Mutational signature modelling in vitro recapitulates bladder cancer initiation

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Background

Smoking is the best established risk factor for bladder cancer and direct mutagenesis by smoke carcinogens on the cellular genome can be described in clonal populations as mutational signatures. A mutational signature summarises a lifetime of DNA-damage (caused by interaction between mutagenic processes, tissue-specific gene transcription, and the DNA-repair machinery) as mutational classes in the context of their local genomic chemistry. Our aim was to establish a novel experimental model for understanding carcinogenic initiation in normal human urothelium.

Methods

Finite normal human urothelial cell cultures were established in vitro and differentiated to form functional barrier epithelia capable of activating the smoke-derived procarcinogen benzo[a]pyrene (BaP), via CYP1A1, into mutagenic metabolites. New in vitro approaches were developed allowing chronic BaP-exposure, subsequent clonal expansion of NHU cells for whole genome DNAseq and mutational signature derivation. This approach retains tissue heterogeneity during the carcinogenic exposure, modelling the early selection pressures in urothelium that lead to clonal expansion and cancer.

Results

The single-base (SBS) and double-base signatures (DBS) derived from BaP-exposed urothelial tissues were dominated by C>A and CC>AA transversions, respectively. The InDel (ID) signature described cytosine deletions in homopolymer regions. Comparison to the Catalogue of Somatic Mutations in Cancer (COSMIC) using the Signal pipeline showed homology with SBS4, DBS2 and ID3, respectively; which have been detected by others in bladder cancer patients. Mutation enrichment in specific genes (eg *KMT2D* and *CDKN1A*) mirrored in situ observations by others; effectively modelling the competitive advantage mutations convey in the carcinogenic tissue environment.

Conclusions

BaP is detected in urine, metabolically activated by urothelial CYP1A1, and therefore may be a bladder mutagen. However, C>A transversions contribute a minor fraction of all mutations recorded in bladder tumours. Widespread DNA damage caused by chronic BaP exposure did not trigger any of the APOBEC-like mutational processes (SBS2/13) that dominate bladder tumour genomes, indicating a missing factor that the new model system is well positioned to identify.