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Original contribution

Prognostic comparison of proliferation markers and World Health Organization 1973/2004 grades in urothelial carcinomas of the urinary bladder☆

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Summary European treatment guidelines of non–muscle-invasive urothelial carcinoma of the urinary bladder are strongly dependent on grade, but grading reproducibility is wanting. Protocolized proliferation features such as Mitotic Activity Index (MAI), Ki-67, and phosphohistone H3 are prognostic and reproducible. The objective of this population-based study was to compare proliferation biomarkers with each other and with World Health Organization (WHO) 1973/2004 grades with regard to prediction of stage progression. A total of 193 primary non–muscle-invasive urothelial carcinomas were analyzed using WHO73/04 grades and measurement of the proliferation markers mentioned above. Sensitivities, specificities, and positive and negative predictive values with confidence intervals (CIs) were estimated with regard to progression prediction. Kaplan-Meier survival curves were made, and the hazard ratio and Harrell's C-index with 95% CIs, *P* values, and adjusted C-index for stage progression or not of WHO73, WHO04, and the proliferation markers were calculated. The median follow-up time was 75 months (range, 1-127). A total of 111 patients (52%) experienced recurrence within 5 years, and 14 patients (7%) progressed. High values of MAI predicted stage progression with a positive predictive value of 0.22 (95% CI, 0.12-0.37). The positive predictive value of Ki-67 and phosphohistone H3 were 0.15 (both 95% CIs, 0.07-0.29) and comparable to that of the WHO04. The prognostic value of MAI was strongest, exceeding that of the other proliferation markers and the WHO grading systems. In conclusion, in non–muscle-invasive urinary bladder urothelial carcinomas, proliferation biomarkers have prognostic value, possibly exceeding that of the WHO classifications.

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1. Introduction

Urothelial carcinoma of the urinary bladder is the second most frequent urologic cancer in the Western world [1,2]. Approximately 70% of non–muscle-invasive (pTa, pT1) urothelial carcinomas recur, and 8% to 30% progress to a higher T stage [3]. Early identification of high-risk patients may allow better patient-tailored therapy preventing further disease development [3,4]. Currently, European guidelines for treatment and follow-up surveillance of non–muscle-invasive urothelial carcinomas are based on the 1973 World Health Organization (WHO73) grading system [5]. The WHO73 distinguishes 3 different grades and evaluates microscopic features related to the degree of cellular anaplasia and mitotic activity. The features used by the newer 2-tiered classification system (WHO04) are quite similar, aiming to develop a universally acceptable classification system [1,6]. Unfortunately, reproducibility of both grading systems is suboptimal [7-9], and other biomarkers should be investigated with regard to prediction of prognosis.

DNA aneuploidy and giant nuclei measured by flow or image cytometry are well-known characteristics of grade 3/high-grade tumors. These features reflect cell clones with abnormal cell division, which are also often highly proliferative. A high mitotic rate is currently regarded as an important diagnostic and prognostic sign in tumors of many different organ sites, including the urinary bladder [10-13].

Here, we describe the results of a long-term study on the prognostic value (for stage progression) of different proliferation features. The classic mitosis count was investigated by using a previously evaluated Mitotic Activity Index (MAI) protocol. We also assessed the percentage of Ki-67 positivity and the number of phosphohistone H3 (PPH3)–positive tumor cells.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Norwegian Regional Ethics Committee (REK Vest, no. 106/09) before the start of the study.

2.2. Patients

Two hundred forty-nine consecutive cases of primary non–muscle-invasive urothelial carcinoma of the urinary bladder were diagnosed at the Departments of Urology and Pathology, Stavanger University Hospital (SUH), between January 1, 2002, and December 31, 2006. All the specimens were originally graded according to the WHO73 classification as grades 1 to 3. Fifty-six cases were lost to follow-up or had inadequate sample quality for further analysis, leaving 193 patients to be included in the study. There were no differences in sex, age, stage, initial diagnosis (grade), or occurrence of carcinoma in situ (CIS) between the excluded 56 and remaining 193 patients.

The patients were uniformly treated according to the national guidelines at the time of diagnosis. There was no change in the treatment protocol during this period. All patients underwent transurethral resection followed by a single instillation of a cytotoxic agent (normally 40 mg mitomycin C). High-risk patients were treated with Bacillus Calmette-Guérin (BCG) instillations (alternatively chemotherapy) over 1 to 3 years. High-risk patients included Ta grade 3 tumors, T1 grade 2 or 3 tumors, or concurrent multifocal CIS. Follow-up data were retrieved from medical records and from any available new specimens at the Department of Pathology, SUH.

Recurrence was defined as the reappearance of histopathologically confirmed urothelial carcinoma in the bladder. *Progression* was defined as an advance in stage or histologically proven metastasis.

2.3. Pathology and grading

Tumor tissue was obtained by transurethral resection or biopsy at the Department of Urology, SUH. The tumor tissue was fixed in 4% buffered formaldehyde, dehydrated, and embedded in paraffin. Four-micrometer-thick sections stained with hematoxylin-erythrosine-saffron (HES) were used for routine diagnostics.

All specimens were independently reviewed according to the WHO73 classification (grades 1 through 3) and WHO04 (low or high grade) by experienced pathologists. The pathologists did their evaluations without prior knowledge of the original grade, treatment, or follow-up of the patients. In case of discrepancies between the reviewers, consensus was reached after discussion using a multihead microscope.

2.4. Immunohistochemistry

Immunohistochemistry (IHC), antigen retrieval, and antibody dilution were optimized before the study onset. Four-micrometer paraffin sections adjacent to the HES sections used for histologic assessment were mounted onto Superfrost Plus slides (Menzel, Braunschweig, Germany), dried overnight at 37°C followed by an hour at 60°C, then deparaffinized in xylene, and rehydrated in decreasing concentrations of alcohol. Antigen retrieval was performed in 10 mM Tris/1 mM EDTA buffer (pH 9.0). The sections were heated for 3 minutes at 110°C, then 10 minutes at 95°C, and cooled to 20°C. The Ki-67 and PPH3 antibodies have been described in detail before [14,15]. All IHC staining procedures were performed using Dako Autostainer Link 48 (Glostrup, Denmark).