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Platinum Priority – Bladder Cancer

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Next-generation Sequencing of Nonmuscle Invasive Bladder Cancer Reveals Potential Biomarkers and Rational Therapeutic Targets

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Abstract

Background: Molecular characterization of nonmuscle invasive bladder cancer (NMIBC) may provide a biologic rationale for treatment response and novel therapeutic strategies.
Objective: To identify genetic alterations with potential clinical implications in NMIBC.
Design, setting, and participants: Pretreatment index tumors and matched germline DNA from 105 patients with NMIBC on a prospective Institutional Review Board-approved protocol underwent targeted exon sequencing analysis in a Clinical Laboratory Improvement Amendments-certified clinical laboratory.
Outcome measurements and statistical analysis: Comutation patterns and copy number alterations were compared across stage and grade. Associations between genomic alterations and recurrence after intravesical bacillus Calmette-Guérin (BCG) were estimated using Kaplan-Meier and Cox regression analyses.
Results and limitations: *TERT* promoter mutations (73%) and chromatin-modifying gene alterations (69%) were highly prevalent across grade and stage, suggesting these events occur early in tumorigenesis. *ERBB2* or *FGFR3* alterations were present in 57% of high-grade NMIBC tumors in a mutually exclusive pattern. DNA damage repair (DDR) gene alterations were seen in 30% (25/82) of high-grade NMIBC tumors, a rate similar to MIBC, and were associated with a higher mutational burden compared with tumors with intact DDR genes ($p < 0.001$). *ARID1A* mutations were associated with an increased risk of recurrence after BCG (hazard ratio = 3.14, 95% confidence interval: 1.51–6.51, $p = 0.002$).
Conclusions: Next-generation sequencing of treatment-naive index NMIBC tumors demonstrated that the majority of NMIBC tumors had at least one potentially actionable alteration that could serve as a target in rationally designed trials of intravesical or systemic therapy. DDR gene alterations were frequent in high-grade NMIBC and were

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associated with increased mutational load, which may have therapeutic implications for BCG immunotherapy and ongoing trials of systemic checkpoint inhibitors. *ARID1A* mutations were associated with an increased risk of recurrence after BCG therapy. Whether *ARID1A* mutations represent a predictive biomarker of BCG response or are prognostic in NMIBC patients warrants further investigation.

Patient summary: Analysis of frequently mutated genes in *superficial* bladder cancer suggests potential targets for personalized treatment and predictors of treatment response, and also may help develop noninvasive tumor detection tests.

1. Introduction

Of the estimated 429 000 people to be diagnosed with bladder cancer in the industrialized world each year, 70–80% will have nonmuscle invasive bladder cancer (NMIBC) [1,2]. Half of all NMIBC patients will experience tumor recurrence within 5 yr, and 20–30% will progress to secondary MIBC [3]. Ultimately, as many as 10–15% of patients presenting with NMIBC will die of bladder cancer [4].

Previous investigations into NMIBC genetics have been limited by their inability to comprehensively profile tumors for multiple cancer-associated genes [5]. More recently, MIBC were comprehensively investigated by The Cancer Genome Atlas (TCGA) and other groups using next-generation sequencing (NGS), leading to the identification of potential biomarkers and targets for therapeutic intervention [6,7]. However, very few NMIBC tumors have been examined with NGS methods to date, and these investigations have been limited by a lack of clinical annotation, the absence of restaging transurethral resection (TUR) to ensure appropriate tumor staging, or a failure to differentiate between primary and recurrent tumors [8–11].

In this study, we examined primary treatment-naive index tumors from a cohort of patients with NMIBC using a massively parallel, targeted, exon capture-based NGS platform to define the prevalence of genetic alterations and their potential clinical implications.

2. Patients and methods

2.1. Patients and samples

Targeted NGS with a 341 or updated 410 cancer-associated gene panel was performed on formalin-fixed paraffin embedded sections of treatment-naive index tumors along with matched germline DNA for 105 patients with NMIBC as part of an Institutional Review Board-approved protocol (Supplementary Fig. 1, Supplementary Table 1, Supplementary data) [12]. A board-certified genitourinary pathologist reviewed representative hematoxylin and eosin slides to confirm grade, stage, and urothelial histology. All patients underwent evaluation and TUR by a urologic oncologist at Memorial Sloan Kettering Cancer Center (MSK). All tumors profiled were newly diagnosed and untreated. Patients who received perioperative mitomycin or other adjuvant perioperative therapies were excluded. All high-grade T1 (HGT1) tumors had restaging TUR and confirmation of uninvolved detrusor muscle. Treatment and management was at the discretion of the treating urologic oncologist. Patients managed with TUR were followed at MSK with cystoscopy and urine cytology every 3 mo for the 1st yr, then every 3–6 mo. Recurrence was defined as histological proven cancer on biopsy or TUR. See Supplementary data for additional details.

For comparison purposes, we also evaluated the frequency of genomic alterations seen in 40 pretreatment index tumors in patients with primary MIBC who were sequenced with MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) on the same Institutional Review Board-approved protocol and 98 MIBC specimens from patients in the TCGA study who were reported to have no prior history of NMIBC (Supplementary data).

2.2. Statistical analysis

Alterations in oncogenes were deemed significant if they were recurrent or known functional missense mutations or amplifications (Supplementary data). Alterations in tumor suppressor and DNA damage repair genes were deemed significant if truncating mutations (nonsense, frameshift indels), recurrent missense mutations, or homozygous deletions were present (Supplementary data). Fisher's exact tests were used to analyze categorical associations. Kruskal-Wallis and Wilcoxon tests were used for continuous variables. Cox regression modeling was used to determine the association between genomic alterations and recurrence after bacillus Calmette-Guérin (BCG). The Kaplan-Meier method and log-rank test were used for estimations of recurrence free survival. A p value of <0.05 was considered statistically significant. All analyses were conducted using R v.3.3.1. (https://cran.r-project.org/bin/windows/base/old/3.3.1/)

3. Results

3.1. Patient demographics and treatment

To characterize the genomic landscape of NMIBC, we analyzed 105 tumors across the disease spectrum comprising low-grade Ta (LGTa; $n = 23$), high-grade Tis (HGTis; $n = 12$), high-grade Ta (HGTa; $n = 32$), and HGT1 ($n = 38$) for alterations in 341 cancer-associated genes. Information on patient demographics and treatments are listed in Table 1, Supplementary Table 2, and Supplementary Table 3. The median follow-up for the NMIBC cohort managed by TUR with or without adjuvant intravesical therapy ($n = 100$) was 24.4 mo, with recurrences occurring in 46 patients. Treatment following resection for the 23 low-grade tumors included surveillance only (48%), intravesical mitomycin (39%), and intravesical BCG (13%). The 82 high-grade tumors were treated with intravesical BCG (81%), observation (12%), or immediate radical cystectomy in five patients (4 = pT1N0, 1 = pTaN0). Additional details on the NMIBC cohort and the MIBC samples used for comparison purposes are available in the Supplementary data, Supplementary Table 2, and Supplementary Table 3.