Myopodin Methylation is Associated With Clinical Outcome in Patients With T1G3 Bladder Cancer

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Purpose: Bacillus Calmette-Guerin is standard treatment to decrease tumor recurrence and delay progression of high risk, nonmuscle invasive bladder tumors. However, it is not yet clear which T1G3 cases are more prone to more aggressive clinical behavior or susceptible to respond to bacillus Calmette-Guerin. We evaluated the role of myopodin methylation as a clinical outcome prognosticator and predictive biomarker for the bacillus Calmette-Guerin response in patients with T1G3 bladder tumors.

Materials and Methods: We analyzed the methylation status of myopodin in tumor specimens from 170 patients with T1G3 bladder cancer, including a subset of 108 who underwent bacillus Calmette-Guerin treatment. Myopodin methylation was assessed by methylation specific polymerase chain reactions. Recurrence, progression to muscle invasive tumors and disease specific overall survival were analyzed using competing risks regression analysis.

Results: Of the 170 cases analyzed 72 recurred (42.4%) and 36 progressed (21.2%). A total of 24 patients (14.1%) died of the disease. Univariate and multivariate survival analysis revealed that myopodin methylation was significantly associated with an increased recurrence rate ($p = 0.004$), progression ($p = 0.002$) and shorter disease specific overall survival ($p = 0.020$). In a subset treated with bacillus Calmette-Guerin myopodin methylation was also related to an increased recurrence rate ($p = 0.011$), progression ($p = 0.030$) and shorter disease specific overall survival ($p = 0.028$).

Conclusions: Epigenetic analysis revealed that myopodin methylation was associated with tumor aggressiveness and clinical outcome in patients with T1G3 disease. Myopodin methylation distinguished patients responding to bacillus Calmette-Guerin from those who may require a more aggressive therapeutic approach.

Key Words: urinary bladder; urinary bladder neoplasms; Mycobacterium bovis; SYNPO2 protein, human; methylation

Abbreviations and Acronyms

BCG = bacillus Calmette-Guerin
CIS = carcinoma in situ
MMC = mitomycin C
NED = no evidence of disease
TUR = transurethral resection

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BLADDER cancer, the fourth most common neoplasm in men, is clinically characterized by a high recurrence rate and poor prognosis once tumors invade the muscularis propia.[1,2] Urothelial carcinoma presenting as T1G3 nonmuscle invasive lesions carries high risk due to the possibility of recurrence and progression to muscle invasive or metastatic disease.[3–7] Intravesical BCG immunotherapy is a highly successful therapy for nonmuscle invasive disease[3–6] with the benefits of decreased recurrence and progression risks.[7,8]

Despite the superior efficacy of BCG over TUR alone or TUR plus intravesical chemotherapy eventually

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more than 50% of nonmuscle invasive bladder cancers persist or recur. This is a particularly acute problem in patients with high risk disease, such as CIS, submucosal invasion (stage T1) and high grade papillary disease, since the risk of progression increases proportionally as the window of opportunity narrows for conservative bladder therapy before cystectomy becomes mandatory. A significant number of these cases fail to respond to BCG therapy and tumors persist or recur and may also become invasive or metastatic.[6,7] Despite the promising sensitivity and specificity of several tumor markers, including microsatellite instability, to predict the BCG response in any of these 3 subtypes of patients with bladder cancer at high risk, no predictive biomarker evaluated to date has been fully introduced into clinical routine practice.[2,6,7] Improved prognostic biomarkers are required to ultimately distinguish indolent bladder tumors from those that are potentially lethal so that therapeutic procedures may be tailored to each individual.[2–11]

Myopodin gene methylation was recently identified in bladder cancer.[12] Myopodin is a dual compartment protein that shows actin bundling activity and redistributes between nucleus and cytoplasm in a differentiation dependent, stress induced manner.[13,14] Myopodin gene deletions at 4q26 and the loss of myopodin protein expression were associated with aggressive prostate cancer using in vitro, in vivo and clinical material.[15–18] In bladder cancer cases to our knowledge genomic deletions have not been reported to date while previous studies showed differential nuclear localization of myopodin among bladder cancer cells while sharing structural cytoplasmic expression patterns.[19] In bladder tumors loss of the nuclear myopodin counterpart is associated with increased tumor staging and poor clinical outcome.[19] The impact of myopodin in nuclear transport was also described in nonneoplastic cells,[20–23] including myocytes.[22] Epigenetic analysis provides a mechanistic explanation for the observed loss of myopodin in uroepithelial malignancies by epigenetic silencing.[12] In that report the impact and clinical relevance of myopodin methylation on bladder cancer progression was evaluated using in vitro strategies and a large series of tissue specimens. Hypermethylation emerged as a strong indicator of tumor progression in patients with bladder cancer. Myopodin hypermethylation was noted to be potentially useful as a tumor stratification biomarker and clinical outcome prognosticator in patients with bladder cancer.

We performed methylation analysis in pT1G3 bladder tumors to evaluate the role of myopodin methylation as a prognosticator of bladder cancer aggressiveness. A subset of these cases treated with BCG allowed the assessment of myopodin methylation as a therapeutic predictive marker of the BCG response in T1G3 disease.

METHODS

Patient Population

At the Hospital Central de Asturias 170 patients were treated for primary stage T1 grade 3 urothelial carcinoma with complete TUR between 1989 and 2008. Tumors were collected according to guidelines for the protection of human subjects, who were recruited under institutional review board approved protocols. Study inclusion criteria were no prior intravesical treatment and a similar histopathological diagnosis (pT1G3 transitional cell carcinoma), as defined by standard criteria,[24] with wide, deep primary resection with muscle in the specimen and paraffin embedded tissue material adequate for analysis. Patients who previously presented with an upper urinary tract tumor were excluded from study. Overall we identified and selected 170 patients with a median age of 71.5 years (range 28 to 94) for analysis.

A subset of 108 patients with a mean age of 71 years (range 28 to 90) received a 6-week BCG course after up to a year of maintenance therapy. Induction and maintenance courses were performed in all BCG treated patients. Instillation 1 (Connaught strain BCG) was given 14 days after TUR, repeated weekly for 6 consecutive weeks and thereafter every 2 weeks 6 more times within 5 to 6 months after surgical intervention. The 62 patients who did not receive BCG were treated with MMC starting 14 to 21 days after TUR. The regimen consisted of weekly MMC instillations (40 mg/50 ml) for 6 weeks and every 2 weeks thereafter for 6 months. Followup consisted of cystoscopy with cytology every 3 months for the first 2 years and every 6 months thereafter. Progression was defined as muscular invasion (stage T2 or higher) or metastatic disease. Patients with recurrence were treated with another course of BCG or cystectomy when disease progressed.

Myopodin Promoter Methylation Analysis

Paraffin embedded bladder tumors were macrodissected based on hematoxylin and eosin evaluation to ensure a minimum of 75% of tumor cells[25] before DNA extraction was done using standard methods.[25,26] Myopodin methylation was analyzed by methylation specific polymerase chain reactions of bisulfite modified genomic DNA using primers specific for methylated or modified unmethylated DNA, as previously reported.[12,26]

Statistical Analysis

We used all 170 cases to analyze associations between myopodin methylation and clinicopathological variables, such as age, gender, tumor size, multifocality and associated CIS, which were evaluated using the nonparametric Wilcoxon-Mann-Whitney and Kruskal-Wallis tests.[27] A subset of 108 cases treated with BCG was used for predictive analysis. Control groups of patients with PT1G3 and NED allowed evaluation of the usefulness of myopodin methylation for prognosis in 98 NED cases of the series of 170 with a median followup of 58 months and prediction in