
Comprehensive genomic characterization of early-stage bladder cancer

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Supplementary Note: Additional Results

Mutational signatures

Apolipoprotein B mRNA Editing Catalytic Polypeptide-like (APOBEC)-related mutagenesis has been associated with both worse and improved outcomes in non-muscle-invasive bladder cancer (NMIBC)¹⁻³. Here, we observed no association between APOBEC-related mutagenesis and the UROMOL2021 classes or patient outcome when applying whole-exome sequencing (WES) data for mutational signature calling. In the previous UROMOL study, mutational signatures were inferred from RNA-seq data¹. Thus, the deviation may be caused by the inherent differences of calling mutational signatures from DNA and RNA or biological differences, such as APOBEC enzymatic activity on RNA or RNA editing processes that may potentially contribute to additional APOBEC-related biological activity in RNA. By comparative analysis of RNA- and DNA-based mutation calls (**Supplementary Table 10**), we observed a higher validation of the RNA mutation calls in an APOBEC context (T[C to T/G]A/T) compared to mutations in a CpG context or other contexts (percentage of validated mutations: 78.2% APOBEC context vs 24.5% CpG context vs 19.2% others), indicating trustworthy calling of APOBEC-related mutations from RNA.

Loss of chromosome Y

Loss of chromosome (chr) Y has recently been linked to poor outcome and immune exhaustion in bladder cancer⁴. Here, loss of chrY was observed in both diploid tumors (21%) and tumors with whole-genome doubling (WGD; 28%), but was neither associated with an increased risk of progression ($p=0.94$) nor with a higher fraction of cells expressing the T cell exhaustion marker PD-1 (assessed by immunohistochemistry; $n=149$; $p=0.51$).

Somatic structural variants in NMIBC

To explore genomic alterations further, we performed long read sequencing of DNA from two tumors with WGD and highly abnormal copy number profiles and two tumors with almost normal copy number profiles (**Extended Data Fig. 9a**). The eight samples (tumor and germline) were sequenced to a median coverage of 31x (18.9x-43.3x) with a median of 3450 base pair read length (1800-4600 base pairs). As expected, more structural variants were observed in the tumors with WGD (**Extended Data Fig. 9b-d**). Of note, the two tumors with WGD were especially enriched for small deletion-like events scattered over all chromosomes, highlighting the high instability of their genomes (**Extended Data Fig. 9c**). Moreover, one of the two tumors with WGD had a high number of structural variants involving different chromosomes (**Extended Data Fig. 9b-d**).

