlated with earlier recurrence of disease (hazard ratio [HR]:1.99; $p < 0.001$) and reduced patient survival (HR: 1.53; $p = 0.04$). Unbiased Ingenuity analysis comparing cancer and normal prostate suggests migratory propensities in PCa. Indeed, overexpression of LMWPTP increases PCa cell migration, anoikis resistance, and reduces activation of focal adhesion kinase/paxillin, corresponding to decreased adherence.
Conclusions: Overexpression of LMWPTP in PCa confers a malignant phenotype with worse clinical outcome. Prospective follow-up should determine the clinical potential of LMWPTP overexpression.
Patient summary: These findings implicate low-molecular-weight protein tyrosine phosphatase as a novel oncogene in prostate cancer and could offer the possibility of using this protein as biomarker or target for treatment of this disease.

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1. Introduction

Kinases and phosphatases that control intracellular phosphorylation are critical regulators of cell proliferation, adhesion, migration, and death. Deregulation of oncogenic kinases has been shown to contribute to cancer development. In general, it is assumed that phosphatases, by counteracting kinase activities, act as tumor suppressors. However, a dual role for the low-molecular-weight protein tyrosine phosphatase (LMWPTP) in tumor cell biology is emerging. Enhanced expression of LMWPTP has been correlated to poor prognosis for some human tumors [1]. This may be related to the fact that this 18-kDa protein tyrosine phosphatase interacts with cancer-related molecules such as platelet-derived growth factor receptor, β-catenin, ephrin type-A receptor 2 (EphA2), Janus kinase 2 (JAK2), and signal transducer and activator of transcription 5 (STAT5), resulting in a positive effect on cell growth and proliferation signaling [2–7].

Prostate cancer (PCa) is one of the most frequently diagnosed cancers in men [8]. There are several treatment strategies available for nonmetastasized primary prostate tumors, including radical prostatectomy (RP), radiation therapy (either external beam or brachytherapy), and active surveillance. Although several treatment guidelines are available, prognostic biomarkers to aid clinical decision making are warranted. Furthermore, since PCa is highly metastatic to the bone and lymph nodes, new treatments should be directed at avoiding this progression toward a metastatic clinical state. The aim of this study was to examine expression levels of LMWPTP in primary PCa, correlate these to clinical phenotype, and study the role of LMWPTP in prostate tumor cell proliferation, migration, and stromal cell interaction.

2. Materials and methods

2.1. Gene expression profiling and pathway analysis

Use of clinical samples was approved by the Erasmus Medical Center medical ethics committee, according to the Medical Research Involving Human Subjects Act in protocol MEC-2004-261. Samples and Affymetrix analysis (Affymetrix Inc, Santa Clara, CA, USA) are described by Hendriksen et al [9]. Differentially regulated genes, their log2 fold change, and associated p values for lymph node metastasis of PCa (LN-PCa), transurethral resection of the prostate (TURP), and PCa, as compared with normal adjacent prostate (NAP), were filtered for a p value ≤ 0.05. Then the corresponding expression and p values were imported into Ingenuity Pathway Analysis (IPA) software (Qiagen, Venlo, Limburg, The Netherlands) for an unbiased analysis of the functional role of acid phosphatase 1, soluble (*ACP1*) in canonical pathways (Supplement).

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections from 7 patients without PCa and 15 patients with PCa and a Gleason score of at least 4 + 3 = 7, who had undergone radical resection, were immunohistochemically stained for LMWPTP (ACP1 antibody sc-100343; Santa Cruz Biotechnologies, Dallas, TX, USA) (Supplement) [10]. In addition, a tissue microarray (TMA) of material from 481 PCa patients [11] was stained. All patients had undergone RP for their disease without previous adjuvant therapy and were evaluated for Gleason score, pathologic (pT) stage, and surgical margins. Biochemical recurrence was defined as an increase in serum prostate-specific antigen (PSA) level after two different measurements at least 3 mo apart. In a subpopulation of these patients, local recurrence, as suspected by clinical presentation, was confirmed by needle biopsy. Death and death due to disease were registered by the physician who last treated the patient. To test the hypothesis that increased LMWPTP status is related to a more invasive tumor (Gleason score and pT stage), we used rank-sum and chi-square tests, and to determine if increased LMWPTP expression is predictive of worse clinical outcome, we used Cox regression analysis with the following covariates: age and PSA level at diagnosis, Gleason score, pT stage, surgical margins, and LMWPTP status. Patient selection and statistics were performed as described in the Supplement.

2.3. Cell culture and transfections

Cells (PC3, DU145, PNT2C2, MG63) were cultured as described in the Supplement. Overexpression plasmid pCS2+MT-LMWPTP was a kind gift from Prof. J. den Hertog, Hubrecht Institute, Utrecht, The Netherlands. Transfection of the LMWPTP construct was performed using Lipofectamine 2000 from Invitrogen (Bleiswijk, The Netherlands) according to the manufacturer's directions (Supplement). Analyses were performed 48 h after transfection.

2.4. Assays

2.4.1. Low-molecular-weight protein tyrosine phosphatase immunoprecipitation and phosphatase assay

LMWPTP activity assays were performed as described by Ruela-de-Sousa et al [12] and in the Supplement.

2.4.2. Migration assays

Transwell migration assay was performed as described by Bijlsma et al [13] and in the Supplement. In scratch-wound assays, cell monolayers were scratched with a pipette tip, washed twice, and photographs were taken (Axiovert200 M microscope; Carl Zeiss BV, Sliedrecht, The Netherlands) to analyze the percentage of open wound area at 24 h (ImageJ software; US National Institutes of Health, Bethesda, MD, USA).

2.4.3. Adhesion assay

Cells in serum-free medium were allowed to adhere to rat-tail type I collagen-coated (Sigma-Aldrich, St. Louis, MO, USA), bovine serum albumin-blocked plates for 10, 30, and 60 min. The attached cells were stained with crystal violet, and absorbance after extraction with 10% acetic acid was measured.

2.5. Fluorescence immunohistochemistry

PC3 cells were grown on coverslips, transfected, and fixed with 3.7% formaldehyde. Fixed cells were permeabilized and blocked in phosphate-buffered saline/0.1% Triton X-100/10% fetal bovine serum, stained with 200 ng/ml 4′,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich), and actin filaments were visualized with 10 μg/mL phalloidin-tetramethyl rhodamine isothiocyanate (Sigma-Aldrich). On mounting in Mowiol DABCO aqueous mounting medium (Vector Laboratories, Burlingame, CA, USA), images were acquired using an epifluorescence microscope (Axiovert200 M; Carl Zeiss BV, Sliedrecht, The Netherlands).

2.6. Western blotting

Protein extraction and blotting were performed as described in Fuhler et al [14] and in the Supplement.

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