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Genomic Predictors of Survival in Patients with High-Grade Urothelial Carcinoma of the Bladder

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Abstract

Urothelial carcinoma of the bladder (UCB) is genomically heterogeneous, with frequent alterations in genes regulating chromatin state, cell cycle control, and receptor kinase signaling. To identify prognostic genomic markers in high-grade UCB, we utilized capture-based massively-parallel sequencing to analyze 109 tumors.

Mutations were detected in 240 genes, with 23 genes mutated in 5% of cases. The presence of a recurrent *PIK3CA* mutation was associated with improved recurrence-free survival (RFS; HR=0.35, $p=0.014$) and cancer-specific survival (CSS; HR=0.35, $p=0.040$) in patients treated with radical cystectomy. In multivariable analyses controlling for pT and pN stages, *PIK3CA* mutation remained associated with RFS (HR=0.39, $p=0.032$). The most frequent alteration, *TP53* mutation (57%), was more common in extravesical (69% vs. 32%, $p=0.005$) and lymph node-positive (77% vs. 56%, $p=0.025$) disease. Patients with *CDKN2A* altered tumors experienced worse RFS

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(HR=5.76, $p<0.001$) and CSS (HR=2.94, $p=0.029$) in multivariable analyses. Mutations in chromatin modifying genes were highly prevalent but not associated with outcomes.

In UCB patients treated with radical cystectomy, *PIK3CA* mutations are associated with favorable outcomes whereas *TP53* and *CDKN2A* alterations are associated with poor outcomes. Genomic profiling may aid in the identification of UCB patients at highest risk following radical cystectomy.

Keywords

bladder cancer; genomics; clinical outcomes; *PIK3CA*; mutation

Despite multimodality treatment, patients with bladder cancer who undergo radical cystectomy (RC) remain at high risk for recurrence. We hypothesized that the significant variability in outcomes of UCB patients partly results from differences in the genetic changes mediating UCB development and progression [1-4]. With the goal of identifying prognostic genomic alterations, we utilized capture-based next-generation sequencing to analyze high-grade UCB tumors for somatic mutations in cancer-associated genes.

Tissue samples and matched germline blood from 109 patients with high-grade UCB were sequenced using MSK-IMPACT as previously described (details in Supplementary Methods) [5]. Cohort characteristics are listed in Table S1. Average sequence coverage was 579x across all targeted exons. An average of 10 mutations was detected per tumor (range 0-46). Overall, mutations were detected in 240 genes, with 23 genes mutated in 5% of cases (Figure 1). As validation, we re-analyzed all samples using an orthogonal sequencing platform including all coding exons of *STAG2*, *KDM6A*, *ARID1A*, and *KMT2D* (*MLL2*). Using this method, the vast majority of mutations in these genes identified by MSK-IMPACT were confirmed (99.3%).

We analyzed mutations and copy number alterations independently and in the context of cancer-related pathways. The most common alteration was *TP53* mutation, identified in 62 patients (57%) (Figure S1A). Alterations in genes that regulate entry into S phase of the cell cycle were also prevalent (46%) (Figure S1B). Consistent with previous reports, mutations in chromatin modifying genes (CMGs) were highly prevalent, occurring in 83% of patients (Figure S1C) [1-3]. *KDM6A* was mutated in 45 patients (41%), with 41/45 truncating mutations. *ARID1A* alterations (28%), similarly enriched for truncating mutations (30/31), were mutually exclusive with *SMARCA4* alterations, suggesting potential overlapping functionality. The histone acetyltransferase genes *CREBBP* (13%) and *EP300* (15%) also exhibited a pattern of mutually exclusive truncating mutations.

