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Original contribution

βIII-tubulin overexpression is linked to aggressive tumor features and genetic instability in urinary bladder cancer☆,☆☆

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Summary Development of genetic instability is a hallmark of tumor progression. Type III β-tubulin (TUBB3) is a component of microtubules involved in chromosome segregation. Its overexpression has been linked to adverse features of urinary bladder cancer. To investigate the role of TUBB3 for development of genetic instability, we compared TUBB3 expression with histopathological features and surrogate markers of genetic instability and tumor aggressiveness; copy number changes of *HER2*, *TOP2A*, *CCND1*, *RAF1*, and *FGFR1*; nuclear accumulation of p53, and cell proliferation in a tissue microarray (TMA) with more than 700 bladder cancers. TUBB3 expression was linked to high-grade and advanced-stage cancers ($P < .0001$), rapid cell proliferation ($P < .0001$), presence of multiple gene copy number alterations ($P = .0008$), and nuclear accumulation of p53 ($P = .0008$). Strong TUBB3 staining was found in 43% of urothelial cancers harboring copy number alterations as compared with 28% of genetically stable cancers, and in 50% of p53-positive cancers as compared with 30% of p53-negative tumors. The fraction of tumors with concomitant TUBB3 and p53 positivity increased with tumor stage and grade: 2% in pTaG1-2, 11% in pTaG3, 17% in pT1G2, 23% in pT1G3, and 32% in pT2-4 cancers ($P < .0001$). Importantly, strong TUBB3 overexpression was detectable in about 20% of low-grade, noninvasive cancers. In summary, our study demonstrates that TUBB3 overexpression is linked to an aggressive subtype of urinary bladder cancers, which is characterized by increased genetic instability,

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p53 alterations, and rapid cell proliferation. Detection of TUBB3 overexpression in genetically stable, low-grade, and noninvasive bladder cancers may be clinically useful to identify patients requiring particular close monitoring.

1. Introduction

Urinary bladder cancer is the fifth most common malignant tumor type in Western societies [1]. About 80% of patients present with low-grade, noninvasive (pTa) cancers or minimally invasive (pT1) stage cancers, which are charaterized by a good prognosis and can be removed by transurethral resection. However, although almost all of these tumors recur, and about 20% will further progress to life-threatening, muscle-invasive disease requiring surgical removal of the bladder [2], regular follow-up examination at close intervals is obligatory. The fact that noninvasive and invase bladder cancer represent 2 distinct molecular subgroups that are characterized by different levels of genetic instability [3] has eventually led to the development of a diagnostic multicolor fluorescence in situ hybridization (FISH) assay for identifying patients at risk of progression [4]. However, the molecular mechanisms underlying development of genetic instability are still poorly understood, and molecular markers capable of predicting genetic instability even before it occurs may further help to improve clinical bladder cancer management [5].

Several lines of evidence suggest that development of genetic instability is accompanied by specific alterations in the microtubule architecture of cancer cells [6]. Microtubules are cytoskeletal proteins composed of polymers of α- and β-tubulin subunits that contribute to cell shaping, motility, vesicle transport, and chromosome segregation. Both α- and β-tubulins exist as multiple isotypes with variable expression patterns across different normal tissues (reviewed by Orr et al [7]). It has been shown that the particular isotype composition of individual microtubules determines their stability and dynamics, with class III β-tubulin (TUBB3) rendering microtubules most dynamic [8]. Abnormal TUBB3 content may also be relevant for development of chromosomal instability, as it is believed to contribute to centrosomal amplification, multipolar spindle poles, and missegregation of chromosomes [9]. In fact, overexpression of TUBB3 is associated with adverse clinical features and/or poor outcome in many epithelial tumor types such as non–small cell lung, gastric, breast, colon, kidney, laryngeal, and ovarian cancer [10,11]. This is also true for urinary bladder cancer, where TUBB3 overexpression has been linked to high-grade disease and shortened recurrence-free survival of patients undergoing radical cystectomy [12].

To better understand the role of the cellular TUBB3 content for development of genetic instability in bladder cancer, we studied the relationship between TUBB3 expression and typical markers of genetic instability, including alterations of the p53 tumor suppressor, oncogene copy number changes, and cell proliferation, in a TMA containing more than 750 bladder cancer samples. The results of this study demonstrate a gradual increase of genetic instability with rising levels of TUBB3.

2. Materials and methods

2.1. Tissues

A preexisting bladder cancer TMA containing 776 urinary bladder tumors, including 686 urothelial cancers, 11 adenocarcinomas, 29 squamous cell carcinomas, 19 small cell carcinomas, 14 sarcomatoid carcinomas, and 8 inverted papillomas, was used [13]. One 0.6-mm tissue core had been punched out from each case, and transferred into a TMA format. Tumor stage and grade were defined according to Union Internationale Contre le Cancer and earlier World Health Organization classification [14,15] (the tumors were not classified according to current World Health Organization classification because the tumors for the TMA construction were reviewed before 2004). Ten cancers with stromal invasion but absence of muscular bladder wall in the biopsy were classified as “at least pT1” and added to the pT2-4 cancers for statistical analysis. A papillary tumor growth was assumed if at least 1 unequivocal papilla with similar atypia to the invasive tumor area was present. Follow-up data were available from 682 patients covering a median follow-up period of 39 months (range, 1-120 months). Time to recurrence was selected as study end point for pTa tumors, and time to progression (to stage pT2 or higher) was selected as study end point for pT1 tumors. *Recurrences* were defined as cystoscopically visible tumors. *Tumor progression* was defined as the presence of muscle invasion (stage pT2 or higher) in a subsequent biopsy. The pathological parameters of the arrayed cancers are described in Table 1. Analysis of patient and corresponding histopathological data for research purposes and construction of TMAs from archived diagnostic left-over tissues was approved by local laws (Hamburgisches Krankenhausgesetz, §12,1) and by the local ethics committee (ethics commission Hamburg, WF-049/09, and PV3652). All work was carried out in compliance with the Helsinki Declaration.

2.2. Immunohistochemistry

Freshly cut TMA sections were immunostained on 1 day and in 1 experiment. For TUBB3 analysis, primary antibody specific for βIII-tubulin (rabbit monoclonal antibody; Epitomics, Burlingame, CA; dilution 1:150) was applied, slides were