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Original article

Muscle-invasive bladder cancer is characterized by overexpression of thymidine kinase 1

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Abstract

Objective: Thymidine kinases have an important role in the synthesis of DNA and exhibit high activity in rapidly proliferating cells. Thymidine kinase 1 (TK1) activity has been shown to be increased in various cancer types and proposed as a prognostic parameter. Aim of the present study was to investigate TK1 in muscle-invasive urothelial carcinoma (UC).

Methods: Corresponding UC and benign samples from paraffin embedded tissue of 111 patients treated with cystectomy for invasive UC from 1996 to 2006 were immunohistochemically (IHC) assessed for TK1. IHC expression patterns were evaluated in a semiquantitative fashion by 2 independent reviewers. Localization of staining was categorized into pure nuclear and additional cytoplasmic localization. Uni- and multivariate analyses were performed to assess differential expression in normal and UC tissue and to evaluate the diagnostic and predictive capability of TK1 by correlation to clinical data. To correlate TK1 expression with molecular subtypes of UC, analysis of TK1 RNA expression levels of the Cancer Genome Atlas UC cohort was performed.

Results: TK1 was significantly overexpressed in invasive UC, compared to benign urothelium ($P < 0.0001$), and cytoplasmic expression was more often found in cancer tissue than in benign tissue ($P = 0.0001$). No correlations of TK1 protein expression patterns to standard histopathological determinants were detected. In univariate analysis, TK1 nuclear and cytoplasmic expression was associated with improved cancer-specific survival ($P = 0.0119$). However, only metastasis status and histologic grade were identified as independent predictors of cancer-specific survival in multivariate analysis. TK1 expression was merely found in the basal layers of benign urothelium. RNA overexpression of TK1 could be correlated to the biologically more aggressive basal UC subtype.

Conclusions: TK1 expression is significantly different in invasive UC and benign urothelium, which underlines its potential as a diagnostic marker. Although TK1 is considered to be a marker of proliferation, and TK1 RNA overexpression is associated with an aggressive UC subtype, its capability as a predictive IHC biomarker for invasive UC remains limited.

Keywords: Bladder cancer; Biomarker; Thymidine kinase 1; Proliferation; Molecular subtypes

1. Introduction

Bladder cancer is a heterogeneous disease that comprises papillary low grade noninvasive neoplasms as well as highly aggressive invasive cancer [1]. The latter is associated with a high risk of cancer recurrence and cancer associated mortality despite surgical treatment with radial cystectomy [2]. Ongoing research is focused on the identification of molecular biomarkers that help to distinguish tumors with favorable and dismal characteristics to allow more precise risk stratification and pave the way to individual adjuvant treatment and follow-up regimen. The putative clinical utility of incorporating immunohistochemical (IHC) parameters into postoperative urothelial cancer (UC) risk estimation was outlined in a study by Lotan et al., who performed IHC

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staining for cell cycle–related biomarkers (p53, p21, p27, cyclin E1, and Ki-67) and evaluated them as putative predictors of recurrence and cancer death after radical cystectomy. Lymphovascular invasion (LVI) and the number of altered biomarkers were identified as independent predictors of recurrence and cancer-specific survival (CSS) [3]. A recently introduced alternative IHC proliferation marker that is discussed to be of diagnostic and predictive relevance in cancer is thymidine kinase 1 (TK1). TK1 has been shown to be highly expressed by activated G1-phase cells in vitro [4].

TK1 is well known for its function in the pyrimidine salvage pathway, where it is involved in DNA synthesis and repair [5]. TK1 expression is reported not to be restricted to the nucleus and is also expressed in the cytoplasm. Moreover, TK1 may be measured in serum samples, which underlines its putative role as a noninvasive biomarker [6].

Although earlier studies have documented the relevance of TK1 protein expression patterns as pathological and clinical predictive markers in cancer entities like renal cell carcinoma [7,8], cancer of the uterine cervix [9], lung [10], and prostate cancer [11], the relevance of TK1 expression in muscle-invasive bladder cancer is unclear.