Alterations in the PI3K/AKT signaling pathway, mutationally activated in many cancers, are potential therapeutic targets in UCB [4]. We identified PI3K/AKT pathway alterations in 38 patients (35%) (Figure S2A). Recurrent missense mutations in *PIK3CA*, which encodes the alpha subunit of PI3-kinase, were detected in 23 patients (21%), the vast majority of which were functionally-validated activating helical domain mutations (Figure S2B). Additional

recurrently mutated PI3K/AKT pathway genes included *PTEN* (6%), *AKT1* (2%) and *TSC1* (6%).

To identify prognostic genomic markers, we analyzed associations between alteration status, clinicopathologic characteristics, and outcomes in 89 patients whose tumors were obtained at RC. For these analyses, we included 22 alterations (Table S2) with putative functional significance (oncogenes: recurrent missense mutations and amplifications; tumor suppressors: nonsense mutations, frameshift indels, and deletions). *TP53* mutations were more associated common in extravesical than organ-confined disease (69% vs. 32%, $p=0.005$) and p53 pathway alterations were more common in node-positive disease (77% vs. 56%, $p=0.025$) (Table S2).

The presence of a recurrent missense mutation in *PIK3CA* was associated with significantly improved RFS and CSS ($p=0.014$ and 0.040 , respectively) (Figure 2A). A similar improvement was observed upon analyzing PI3K/AKT pathway genes as a functionally related group (HR=0.34, $p=0.006$ and HR=0.29, $p=0.01$; Figure 2B). Furthermore, after controlling for pT stage and nodal involvement, *PIK3CA* mutation and PI3K/AKT pathway alteration remained significant predictors of improved RFS (HR=0.39, $p=0.032$ and HR=0.37, $p=0.017$, respectively; Table S3). Notably, *PIK3CA* alterations have been associated with favorable disease-specific outcomes in other malignancies [6, 7]. Although relatively favorable, UCB patients with *PIK3CA* mutant tumors still experienced a significant rate of disease recurrence (44% at 5 years). Adjuvant trials of selective PI3-kinase inhibitors in this genetically defined subset of patients would thus be rational.

Patients with p53 pathway alterations had worse RFS (HR=1.92, $p=0.048$) and CSS (HR=2.94, $p=0.013$), but these did not remain statistically significant after adjustment for pT and pN stages (Table S3). Patients with *CDKN2A* alterations experienced worse RFS and CSS in multivariable analyses (HR=5.76, $p<0.001$ and HR=2.94, $p=0.029$, respectively). The significantly poor outcome of patients with *CDKN2A* altered tumors provides rationale for the study of selective cdk4 inhibitors in this genomic subgroup.

We identified higher CMG mutation rates than previously reported [1]. Methodological differences may account for this, as our deep sequencing approach (mean coverage 579x) likely provided greater sensitivity. However, we did not find an association between CMG mutations and outcomes. Given their presence in nearly all UCB tumors (83% in this series), we speculate that epigenetic alterations may be necessary events in UCB pathogenesis and thus CMG mutations are not associated with outcomes.

Studies investigating the prognostic significance of alterations in the cohesin subunit *STAG2*, which regulates sister chromatid cohesion and segregation, in UCB have reported contradictory findings. In one study, *STAG2* alterations were associated with worse survival [2], while another found that loss of *STAG2* expression was associated with worse disease characteristics and outcomes following RC [8]. In contrast, a third study reported that loss of *STAG2* expression was associated with improved outcomes [9]. In our study, we observed no association between *STAG2* mutations and outcomes.

Given the genomic heterogeneity of bladder cancer, one limitation of the current study is its sample size. We may have also underestimated the genetic variation present within individual tumors by sampling a single site. Further, although our assay included all highly mutated genes identified by recent whole exome studies, less frequently mutated genes and structural alterations were not detectable with our capture-based approach. Finally, our targeted DNA sequencing approach does not address epigenetic differences and/or differences in gene expression [10]. Given these limitations of the current study, a larger and more comprehensive analysis may identify co-mutation patterns that are more predictive of disease outcome.

