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Original article

Prognostic role of N-cadherin expression in patients with non–muscle-invasive bladder cancer

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Abstract

Purpose: To assess the role of N-cadherin as a prognostic biomarker in patients with non–muscle-invasive bladder cancer (NMIBC) treated with transurethral resection with or without adjuvant intravesical therapy.

Patients and methods: Immunohistochemistry using monoclonal mouse antibody was used to evaluate the expression status of N-cadherin in 827 patients with NMIBC. N-cadherin was considered positive if any immunoreactivity with membranous staining was detected. Multivariable Cox regression models were performed to evaluate the prognostic effect of N-cadherin on survival outcomes.

Results: N-cadherin expression was observed in 333 patients (40.3%); it was associated with pT1 stage and high tumor grade (both were $P < 0.001$). Median follow-up was 55 months (interquartile range: 18–106). On multivariable Cox regression analyses that adjusted for the effect of the standard clinicopathologic features, N-cadherin expression remained associated with recurrence-free survival ($P = 0.007$) but not progression-free survival ($P = 0.3$), cancer-specific survival ($P = 0.2$), or overall survival ($P = 0.9$). Adding N-cadherin to a model for prediction of disease recurrence modestly improved its discrimination from 72.8% to 73.4%.

Conclusion: N-cadherin is expressed in approximately 2/5 patients with NMIBC. Its expression is associated with adverse pathological features and higher risk of disease recurrence but not progression. N-cadherin could be incorporated in predictive tools to assist in recurrence prediction helping thereby in patient selection regarding adjuvant therapies and follow-up planning.

Keywords: N-cadherin; Non–muscle-invasive bladder cancer; Urothelial tumor; Transurethral resection; Prognosis; Prediction; Progression; Recurrence; Survival

1. Introduction

Worldwide, bladder cancer (BCa) is the ninth most commonly diagnosed cancer and ranks 13th among the causes of cancer mortality [1]. Approximately three-fourths of patients with newly diagnosed BCa present with non–muscle-invasive bladder cancer (NMIBC) in Western countries that include stage Ta, T1, and carcinoma in situ (CIS) [2,3]. Transurethral resection of bladder (TURB) is the standard of care for proper diagnosis, staging, and therapy [2]. NMIBC is a highly heterogeneous disease with a varied risk and of disease recurrence and progression. For example, within the first year after TURB, the probability of

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disease recurrence ranges from 15% to 60%, whereas the probability of disease progression at 5 years ranges from 7% to 40% [2,4]. Delivery of adjuvant intravesical chemotherapy and immunotherapy reduces the risk of tumor recurrence and progression for immunotherapy, respectively [5–8].

Predicting individual patient risk for both disease recurrence and progression is necessary to help physicians and patients in determining treatment and follow-up. For example, identification of the best candidates for adjuvant therapies or early radical cystectomy or both may help improve outcomes. Toward this end, multiple models have been developed to help in stratification of the risk of disease recurrence and progression These models were, however, solely based on patient and pathological tumor characteristics [4,9,10]. Integrating relevant molecular biomarkers into such tools is highly likely to improve their optimal predictive accuracy by harnessing the necessary biological information [11].

N-cadherin is a member of cadherin superfamily that mediates cell-cell interaction in epithelial tissue [12]. Dysregulation of E-, P-, and N-cadherins has been linked to tumor invasiveness in various epithelial malignancies. For example, N-cadherin expression has been linked to more aggressive phenotype in breast, prostate, and colon cancers [12–15]. Few investigators addressed the association of N-cadherin in NMIBC, with only 1 study evaluating its association with intravesical recurrence. Therefore, our aim was to assess the prognostic role of N-cadherin expression in a large multicentre cohort of patients with NMIBC treated with TURB with or without adjuvant therapies. We hypothesized that N-cadherin, a molecular biomarker, is associated with adverse pathological features as well as higher probabilities of disease recurrence and progression.

2. Material and methods

2.1. Patient selection

This was a retrospective multicentre study that included 827 patients with pathologically proven NMIBC. No patient had upper tract or prostatic involvement, evidence or suspicion of metastasis. Institutional review board approval was obtained. All participating centers provided institutional data-sharing agreements before the initiation of the study.

2.2. Data collection and pathological evaluation

Demographic, clinical, intraoperative, and pathological data were collected from patient medical records. All tumors were urothelial carcinomas. Tumor stage was coded according to the 2002 American Joint Committee on Cancer (AJCC) TNM Staging system. Tumor grade was coded according to the 1973 WHO system. All slides were re-reviewed by 2 experienced uropathologists in each center who were blinded to clinical outcomes. Concomitant CIS was defined as CIS in the presence of any other urothelial tumor in the bladder [8].

2.3. Immunohistochemical staining

Immunohistochemical staining was scored by 2 experienced uropathologists who were blinded to clinicopathological outcomes. Staining was performed on 4 μm formalin-fixed tissue microarray sections with an automated immunohistochemical processor (Ventana, Benchmark ultra) using the primary monoclonal anti-N-CD mouse antibody (Dako, Clone 6G11) at a dilution 1:50 for 32 minute and a pretreatment CC1 for 36 minute. Any membranous immunoreactivity was considered positive because normal urothelial tissue does not express N-cadherin. Myocardium tissue was used as a positive control, as it is known to possess N-cadherin expression [12,16]. E-cadherin staining was also performed with the same immunohistochemical processor, namely with primary anti-E-Cadherin monoclonal mouse antibody (Dako, Clone NCH-38) at a dilution of 1:2 for 32 minute after pretreatment CC1 for 60 minute. Normal urothelium was used as positive control, as normal urothelium express E-cadherin. Sections processed without incubation of primary antibody were used as negative controls as described earlier [17].

2.4. Management and follow-up

TURB was performed in all patients and was followed by either single, immediate postoperative intravesical instillation (mitomycin C 40 mg, epirubicin 80 mg, or doxorubicin 50 mg), adjuvant chemotherapy with mitomycin C, or adjuvant Bacillus Calmette-Guérin immunotherapy. The first adjuvant intravesical therapy was given 7 to 21 days after TURB, unless contraindicated. Follow-up consisted of history, physical examination, urine analysis, urine cytology, and cystourethroscopy. The frequency of cystourethroscopy was individualized and tailored to patient risk, according to international guidelines [2]. Imaging of the upper tract was done at the time of diagnosis and then annually or when clinically indicated, for example, in the presence of positive urine cytology. Disease recurrence was defined as the first pathologically proven tumor relapse of any stage or grade, whereas disease progression was defined as muscle-invasive tumor relapse. Causes of death were coded according to chart review or death certificate or both [18].

2.5. Statistical analysis

Categorical and continuous variables were compared between patient populations using chi-square and Mann-Whitney *U* tests, respectively. Estimates of survival outcomes such as recurrence-free survival (RFS), progression-free survival (PFS), cancer-specific survival (CSS), and overall survival (OS) were evaluated using the