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Original contribution

Overexpression of the chromatin remodeler death-domain–associated protein in prostate cancer is an independent predictor of early prostate-specific antigen recurrence☆

Maria Christina Tsourlakis MD[a,1], Michael Schoop PhD[a,1], Christoph Plass PhD[b], Hartwig Huland MD[c], Markus Graefen MD[c], Thomas Steuber MD[c], Thorsten Schlomm MD[c], Ronald Simon PhD[a], Guido Sauter MD[a], Hüseyin Sirma MD[a], Sarah Minner MD[a,*]

[a]*Institute of Pathology, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany*
[b]*Division of Epigenomics and Cancer Risk Factors, DKFZ, 69120 Heidelberg, Germany*
[c]*Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany*

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Summary Molecular markers reliably predicting the aggressiveness of prostate cancer are currently lacking. Death-domain–associated protein (DAXX) has been implicated in the regulation of chromatin remodeling, transcription, and apoptosis that are integral to oncogenesis and cancer progression. DAXX expression was analyzed by immunohistochemistry on a tissue microarray containing 7478 prostate cancer specimens. Results were compared with tumor phenotype, biochemical recurrence, and v-ets erythroblastosis virus E26 oncogene homolog (ERG) status. DAXX expression was predominantly seen in the nucleus. DAXX expression was detectable in 4609 (80.6%) of 5718 interpretable cancers and considered strong in 5.9%, moderate in 45.8%, and weak in 28.9%. Strong DAXX expression was associated with both *transmembrane protease, serine 2* (*TMPRSS2*)*/ERG* rearrangement and ERG expression ($P < .0001$ each). Strong DAXX expression was tightly linked to high Gleason grade, advanced pT stage, increased cell proliferation index, and early prostate-specific antigen recurrence ($P < .0001$ each). The prognostic role of DAXX expression was independent of Gleason grade, pT stage, and pN stage. Our study establishes DAXX as a novel independent prognosticator in prostate cancer and suggests an important role of DAXX expression for both prostate cancer development and progression. Furthermore, DAXX appears to exert biologically different effects in ERG-positive and ERG-negative prostate cancers.

1. Introduction

Prostate cancer is a leading cause of cancer-related mortality in males. About 50% of males will develop prostate cancer during their lifetime, but it is estimated that less than

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* Corresponding author.
E-mail address: s.minner@uke.uni-hamburg.de (S. Minner).
[1] These authors have contributed equally to the study.

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half of them will have potential life-threatening disease that requires treatment [1,2]. Despite recent advances, the only established pretreatment prognostic parameters currently include Gleason grade and tumor extent on biopsies, preoperative prostate-specific antigen (PSA), and clinical stage. Because these data are statistically powerful but not sufficient for optimal individual treatment decisions, it can be hoped that a better understanding of the biology of the disease will eventually lead to the identification of clinically applicable molecular markers that enable reliable prediction of prostate cancer aggressiveness.

Growing evidence indicates that chromatin conformation plays a key role in maintenance of genomic integrity and prevention of prostate cancer development [3]. Whole-genome DNA sequencing by us and others have repeatedly implicated alterations of various genes involved in chromatin remodeling, such as *chromodomain helicase DNA binding protein* (*CHD*) *1 and 6*, *death-domain–associated protein* (*DAXX*), *alpha thalassemia/mental retardation syndrome X-linked protein* (*ATRX*), and *AT-rich interactive domain 1A protein* (*ARID1A*) [4–6] in cancer development and progression. It was suggested that alterations of such genes may contribute to the high rate of chromosomal rearrangements in these prostate cancers, which typically occur at regions where the conformation of androgen regulated genes is influenced by these chromatin-modifying genes. DAXX is a chromatin-modifying protein, for which a high prevalence of somatic mutations was found in multiple cancer types including pancreatic neuroendocrine tumors and glioblastomas [6–8]. Because DAXX inhibits the androgen receptor (AR)–dependent transcription, we hypothesized that DAXX may also be involved in prostate carcinogenesis [9].

In the present study, we used a set of tissue microarrays (TMAs) harboring samples from 7478 patients with prostate cancer to learn more about the impact of DAXX expression in prostate cancer. The data suggest a strong prognostic relevance of DAXX protein levels, particularly in the subgroup of v-ets erythroblastosis virus E26 oncogene homolog (ERG)–negative prostate cancers.

2. Materials and methods

2.1. Patients

Radical prostatectomy specimens were available from 7478 patients undergoing surgery at the Department of Urology and the Martini Clinic at the University Medical Center Hamburg-Eppendorf and were distributed among 16 TMA blocks, each containing 144 to 522 tumor samples. One 0.6-mm core was taken from a representative tissue block from each patient. For internal controls, each TMA block also contained various control tissues including normal prostate tissue. Follow-up data were available for a total of 6843 patients, with a median follow-up of 36.6 (range, 1-219) months (Table 1). PSA values were measured after surgery, and PSA recurrence was defined as a postoperative PSA of 0.2 ng/mL and increasing at first of appearance. The molecular database attached to this TMA contained results on ERG expression in 4266, *ERG* breakapart fluorescence in situ hybridization (FISH) analysis in 1191 (expanded from Minner et al [10]), and Ki-67 expression in 2629 cancers [11].

2.2. Immunohistochemistry

Freshly cut TMA sections were immunostained on 1 day and in 1 experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C, pH 7.8. DAXX immunostaining was performed using a well-established polyclonal antibody (HPA008736; Sigma-Aldrich, St Louis, MO; dilution 1:100). The Envision system (DAKO, Glostrup, Denmark) was used to visualize the immunostaining. Stromal cells and endothelial cells were regarded as internal positive controls. Because the intensity of nuclear immunostaining was largely homogeneous throughout the tissue samples, nuclear staining was arbitrarily estimated in a 4-step intensity scale (intensity: 0, negative; 1+, weak staining; 2+, moderate staining; 3+, strong staining). Scoring was validated by intraobserver and interobserver variation assessment. A total of 1500 consecutive samples were also reviewed for DAXX staining in high-grade prostatic intraepithelial neoplasia (HGPIN) adjacent to cancer. This analysis identified 20 samples with unequivocal HGPIN and cancer on the same tissue spot.

2.3. Statistics

For statistical analysis, the JMP 9.0 software (SAS Institute Inc, Cary, NC) was used. Contingency tables were calculated to study the association between DAXX immunoreactivity and clinicopathologic variables. The χ^2 (likelihood) test was used to find significant relationships. Kaplan-Meier curves were generated for PSA recurrence-free survival. The log-rank test was applied to test the significance of differences between stratified survival functions. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathologic, molecular, and clinical variables.

3. Results

3.1. Technical issues

A total of 5718 (76.5%) tumor samples were interpretable in our TMA analysis. Reasons for noninformative cases (1760 spots; 23.5%) included lack of tissue samples or