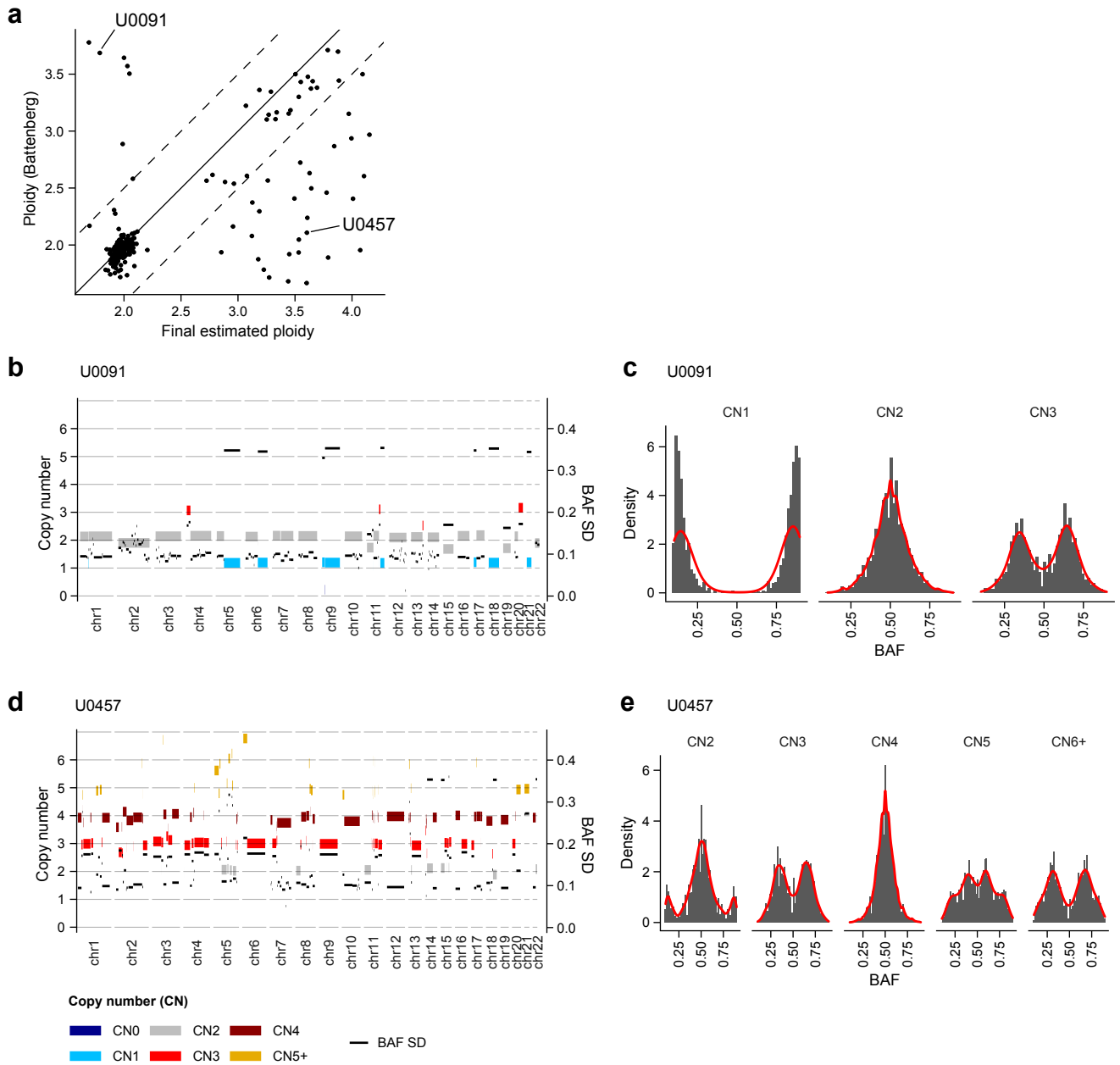
Using total RNA-sequencing data, a total of 58 fusion genes were called in the four tumors. Somatic breakpoints in the DNA could explain 22.5% (13/58) of these. Eight of them were called in U1171, a tumor with WGD, including a fusion gene between *RGS17* on chr6 and *DNAH7* on chr2 and a gene fusion between *GABPB2* and *RPRD2* on chr1 (**Extended Data Fig. 9e**).

microRNA activity in tumors with WGD

Additionally, we compared predicted microRNA (miRNA) activity across diploid tumors and tumors with WGD and found 101 miRNAs with differential activities (family-wise error rate (FWER)<0.1; **Extended Data Fig. 10a, Supplementary Table 11**). This included several oncogenic and tumor suppressive miRNAs that have previously been associated with urothelial carcinoma, such as miR-21, miR-29a, miR-203, miR-9, and miR-34c (FWER<2.4x10⁻²)^{5,6}. miR-29a has been shown to be down-regulated in progressing NMIBCs⁵, and here we found a lower median activity in tumors with WGD compared to diploid tumors (**Extended Data Fig. 10b**). The target genes (n=2,107) of miRNAs with perturbed activity between diploid tumors and tumors with WGD showed high enrichment for genes involved in regulation of metabolic and cellular processes (FWER=<2x10⁻¹⁶). Enriched gene sets also included *DNA damage response* (FWER=5.4x10⁻¹²), and *cell cycle* genes (FWER=3.3x10⁻⁹), which included *RBL2*, *E2F1* and *FOXM1* (**Supplementary Table 12**).

References

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Supplementary Figure 1. Copy number estimation

a. Correlation between the ploidy estimated by Battenberg and the final ploidy estimation in our analysis. The solid line shows a slope of 1 and dashed lines a corresponding deviation of 0.5. **b.** Inferred total copy number (CN) profile for tumor U0091. The thick coloured lines show the CN and the thin black lines show the B-allele frequency (BAF) standard deviation (SD) for corresponding copy number segments. **c.** BAF in segments with CN1, CN2 and CN3 in tumor U0091. **d.** Inferred total CN profile for tumor U0457. The thick coloured lines show the CN and the thin black lines show the BAF SD for corresponding copy number segments. **e.** BAF in segments with CN1, CN2 and CN3 in tumor U0457.