To further identify the diagnostic and prognostic effect of TK1 in muscle-invasive UC, we evaluated expression patterns and clinical parameters of patients undergoing cystectomy for muscle-invasive UC of the bladder.

2. Patients and methods

Based on our institutional database, patients undergoing cystectomy for UC from January 1996 to December 2006 were identified. Patients with high-risk non–muscle-invasive UC or non–urothelial histology were excluded from analysis. Clinical and histopathological staging was performed according to the American Joint Committee on Cancer TNM classification (2002 Version) [12]. Patient characteristics and clinical follow-up were recorded. The individual follow-up regimen was performed at the discretion of the referring physician, but in general consisted in postoperative visits every 3 to 4 months in the first year, semiannually in for the second and third year, and annually thereafter.

From cystectomy specimen, a tissue microarray (TMA) was created based on the identification of representative sections of tumor and normal tissue in hematoxylin and eosin staining. Benign cores were taken from blocks without cancer infiltration Cores of two or more tumor areas and corresponding benign tissue were integrated. The final TMA consisted of 363 tissue cores.

2.1. Immunohistochemistry

For TK1-IHC staining, a mouse-monoclonal antibody (TK1 XPA-210, SSTK, clone 5, AroCell, Uppsala, Sweden) in a dilution of 1:200 was applied (diluent: Dako S3022, Dako, Glostrup, Denmark). Incubation with the primary antibody was performed for 60 minutes at room temperature. Visualization was performed using a commercially available system (EnVision, G/2 System AP, Rabbit/Mouse Permanent Red, Dako, Glostrup, Denmark), whereas hematoxyline was applied for counterstaining.

TMA evaluation was performed in a blinded fashion by 2 independent reviewers, divergent results were rechecked. Evaluation of XPA-210/TK1 staining was undertaken in a semiquantitative manner. Microscopic analysis was performed at $\times 100$ magnification. The number of stained cells was related to the number of total cells per core. For categorical analysis, TK1 positive cell count was defined as low or high according to comparison with the median positive cell count. In addition, localization of staining was categorized as pure nuclear (0), nuclear and low cytoplasmic staining (1), and nuclear and intensive/high cytoplasmic staining (2).

To rule out cancer in the benign reference cores, IHC for cytokeratin 20 and p53 was performed.

2.1.1. Analysis of TK1 RNA expression

To evaluate representative RNA expression patterns of TK1, we analyzed the public muscle-invasive UC RNA-seq V2 data set from the Cancer Genome Atlas (TCGA) [13] and compared RNA expression of TK1 between the 4 distinctive molecular subtypes [13]. Expression data of discriminative marker genes for the respective subtypes (basal: FGFR3, FOXA1, GATA3, KRT20 and PPARG; luminal: KRT14, KRT6A, KRT6B and KRT6C) were compared with TK1 RNA expression levels, with low TK1—defined as the quartile of patients with lowest expression, and high TK1—defined as the quartile with the highest TK1 expression.

2.1.2. Statistical analysis

Statistical analysis was performed using MedCalc (Version 12.5, Ostend, Belgium).

Univariate analyses were performed using Chi-square test and univariate Cox regression analyses. For the comparison of expression patterns in normal tissue and UC, a Wilcoxon Kruskal-Wallis test was applied. Kaplan-Meier analyses were performed to estimate recurrence-free survival, CSS, and overall survival (OS), differences between subgroups were evaluated using Log-rank tests. Multivariate testing was performed using Cox regression analyses. P values < 0.05 were considered as stastically significant.

The study was approved by the institutional review board of the University of Tubingen (279/2013BO2).

3. Results

A total of 111 patients and the respective surgical specimen were eligible for the study. Of these, 85 (76.7%) were men, and 26 (23.4%) were women. Median age at diagnosis was 68