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Bladder Cancer

FGFR3 Mutations and a Normal CK20 Staining Pattern Define Low-Grade Noninvasive Urothelial Bladder Tumours

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Abstract

Objectives: Molecular markers superior to conventional clinicopathologic parameters are needed to predict disease courses in bladder cancer patients. In this study, we investigated four markers (Ki-67, TP53, CK20, *FGFR3*) in primary urothelial bladder tumours and compared them with traditional pathologic features.
Methods: Tissue microarrays were used to analyse CK20, TP53, and Ki-67 expression immunohistochemically in 255 unselected patients. *FGFR3* mutations were detected by SNaPshot analysis.
Results: Abnormal CK20 expression was strongly associated with higher tumour grades and stages ($p < 0.001$); however, 65% of pTa tumours revealed an abnormal CK20 pattern. In the group of pTaG1 tumours, 59% presented with an abnormal CK20 pattern, whereas 82% carried the *FGFR3* mutation. In the group of bladder tumours with normal CK20 pattern, the *FGFR3* gene was mutated in 89%, whereas a mutated *FGFR3* gene was found in only 37% of cases with abnormal CK20 expression ($p < 0.001$). All markers proved to be strong predictors of disease-specific survival in univariate studies. However, in multivariate analyses they were not independent from classical pathologic parameters. None of the molecular markers was significantly associated with tumour recurrence.
Conclusions: Dysregulation of CK20 expression is an early event in the carcinogenesis of papillary noninvasive bladder cancer, but occurs later than *FGFR3* mutations. The group of low-grade noninvasive papillary tumours is defined by the presence of an *FGFR3* mutation and a normal CK20 expression pattern.

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1. Introduction

Most bladder tumour patients (75–80%) present with papillary noninvasive (pTa) or superficially invasive (pT1) urothelial tumours at first presentation, whereas the remaining 20–25% of primary tumours are already muscle-invasive (≥pT2). pTa and pT1 tumours can be removed by transurethral resection (TUR); however, 70% of patients will have one or more recurrent tumours, and up to 25% will eventually develop muscle-invasive disease. Bladder cancer patients therefore have to be monitored thoroughly for disease recurrence and progression. Recent attempts in predicting prognosis include image analysis [1] and uPAR expression analysis [2]; still there are no established markers, molecular or classical, that are able to predict which tumours will progress and which will not.

Mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene are very frequent in pTa bladder tumours (about 75%) [3–5], less frequent in pT1G3 tumours [6,7], and rare in carcinoma *in situ* (pTis) [3,7]. Furthermore, patients with primary bladder cancers with an *FGFR3* mutation were shown to have a significantly better prognosis than patients without a mutation [5].

Increased proliferative activity using Ki-67 immunohistochemistry (IHC) and *TP53* mutations are both markers that indicate an unfavourable disease course [8,9]. However, bladder tumours with an increased immunoreactivity for Ki-67 and TP53 progress less often when an *FGFR3* mutation is present [5]. *FGFR3* and *TP53* are almost mutually exclusive, and represent different pathways in bladder cancer development [5,10,11]. The combination of *FGFR3* mutation analysis and Ki-67 IHC, defined as molecular grading, proved to be superior to other parameters in the prediction of progression and survival of bladder cancer patients [5].

Cytokeratin 20 (CK20) is another marker for tumours of low stage and grade, and is expressed in the umbrella cells of normal urothelium [12] and reactive atypia. When CK20 expression in bladder tumours is limited to the umbrella cells, it is associated with a mild disease course, while expression in the entire urothelium in more than 10% of the tumour cells is associated with higher tumour grade [13] and an increased risk of progression and recurrence [14,15]. In urothelial carcinoma *in situ*, intense CK20 expression is found in the majority of malignant cells [16,17].

To study the possible prognostic value of these four molecular markers and their relation to each other in the pathogenesis of bladder cancer, we analysed a large series of unselected primary urothelial bladder tumours for *FGFR3* mutations, and for expression of CK20, TP53, and Ki-67 in relation to tumour stage, grade, multifocality, adjacent carcinoma *in situ*, and prognosis in these patients.

2. Methods

2.1. Bladder cancer tissue microarray

A tissue microarray was constructed [18] from 255 consecutive, formalin-fixed, paraffin-embedded, primary urothelial bladder cancer tissues (Institute of Pathology, University of Regensburg, Regensburg, Germany). Clinical data were obtained from the Central Tumour Registry, Regensburg, Germany, and by telephone interviews (M.B., S.D.) in case of missing data. The Institutional Review Board of the University of Regensburg approved analysis of tissues from human subjects.

Haematoxylin-eosin–stained slides of all tumours were evaluated by a single surgical pathologist (A.H.). Tumour stage and grade were assigned according to Union Internationale Contre le Cancer (UICC) and World Health Organization (WHO) criteria. Growth pattern was determined for all invasive tumours (≥pT1). Papillary growth was defined by the presence of a papillary tumour component (≥20%) with a histologic grade identical to the invasive tumour. All other tumours were considered to have a solid growth pattern. Clinicopathologic data are summarised in Table 1. Retrospective clinical follow-up data were available regarding the end points, recurrence-free survival, and overall survival for all patients with a median follow-up period of 75 mo (range: 0–147 mo). The median follow-up for censored patients was 81 mo. Recurrences were defined as cystoscopically visible tumours with histologic verification. Data on progression-free survival were not available.

2.2. Immunohistochemistry

Immunohistochemical studies utilised an avidin-biotin peroxidase method with a diaminobenzidine (DAB) chromatogen. After antigen retrieval (microwave oven for 30 min at 250 W), immunohistochemistry was carried out in a NEXES immunostainer (Ventana, Tucson, AZ, USA) following manufacturer's instructions. The following primary antibodies were used: anti-TP53 (mouse monoclonal IgG, clone Bp53-12 (sc-263); Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA; dilution 1:1000), anti-CK20 (mouse monoclonal IgG2a, clone IT-Ks20.8 (61026); Progen Biotechnik GmbH, Heidelberg, Germany; dilution 1:10), and anti-Ki-67 (mouse monoclonal IgG1, clone MIB-1 (M7240); Dako, Glostrup, Denmark; dilution 1:50). One surgical pathologist (A.H.) performed a blinded evaluation of the slides. TP53 positivity was defined as strong nuclear staining in more than 10% of the tumour cells. The percentage of Ki-67 positive cells of each specimen was determined as described previously [19]. High Ki-67 labelling index was defined if more than 25% of the tumour cells were positive [5]. CK20 staining was