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Bladder Cancer

Expression of the Endothelin Axis in Noninvasive and Superficially Invasive Bladder Cancer: Relation to Clinicopathologic and Molecular Prognostic Parameters

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Abstract

Background: The endothelin (ET) axis plays a role in cancer biology and plays a potential role as a target for molecular therapy in urogenital tumours. Alterations of several proteins of the ET axis were detected in invasive bladder cancer.
Objectives: To examine the potential role of the expression of ET axis proteins compared to other prognostic parameters (kinase inhibitor 67 [Ki-67], tumour protein 53 [TP53], and fibroblast growth factor receptor 3 gene [*FGFR3*] mutations) in noninvasive and invasive bladder cancer.
Design, setting, and participants: Tissue microarrays from 154 consecutive patients with pTa–pT2 urothelial bladder cancer were immunohistochemically stained for endothelin 1 (ET-1), endothelin A and B receptors (ET_AR, ET_BR), TP53, and Ki-67. *FGFR3* mutations were detected by SNaPshot analysis.
Measurements: The results were correlated with clinicopathologic parameters and disease-specific survival, overall survival, and recurrence-free survival.
Results and limitations: Proteins of the ET axis were frequently expressed in bladder cancer (ET-1 in 62% of tumours, ET_AR in 93% of tumours, and ET_BR in 84% of tumours). ET-1 expression was strongly correlated with tumour stage ($p = 0.015$), histologic grade ($p = 0.008$), and low proliferation status ($p = 0.003$). ET_AR immunostaining was only associated with low proliferation status ($p = 0.015$). Kaplan-Meier survival analysis showed a significantly longer overall survival for patients with ET-1-expressing tumours ($p = 0.007$). A significantly longer disease-free survival was found in patients with ET_AR-expressing tumours ($p = 0.040$), whereas ET_BR expression was significantly correlated to a longer disease-free survival only in subgroups of patients with multifocal tumours ($p = 0.031$), low proliferation index (Ki-67 $\leq$10; $p = 0.050$), low TP53 expression ($\leq$10; $p = 0.018$), and tumours with an *FGFR3* mutation ($p = 0.026$). In the global model for recurrence-free survival, only high-grade ($p = 0.048$) and negative ET_AR immunoreactivity ($p = 0.048$) were correlated with poor prognosis.
Conclusions: In addition to other factors, particularly age at diagnosis and growth pattern, lack of ET-1 expression may be an independent negative prognostic factor for the overall-survival probability of bladder cancer patients. Lack of ET_AR expression may be an independent negative marker for recurrence-free survival.

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1. Introduction

Bladder cancer is the seventh most frequent malignant tumour [1] and the fourth most frequent malignant tumour in males in Europe [2]. Of all patients diagnosed, up to 25% have muscle-invasive tumours (pT2–pT4) at first presentation, but most patients (75–80%) present with noninvasive and superficially invasive tumours (pTa–pT1) [3]. The therapy of choice is resection of superficial tumours by transurethral resection, whereas radical cystectomy with urinary diversion remains the therapeutic gold standard for muscle-invasive tumours [4]. However, 70% of patients presenting with noninvasive (pTa) or superficially invasive (pT1) urothelial tumours will have recurrent tumours, and up to 25% may develop muscle-invasive disease [3]. Following initial transurethral resection of the bladder tumour, patients at risk for recurrence or progression can be identified by a variety of prognostic factors, including tumour grade and stage, the presence of vascular or lymphatic invasion, early recurrence, the number and size of tumours, and the finding of concomitant dysplasia or carcinoma in situ [5–7]. These clinicopathologic features were summarised in a prognostic algorithm. However, the classical prognostic factors cannot predict recurrence and progressive disease for the individual patient. Therefore, great efforts have been made to find molecular markers of biologic aggressiveness, and the following were proposed: B-cell leukaemia/lymphoma 2 (BCL-2), tumour protein 53 (TP53), BCL-2–associated X protein (BAX), paired box gene 5 (*PAX5*), cyclin D3, cytokeratin 20, kinase inhibitor 67 (Ki-67) expression, and fibroblast growth factor receptor 3 gene (*FGFR3*) mutations, as well as gene expression analyses [8–16]. Among those candidate markers are also endothelin-1 (ET-1) and its receptors, endothelin-A (ET_AR) and endothelin-B (ET_BR), commonly referred to jointly as the *endothelin axis* (ET axis). ET-1 belongs to a group of multifunctional peptides (ET-1, ET-2, and ET-3), was originally isolated from porcine endothelial cells in 1998, and has strong vasoconstrictive effects [17]. In human tumours, it was demonstrated to stimulate tumour cell proliferation [18] and to have antiapoptotic [19] and neoangiogenic [20] effects. Endothelins exert their physiologic effect via two high-affinity, G-protein–coupled receptors, ET_AR and ET_BR. The expressions of the different proteins of the ET axis play different roles in various human tumours. ET-1 is a potentially important prognostic factor in advanced prostate cancer progression [21] and could be detected in prostatic intraepithelial neoplasia [22]. Higher-than-normal levels of ET-1 messenger ribonucleic acid (mRNA) were detected in ovarian cancers [23], and overexpression of ET_BR in tumour endothelial cells from human ovarian cancers was associated with the absence of tumour-infiltrating lymphocytes and short patient survival time [24]. The expression of proteins of the ET axis was associated with more aggressive types of breast cancer, which demonstrated shorter disease-free survival, shorter overall survival, higher vascularity, and higher vascular endothelial growth factor (VEGF) expression [25]. In invasive bladder cancer, a high expression of the ET axis both on mRNA and protein level was found [26]. Both ET receptors were expressed in advanced stages of bladder cancer (pT2–pT4), and ET_BR expression was associated with longer disease-free survival [27].