In summary, we performed capture-based, next-generation sequencing on a large cohort of high-grade UCB patients with the goal of identifying prognostic genomic alterations. Patients with *TP53* mutant tumors experienced worse outcomes, but *TP53* mutations were not significantly associated with outcome in multivariable analyses given their association with locally advanced and/or node-positive disease. In contrast, *PIK3CA* and/or PI3K/AKT pathway alterations were associated with improved disease-specific outcomes independent of tumor and lymph node stage whereas *CDKN2A* alterations were independently associated with worse outcomes. These findings, pending validation, may inform patient selection for novel combined modality approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Gui Y, Guo G, Huang Y, Hu X, Tang A, Gao S, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet.* 2011; 43:875–8. [PubMed: 21822268]
- [2]. Guo G, Sun X, Chen C, Wu S, Huang P, Li Z, et al. Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. *Nat Genet.* 2013; 45:1459–63. [PubMed: 24121792]
- [3]. Cancer Genome Atlas Research N. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature.* 2014; 507:315–22. [PubMed: 24476821]
- [4]. Iyer G, Al-Ahmadie H, Schultz N, Hanrahan AJ, Ostrovskaya I, Balar AV, et al. Prevalence and co-occurrence of actionable genomic alterations in high-grade bladder cancer. *J Clin Oncol.* 2013; 31:3133–40. [PubMed: 23897969]
- [5]. Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov.* 2012; 2:82–93. [PubMed: 22585170]
- [6]. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, et al. PIK3CA mutation associates with improved outcome in breast cancer. *Clin Cancer Res.* 2009; 15:5049–59. [PubMed: 19671852]

- [7]. Shigaki H, Baba Y, Watanabe M, Murata A, Ishimoto T, Iwatsuki M, et al. PIK3CA mutation is associated with a favorable prognosis among patients with curatively resected esophageal squamous cell carcinoma. *Clin Cancer Res.* 2013; 19:2451–9. [PubMed: 23532889]
- [8]. Solomon DA, Kim JS, Bondaruk J, Shariat SF, Wang ZF, Elkahoul AG, et al. Frequent truncating mutations of STAG2 in bladder cancer. *Nat Genet.* 2013; 45:1428–30. [PubMed: 24121789]
- [9]. Balbas-Martinez C, Sagrera A, Carrillo-de-Santa-Pau E, Earl J, Marquez M, Vazquez M, et al. Recurrent inactivation of STAG2 in bladder cancer is not associated with aneuploidy. *Nat Genet.* 2013; 45:1464–9. [PubMed: 24121791]
- [10]. Sjodahl G, Lauss M, Lovgren K, Chebil G, Gudjonsson S, Veerla S, et al. A molecular taxonomy for urothelial carcinoma. *Clin Cancer Res.* 2012; 18:3377–86. [PubMed: 22553347]

Take Home Message

Using next-generation sequencing, we identified genomic subsets of high-grade urothelial bladder cancer associated with favorable (*PIK3CA* mutant) and unfavorable (*CDKN2A* altered) outcomes. These findings may aid in the selection of patients most likely to benefit from novel combined modality approaches.

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Figure 1.

Most commonly altered genes in 109 high-grade urothelial carcinoma of the bladder tumors analyzed by MSK-IMPACT. The number of patients with alterations is depicted on the right. Alterations were categorized by type, as recurrent missense mutations, novel missense mutations, truncating mutations (frameshift, nonsense and splice site), and copy number amplifications or deletions. The oncoprint shows the distribution of alterations across the sequenced samples. The top bar graph illustrates the number of MSK-IMPACT gene alterations per sample.

Figure 2.

Associations with PI3K/AKT pathway alterations and clinical outcomes in 89 urothelial carcinoma of the bladder patients treated with radical cystectomy. Recurrence-free survival and cancer-specific survival stratified by (A) *PIK3CA* mutation status and (B) PI3K/AKT pathway alteration status. P values reflect log-rank test.