Does a novel mutagenic process target *KMT2D* mutation in the most common first event on the path to bladder cancer?

Simon C. Baker\*, Andrew S. Mason & Jennifer Southgate

Jack Birch Unit of Molecular Carcinogenesis, Department of Biology and York Biomedical Research Institute, University of York, Heslington, York YO10 5DD, UK.

Contact Information for Corresponding Author\* (Address as above)

Tel: +44 1904 328706

Fax +44 1904 328704

E-mail address: [simon.baker@york.ac.uk](mailto:simon.baker@york.ac.uk)

In October, two articles in Science highlighted mutations of the *KMT2D* histone H3 lysine 4 methyltransferase gene in “normal” urothelium [1, 2].

Li *et al.* described exome sequencing of “morphologically normal urothelium” from tumour-bearing bladders and reported *KMT2D* mutation in 16 of the 133 regions (12.0%) [2]. Field effects are considered common in bladder cancer, with another recent study finding *KMT2D* to be the most common non-synonymously mutated gene (23%) in histologically normal epithelium from tumour-bearing bladders [3]. It was therefore important that these findings from tumour-bearing bladders were confirmed in normal urothelium without underlying malignant or pre-neoplastic change. Lawson *et al.* studied normal urothelium from deceased organ transplant donors (without known bladder disease) and found *KMT2D* was the gene under greatest positive selection for gain of driver mutations, with 13/15 patients (86.6%) possessing mutations and a single patient harbouring 28 unique mutations in the gene [1].

We recently reported in European Urology the first mutational signatures derived from normal human urothelium in a functionally-differentiated, mitotically-quiescent *in vitro* model [4]. Following specific exposure to benzo[a]pyrene (BaP) as an exemplar pro-carcinogen, *KMT2D* accumulated mutations in all the exposed clones, and more frequently than any other commonly-mutated driver gene, as described by The Cancer Genome Atlas [4].

Across all observed mutations, Lawson *et al*. found extensive inter-patient variation in the prevalentmutation class, Li *et al.* found T>A transversions were dominant in driver mutations (which they related to aristolochic acid exposure) and we found the genomes of BaP-exposed clones to be dominated by the expected C>A transversions. In each of these three studies [1, 2, 4] and largely against the prevailing mutational signatures, the mutations in *KMT2D* were preferentially C>T transitions (Figure 1). Analysis of the combined signature for *KMT2D* mutations across all three studies [1, 2, 4] shows they distinctively occur within an NCA trinucleotide-context (Figure 1), which does not match any existing genome-wide single-base substitution signature. These findings suggest that *KMT2D* in the urothelium is susceptible to damage under conditions of cellular stress, but that the mutations are caused by a process not directly attributable to the stressor.

The process that damages the *KMT2D* gene-body could involve APOBECs but does not reflect their normal context preferences. The mutational signature (Figure 1) appears novel and is therefore most likely confined to specific areas of the genome; however, more data is required to improve the resolution of the signature and identify the damaging process. The *KMT2D* gene is highly spliced with 55 recorded exons (ENST00000301067.12, genome-wide median/transcript=9) which likely make the gene susceptible to splicing error inducing mutations.

*KMT2D* is a recognised tumour suppressor gene in the bladder epithelium. An emerging hypothesis for how KMT2D loss functions in carcinogenesis implicates it as a coordinator of epithelial tissue homeostasis through regulation of p63 target gene expression [5]. These data combine to suggest that *KMT2D* is uniquely susceptible to mutational stress in the normal urothelium and mandates fresh evaluation of the genomic context that underpins this risk.

References

[1] Lawson ARJ, Abascal F, Coorens THH, Hooks Y, O'Neill L, Latimer C, et al. Extensive heterogeneity in somatic mutation and selection in the human bladder. Science. 2020;370:75-82.

[2] Li R, Du Y, Chen Z, Xu D, Lin T, Jin S, et al. Macroscopic somatic clonal expansion in morphologically normal human urothelium. Science. 2020;370:82-9.

[3] Strandgaard T, Nordentoft I, Lamy P, Christensen E, Thomsen MBH, Jensen JB, et al. Mutational Analysis of Field Cancerization in Bladder Cancer. Bladder Cancer. 2020;6:253-64.

[4] Baker SC, Mason AS, Southgate J. Procarcinogen Activation and Mutational Signatures Model the Initiation of Carcinogenesis in Human Urothelial Tissues In Vitro. Eur Urol. 2020;78:143-7.

[5] Lin-Shiao E, Lan Y, Coradin M, Anderson A, Donahue G, Simpson CL, et al. KMT2D regulates p63 target enhancers to coordinate epithelial homeostasis. Genes Dev. 2018;32:181-93.

Figure Legend

Figure 1 – Initial mutational signature derived from *KMT2D* gene mutations reported in three recently published studies of mutations in “normal” urothelium both in patient samples [1, 2] and *in vitro* [4]. The signature highlights that *KMT2D* mutations were preferentially C>T transitions and that they distinctively occur within an NCA trinucleotide-context unlike any signature published to-date.