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Impact of Molecular Subtypes in Muscle-invasive Bladder Cancer on Predicting Response and Survival after Neoadjuvant Chemotherapy

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Article info

Abstract

Background: An early report on the molecular subtyping of muscle-invasive bladder cancer (MIBC) by gene expression suggested that response to neoadjuvant chemotherapy (NAC) varies by subtype.
Objective: To investigate the ability of molecular subtypes to predict pathological downstaging and survival after NAC.
Design, setting, and participants: Whole transcriptome profiling was performed on pre-NAC transurethral resection specimens from 343 patients with MIBC. Samples were classified according to four published molecular subtyping methods. We developed a single-sample genomic subtyping classifier (GSC) to predict consensus subtypes (claudin-low, basal, luminal-infiltrated and luminal) with highest clinical impact in the context of NAC. Overall survival (OS) according to subtype was analyzed and compared with OS in 476 non-NAC cases (published datasets).
Intervention: Gene expression analysis was used to assign subtypes.

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Outcome measurements and statistical analysis: Receiver-operating characteristics were used to determine the accuracy of GSC. The effect of GSC on survival was estimated by Cox proportional hazard regression models.

Results and limitations: The models generated subtype calls in expected ratios with high concordance across subtyping methods. GSC was able to predict four consensus molecular subtypes with high accuracy (73%), and clinical significance of the predicted consensus subtypes could be validated in independent NAC and non-NAC datasets. Luminal tumors had the best OS with and without NAC. Claudin-low tumors were associated with poor OS irrespective of treatment regimen. Basal tumors showed the most improvement in OS with NAC compared with surgery alone. The main limitations of our study are its retrospective design and comparison across datasets.

Conclusions: Molecular subtyping may have an impact on patient benefit to NAC. If validated in additional studies, our results suggest that patients with basal tumors should be prioritized for NAC. We discovered the first single-sample classifier to subtype MIBC, which may be suitable for integration into routine clinical practice.

Patient summary: Different molecular subtypes can be identified in muscle-invasive bladder cancer. Although cisplatin-based neoadjuvant chemotherapy improves patient outcomes, we identified that the benefit is highest in patients with basal tumors. Our newly discovered classifier can identify these molecular subtypes in a single patient and could be integrated into routine clinical practice after further validation.

1. Introduction

Neoadjuvant cisplatin-based chemotherapy (NAC) is the standard treatment in muscle-invasive bladder cancer (MIBC) prior to radical cystectomy [1–3]. Although NAC improves pathological downstaging and patient survival, only approximately 40% of patients experience a major response, defined as absence of muscle-invasive disease and lymph node metastasis (<pT2 and pN0) [4]. Nonresponding patients are unlikely to derive clinical benefit, are exposed to substantial toxicity, and experience a delay in definitive local therapy [2,3]. Identification of molecular markers of non-responsiveness is essential for more precise delivery of care. Recent analyses suggest that specific mutations, especially in *ERCC2*, *ERBB2*, and DNA repair genes, may predict response to NAC [5–8]. Here we aimed to use RNA expression analysis for the development of predictive biomarkers.

Recent identification of molecular gene expression subtypes [9–13] and prior work highlighting the clinical impact of basal MIBC [14] have advanced our understanding of the biology of bladder cancer. Molecular classification provides a framework for further study and has potential implications for the clinical management of MIBC. Four different molecular subtyping schemes have been described [10–13]. Each was developed in different patient populations using unique genomic platforms, and only one was based on integrative multiplatform genomic analysis [10]. Despite these differences, each identifies molecular phenotypes that share many similarities. They represent a division into basal and luminal tumors at a higher level, with different subclassifications that are specific to each system.

Choi et al [11] first introduced the concept that molecular subtypes may predict response to NAC. In three cohorts with a total of 100 patients, a subset classified as having "p53-like" tumors demonstrated a lower response rate to cisplatin-based combination chemotherapy. This finding has not been validated in additional larger patient cohorts and has not been investigated with the other subtyping methods. Furthermore, none of the four subtyping models is suitable for clinical implementation because each requires classification of an entire patient cohort in order to assign an individual patient sample to a subtype.

In this study, we aimed to correlate large multi-institutional patient cohort outcomes after NAC with molecular subtyping of pre-NAC specimens according to four published classification methods: University of North Carolina (UNC), MD Anderson Cancer Center (MDA), The Cancer Genome Atlas (TCGA), and Lund University [10–13,15]. Moreover, we aimed to develop a single-patient assay based on transcriptomic analysis of transurethral resection (TUR) specimens that would be suitable for use in a clinical laboratory setting.

2. Patients and methods

2.1. Patient populations

For the discovery NAC cohort, 250 consecutive patients from five institutions were compiled. MIBC (cT2-4aN0-3M0) was diagnosed by TUR prior to receiving at least three cycles of NAC. For the validation NAC cohort, 93 consecutive patients with MIBC from two institutions were selected, whose characteristics were similar to those of the discovery set.

2.2. Tissue sampling and gene expression profiling

Whole transcriptome analysis was performed on formalin-fixed, paraffin-embedded tumor tissue with GeneChip® Human Exon 1.0 ST Array (Affymetrix) in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory [16]. In total, 223/250 (89%) and 82/93 (88%) of the discovery and validation NAC cohorts, respectively, passed quality control (Supplementary Table 1). Microarray data were normalized and genes summarized using single-channel array normalization [17].

2.3. Datasets from the public domain

For investigation of the prognostic impact of the published methods for molecular subtyping, the 397 patients without chemotherapy prior to sample collection from the TCGA bladder urothelial carcinoma were