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Letter to the Editor

Non-Coding Mutations in Urothelial Bladder Cancer: Biological and Clinical Relevance and Potential Utility as Biomarkers

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Building on previous studies to provide evidence for the inclusion of non-coding elements as potential biomarkers for urothelial bladder cancer (UBC) [2–6], Jeeta et al. [1] recently identified clinically relevant non-coding mutations in five genes (*TBC1D12*, *GPR126*, *PLEKHS1*, *LEPROTL1* and *WDR74*). Clinical relevance was assessed by performing analyses of mutation frequencies across disease stages, association with clinical outcomes, distribution of mutations relative to one another, relative to mutational signatures, their effects on gene expression, and isoform usage. Substantiated by $\chi 2$ tests, logistic regression, and Cox proportional-hazards models, all of these analyses were informative. However, with regard to mutation frequencies, a more direct calculation of their cancer effect size—which accounts for underlying rates of mutation at each site and quantifies the somatic selection for each variant [7]—could strengthen their conclusions regarding sites of genes that should or should not be included in UBC detection panels.

Jeeta et al. found that two non-coding sites immediately upstream of the start codon of *TBC1D12* were mutated in 25.5% of 302 UBCs—either C$\longrightarrow$T at chr10 : 96162368 (43 tumors) or G$\longrightarrow$A at chr10 : 96162370 (41 tumors). They found that non-coding mutations were strongly associated with APOBEC signatures ($P < 0.01$), and inferred that APOBEC activity was the primary cause for early and widespread events in UBC. The frequency of these non-coding somatic variants of *TBC1D12* among 412 UBC tumor exome sequences are publicly available on the Genomic Data Commons. Our analysis of the 24 C$\longrightarrow$T mutations observed among these tumors estimated the underlying C$\longrightarrow$T mutation rate at chr10 : 96162368, providing an estimated cancer effect size of 37241.2, revealing it as the 4th most powerful driver mutation of cancer cell line growth and survival of all recurrent SNVs in TCGA-BLCA dataset: a stronger driver than any

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known SNV in UBC-associated driver genes *KRAS*, *PI3KCA,* or *FGFR3* [8, 9]. The 42 G⟶A changes at chr10 : 96162370 constituted an effect size of 7247.3 (ranked 30th). This calculated difference in cancer effect between the two mutations correlates perfectly with observed functional differences in gene expression: a C⟶T mutation at chr10 : 96162368 downregulates gene expression, but a G⟶A mutation at chr10 : 96162370 does not [4].

The similar frequencies of these mutations despite their difference in effect may seem puzzling. However, the C⟶T mutation rate at chr10 : 96162368 is 1.8×10^{-6} per cancer-competent somatic cell per oncogenesis-to-tumor-resection, whereas the G⟶A rate at chr10 : 96162370 is 1.7×10^{-5}—nearly an order of magnitude greater. Why this large difference in underlying mutation rate? G⟶A at chr10 : 96162370 occurs in a sequence context expected to be subject to mutation by APOBEC, while C⟶T at chr10 : 96162368 does not [4, 10]. Thus, APOBEC-mediated mutagenesis explains much of the high prevalence of chr10 : 96162370, but does not explain the high prevalence of chr10 : 96162368; rather, its high cancer effect size suggests that its prevalence is observed as a consequence of its major role in UBC tumorigenesis. This pattern is remarkable because it stands in some contrast to the idea that APOBEC mutagenesis is particularly prone to be the source of driver mutations in cancer. However, remarkable or not, it may not be unusual: it has been shown in head and neck squamous cell carcinoma that while APOBEC mutagenesis increases the total number of mutations occurring, on average, mutations with an APOBEC signature exhibit a lower cancer effect size [11]. Furthermore, it is not unique to non-coding mutations: it has also been demonstrated in UBC that S249C, the *FGFR3* coding mutation with the highest mutation rate and highest prevalence (due to APOBEC), does not possess the highest effect size of *FGFR3* mutations [12].

TBC1D12 provides an example of the importance of such an analysis to the interpretation of noncoding variant site data—an example in which the inferred cancer effect size is corroborated by experimental data, and for which the relationship between APOBEC-induced mutagenesis and cancer effect size has previously been explored [11]. While the purposes of variant detection panels can be diverse, quantification of the relative roles of sites as passenger or driver mutations assists in their precise and thoughtful design.

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AUTHOR CONTRIBUTIONS

JPT conceived the work; AY performed the work; all authors contributed to interpretation and analysis of data, and to writing the article.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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