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Platinum Priority – Bladder Cancer

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Defects in DNA Repair Genes Predict Response to Neoadjuvant Cisplatin-based Chemotherapy in Muscle-invasive Bladder Cancer

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Abstract

Background: Cisplatin-based neoadjuvant chemotherapy (NAC) before cystectomy is the standard of care for muscle-invasive bladder cancer (MIBC), with 25–50% of patients expected to achieve a pathologic response. Validated biomarkers predictive of response are currently lacking.
Objective: To discover and validate biomarkers predictive of response to NAC for MIBC.
Design, setting, and participants: Pretreatment MIBC samples prospectively collected from patients treated in two separate clinical trials of cisplatin-based NAC provided the discovery and validation sets. DNA from pretreatment tumor tissue was sequenced for all coding exons of 287 cancer-related genes and was analyzed for base substitutions, indels, copy number alterations, and selected rearrangements in a Clinical Laboratory Improvements Amendments–certified laboratory.
Outcome measurements and statistical analysis: The mean number of variants and variant status for each gene were correlated with response. Variant data from the discovery cohort were used to create a classification tree to discriminate responders from nonresponders. The resulting decision rule was then tested in the independent validation set.
Results and limitations: Patients with a pathologic complete response had more alterations than those with residual tumor in both the discovery ($p = 0.024$) and validation ($p = 0.018$) sets. In the discovery set, alteration in one or more of the three DNA repair genes *ATM*, *RB1*, and *FANCC* predicted pathologic response ($p < 0.001$; 87% sensitivity, 100% specificity) and better overall survival ($p = 0.007$). This test remained predictive for pathologic response in the validation set ($p = 0.033$), with a trend towards better overall survival ($p = 0.055$). These results require further validation in additional sample sets.
Conclusions: Genomic alterations in the DNA repair-associated genes *ATM*, *RB1*, and *FANCC* predict response and clinical benefit after cisplatin-based chemotherapy for MIBC. The results suggest that defective DNA repair renders tumors sensitive to cisplatin.

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Patient summary: Chemotherapy given before bladder removal (cystectomy) improves the chance of cure for some but not all patients with muscle-invasive bladder cancer. We found a set of genetic mutations that when present in tumor tissue predict benefit from neoadjuvant chemotherapy, suggesting that testing before chemotherapy may help in selecting patients for whom this approach is recommended.

1. Introduction

Muscle-invasive bladder cancer (MIBC) is characterized by a propensity to metastasize. Currently, neoadjuvant cisplatin-based chemotherapy followed by cystectomy is the standard of care for MIBC on the basis that phase 3 clinical trial data and meta-analyses show better overall survival (OS) with this approach [1,2]. Pathologic response at the time of cystectomy predicts survival [3]. Unfortunately, only approximately a third of patients achieve such a response [1,4]. Genomic profiling is an increasingly useful tool for understanding the molecular etiology of bladder cancer; however, while molecular biomarkers are currently used clinically to guide treatment selection in melanoma (BRAF), lung cancer (EGFR) and colorectal cancer (KRAS), validated genomic biomarkers predictive of response to therapy are currently lacking for bladder cancer [5–7]. We recently reported the results of a clinical trial using three cycles of neoadjuvant accelerated methotrexate, vinblastine, doxorubicin, and cisplatin (AMVAC) in patients with MIBC [4]. Using a discovery set of prospectively collected pretreatment tumor samples from patients treated in this study, we sought to identify potential genomic biomarkers of response, hypothesizing that genomic alterations could be identified that would effectively predict response to DNA-damaging chemotherapy in MIBC. A set of identically collected samples from a follow-up trial of similar design testing three cycles of neoadjuvant dose-dense gemcitabine and cisplatin (DDGC) [8] served as the validation cohort.

2. Patients and methods

2.1. Study design and patients

The discovery (AMVAC) and validation (DDGC) sets consisted of pretreatment tumor samples collected from all MIBC patients treated during two previously reported trials (NCT01031420 [4] and NCT01611662 [8], respectively) who received all three cycles of chemotherapy and for whom adequate pretreatment tissue was available. For each cohort, pretreatment formalin-fixed paraffin-embedded (FFPE) sections were obtained and sequenced as described below. Patients provided informed consent for study treatment and collection and testing of archival tissue, clinical data, demographic data, and follow-up data as part of each trial. We considered two definitions of response: pathologic complete response, defined as no remaining tumor in the specimen (pT0pN0cM0), and tumors that were downstaged to non-MIBC disease ($\leq$pT1pN0cM0). Both have been used as endpoints in clinical trials and correlate with improved progression-free survival (PFS) and OS in our AMVAC clinical trial and others [1,4,8–10].

2.2. Genomic sequencing

Genomic DNA was extracted from 40 μg of FFPE tumor tissue using a Maxwell 16 FFPE Plus LEV DNA Purification kit (Promega, Madison, WI, USA). Samples of 50–200 ng of extracted DNA were sheared to ~100–400 bp by sonication and were then subjected to end-repair, dA-addition, and ligation of indexed Illumina (San Diego, CA, USA) sequencing adaptors [11]. Sequencing libraries were hybridization-captured using a pool of >24 000 individually synthesized 5′-biotinylated DNA oligonucleotides (Integrated DNA Technologies, Coralville, IA, USA). These baits were designed to target ~1.5 MB of the human genome, including 4557 exons of 287 cancer-related genes, 47 introns of 19 genes frequently rearranged in cancer, and 3549 polymorphisms located throughout the genome. DNA sequencing was performed using the HiSeq instrument (Illumina) with 49 × 49 paired-end reads, targeting >500× unique median sequence coverage. Sequence data analysis and quality control measures are described in the Supplementary material, along with gene expression data analysis, support vector machine training with sequence and protein structural features, and ATM protein modeling.

2.3. Statistical analysis

A classification tree was used to identify a parsimonious decision rule to discriminate responders from nonresponders. At each branch point, the relationship between gene (variant vs wild type) and responder status was assessed within the subtree using two-sided Fisher's exact tests. The branch was then split on the gene that resulted in the lowest p value. This process was repeated until it was no longer possible to identify a gene that was significantly ($p \leq 0.05$) associated with response in the subtree. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and two-sided 95% exact confidence intervals (CIs) were computed to characterize the operating characteristics of the resulting *ATM*/*RB1*/*FANCC* decision rule using the discovery and validation data sets with each definition of pathologic response ($\leq$pT1pN0cM0 and pT0pN0cM0). We assessed the significance of the resulting decision rule in the AMVAC discovery set using a permutation-based approach by randomly reordering the responder/nonresponder status of the individuals, keeping variant information fixed. The proportion of reorderings resulting in a misclassification rate at least as small as that for the original tree was taken as an estimate of the overall p value. The number of genomic variants detected in responders (pT0) was compared to that in nonresponders by two-sample t tests separately within the AMVAC and DDGC data sets. OS and PFS were calculated using the Kaplan-Meier method.

3. Results

3.1. Discovery

Of the 44 patients treated on the AMVAC study, 37 received all three cycles of chemotherapy. Three additional patients were excluded because of insufficient pretreatment tissue, yielding a discovery set of 34 for genomic analysis. The