The purpose of the present study was to determine the predictive value of the expression of the proteins of the ET axis in a clinicopathologically and molecularly well-defined, consecutive cohort of patients with primary noninvasive or superficially invasive bladder cancer compared with muscle-invasive (pTa–pT2) disease. In addition, we correlated ET axis expression to other established clinicopathologic and molecular prognostic factors of noninvasive or superficially invasive bladder cancer (ie, TP53 expression, proliferation index [Ki-67], and *FGFR3* mutation status) [15].

2. Methods

2.1. Patients and tumour specimens

A tissue microarray containing 154 consecutive, formalin-fixed, paraffin-embedded, primary urothelial bladder cancer tissues of transurethral resection material obtained from the Institute of Pathology, University of Regensburg, Regensburg, Germany was constructed, as described previously [15]. All markers were successfully investigated in all of the above tissues, except where otherwise indicated. Immunohistochemical analyses of ET1, ET_AR, and ET_BR were carried out in the same specimens. Clinical data were obtained from the Central Tumour Registry, Regensburg, Germany, and by telephone interviews [15]. An experienced pathologist (AH) evaluated haematoxylin and eosin-stained slides of all tumours. Tumour stage and grade were assigned according to Union Internationale Contre le Cancer (UICC) [28] and World Health Organisation [6] criteria. The patients' ages at diagnosis ranged from 35 yr to 95 yr, with a median of 68 yr. All clinicopathologic parameters are summarised in Table 1.

Clinical follow-up data were available for all patients with a median follow-up period of 67.5 mo (range: 1–151 mo). Time to recurrence and time to death were selected as end points in patients with urothelial carcinomas. Recurrences were defined as cystoscopically visible tumours with histologic verification. The University of Regensburg Institutional Review Board granted approval for the study.

2.2. Immunhistochemistry for kinase inhibitor 67, tumour protein 53, endothelin 1, and endothelin receptors A and B

From the tissue microarray (TMA) block, 4-μm sections were cut and mounted on poly-L-lysine-coated glass slides. Staining for Ki-67 and TP53 was carried out in a NexES immunostainer (Ventana, Tucson, AZ, USA) using the avidin-biotin peroxidase method with diaminobenzidine as chromatogen, as previously described [15]. The following primary antibodies were used: anti-TP53 (mouse monoclonal IgG, clone Bp53-12 [sc-263]; Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA; 1:1000) and anti-Ki-67 (mouse monoclonal IgG1, clone MIB-1 [M7240]; Dako Diagnostics, Glostrup, Denmark; 1:50). One surgical pathologist (AH) performed a blind evaluation of the slides. Successful evaluations were carried out on 149 (Ki-67) and 152 (TP53) analysable spots. TP53-positive tissues were those with strong nuclear staining in >10% of the tumour cells. The percentage of Ki-67 positive cells in each specimen was determined, as described previously. An increased Ki-67 labelling index was indicated when >10% of the tumour cells were positive [12,29].

Staining for ET_AR, ET_BR, and ET-1 was also performed in a multistep, semiautomatic procedure (Dako Autostainer; Dako Diagnostics,