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Prostate Cancer

Predictive Value of the Differential Expression of the Urokinase Plasminogen Activation Axis in Radical Prostatectomy Patients

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Abstract

Background: The urokinase plasminogen axis is composed of urokinase plasminogen activator (uPA), its receptor (uPAR), and its inhibitors (PAI-1 and PAI-2). This axis is involved in cell proliferation, angiogenesis, extracellular matrix degradation, invasion, and metastases.
Objective: To assess the relationship of the uPA axis with pathologic features and outcomes in prostate cancer.
Design, setting, and participants: Retrospective study of 230 consecutive patients treated with radical prostatectomy for clinically localized disease.
Interventions: None.
Measurements: Immunohistochemical staining for uPA, uPAR, and PAI-1 were carried out on serial archival tissue microarray specimens. These markers were histologically categorized as normal or overexpressed. Disease recurrence was classified as aggressive if metastases were present, if postrecurrence prostate-specific antigen (PSA) doubling time was <10 mo, or if the patients failed to respond to salvage local radiation therapy.
Results and limitations: The median follow-up was 63 mo. The combined expression of uPA and PAI-1 was associated with extraprostatic extension ($p = 0.01$) and seminal vesicle invasion ($p = 0.008$). On multivariable analysis, the combined uPA/PAI-1 expression was associated with overall (risk ratio [RR]: 2.3; 95% confidence interval [CI]: 1.1–4.8; $p = 0.02$) and aggressive disease recurrence (RR: 9.4; 95% CI: 3.5–25; $p < 0.0001$) but not with nonaggressive disease recurrence. Expression of uPAR was not associated with any of the outcomes. The study is limited by its retrospective nature and lack of long-term follow-up.
Conclusions: Overexpression of both uPA and PAI-1 is associated with adverse pathologic features and higher risk of overall and aggressive disease recurrence in men treated with radical prostatectomy for clinically localized prostate cancer. After validation, these markers may be useful in selecting patients most likely to benefit from adjuvant therapy. These markers should also be considered for addition into postoperative prediction tools.

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1. Introduction

Prostate cancer is the most common malignancy and is the third most common cause of cancer-related deaths in American men [1]. Prostate-specific antigen (PSA) screening has led to the detection of a number of early stage cancers for which curative treatments such as radical prostatectomy can result in durable disease control; however, up to 30% of men develop cancer recurrence after radical prostatectomy [2,3]. The prognosis after cancer recurrence is variable. Some men rapidly develop metastatic disease and die from the cancer, whereas others have a more indolent form of the disease that requires no treatment. Ability to accurately predict the risk of the individual patient developing any, and especially aggressive, disease recurrence after radical prostatectomy is limited. Better prognostic markers are needed to identify men at increased risk for aggressive disease recurrence who may benefit from multimodal therapy such as radiotherapy, androgen deprivation, chemotherapy, or experimental protocols.

Components of the urokinase-type plasminogen activator (uPA) axis are candidate biomarkers that may help identify aggressive prostate cancer phenotype. This axis plays a central role in cell proliferation, angiogenesis, extracellular matrix degradation, invasion, and metastasis [4]. The uPA axis is composed of uPA (a serine protease), its receptor (uPAR), and its two inhibitors: plasminogen activator inhibitor types 1 and 2 (PAI-1 and PAI-2). Overexpression of uPA and PAI-1 has been shown to be associated with adverse prognosis in various malignancies such as breast, gastric, colorectal, renal, and bladder cancers [5–10]. The combined expression uPA and PAI-1 has been used as entry criteria for enrollment of breast cancer patients into trials of adjuvant chemotherapy [11]. In prostate cancer, altered expression of uPA and PAI-1 is associated with adverse pathologic features such as higher Gleason sum and lymph nodal and skeletal metastasis [12,13]; however, the prognostic role of the uPA axis has not been well studied in patients with clinically localized prostate cancer.

In this study, we evaluated the expression of uPA, uPAR, and PAI-1 in consecutive radical prostatectomy specimens and assessed their association with outcomes. We also focused on predicting aggressive prostate cancer recurrence.

2. Methods

2.1. Patient population

All studies were undertaken with the approval and oversight of the Institutional Review Board. The study analyzed 230 consecutive patients treated with radical prostatectomy and bilateral lymphadenectomy for clinically localized prostatic adenocarcinoma (clinical stage T1 to T2) at one of the affiliated hospitals of the University of Texas Southwestern Medical Center during the period from July 12, 1994, through November 13, 1997, and who had archival tissue available. No patient was treated preoperatively with either hormonal or radiation therapy, and none had secondary cancers. The clinical stage was assigned by the operative surgeon according to the 1992 TNM system. Data on patient age were missing for two patients. The median patient age ($n = 228$) in this study was 62.5 yr (interquartile range: 57.6–66.8; range: 39.8–75.5). Serum PSA was measured with the Hybritech Tandem-R assay. Staff pathologists examined all prostatectomy specimens in accordance with the guidelines of the College of American Pathologists [14].

2.2. Immunohistochemistry and scoring

We performed uPA, PAI-1, and uPAR immunohistochemical staining using serial sections from the same paraffin-embedded microarray tissue blocks. Murine IgG1 monoclonal antibodies against uPA (dilution 1:100), PAI-1 (dilution 1:50), and uPAR (dilution 1:100) were used (American Diagnostica, Greenwich, CT). Immunostaining was performed on the Dako Autostainer (Carpinteria, CA). Reagents were used as supplied in the Envision Plus Detection Kit (Carpinteria, CA). Dako Target Retrieval Solution (pH 6.0) was used. Optimum primary antibody dilutions were predetermined using known positive control tissues. Sections were counterstained with hematoxylin and blued in Richard Allen Bluing Reagent. A known positive control section was included in each run to assure proper staining. Tumor sections with the primary antibodies substituted with rabbit immunoglobulin fraction (normal) or IgG1 monoclonal were used as negative controls. Fig. 1 shows representative slides of immunohistochemical staining for all three markers.

We used bright-field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System, ChromaVision Medical Systems Inc., San Juan Capistrano, CA) to detect, classify, and count stained cellular objects based on predetermined color morphology. We obtained the mean, maximum, range, and standard deviation of staining intensity and percent positive nuclei/area measurements by using 10 random hot spots within each specimen. The mean of the triplicate cores was calculated for data analysis. All markers were placed in one of two categories: overexpression or normal. In a preliminary study, we assessed the discriminative value of uPA, PAI-1, and uPAR as categorical variables with serial increments of cut-offs ranging from 5% to 90% positive cells with regard to PSA recurrence (data not shown). Kaplan-Meier analyses revealed that the uPA, PAI-1, and uPAR cut-offs of 30%, 30%, and 50% immunoreactivity, respectively, were the best discriminators for PSA recurrence.

2.3. Postoperative follow-up

Patients underwent a digital rectal examination and PSA measurement postoperatively every 3 mo for the first year,