MUTATION

Clone conflict

It is now widely recognized that during ageing, normal tissues become colonized by clones, which have acquired somatic mutations in common cancer driver genes. Surprisingly, many of these mutated genes confer a strong positive selective advantage, and the progressive accumulation of such mutations throughout life can eventually lead to cancer. However, our knowledge of the in vivo dynamics and evolution of these clones over time as well as the mechanisms underlying the selection of the mutant genes remains limited. To investigate these principles, Colom et al. took advantage of the oesophageal epithelium of mutagen-treated mice as a model system to mimic the mutational landscape of ageing human oesophageal epithelium.

> “the fate of mutagen-exposed cells is governed by the genotype of their neighbours”

To generate a patchwork of mutant clones, mice were orally given diethylnitrosamine (DEN), a mutagen found in tobacco smoke, for two months before characterizing the mutational burden that had evolved in the oesophageal epithelium a year later. To detect clonal expansions, 2 mm^2 biopsy samples were collected from the oesophageal epithelium of control (untreated) and DEN-treated mice on which ultradeep targeted exome sequencing was performed. This revealed a high mutational burden of approximately 24 mutations per megabase in DEN-exposed epithelium compared with approximately 0.3 in control epithelium.

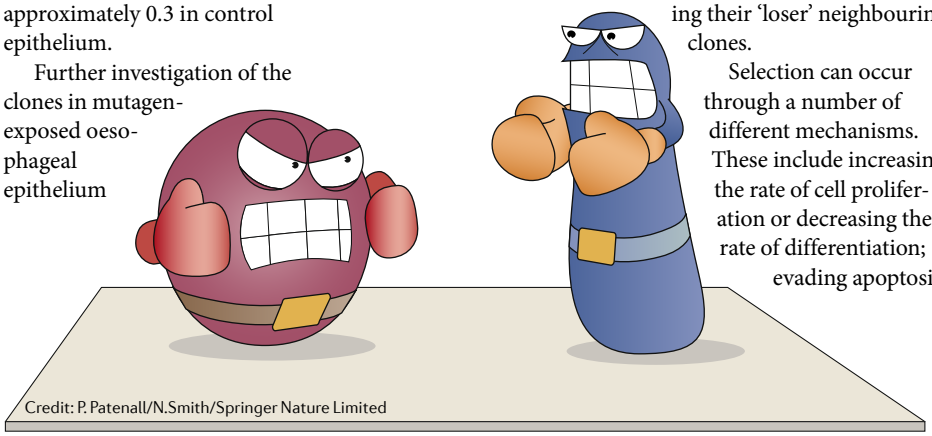
Further investigation of the clones in mutagen-exposed oesophageal epithelium identified eight mutant genes that were strongly positively selected — *Notch1*, *Notch2*, *Trp53*, *Cul3*, *Arid1a*, *Kdm6a*, *Adam10* and *Ripk4*. Sequencing of aged normal human oesophagus demonstrated that of these eight mutant genes, the first five are also positively selected in humans, validating the model system and suggesting that the evolution of mutant genes is convergent in DEN-treated mice and ageing humans. The predominant mutated gene in mutagen-treated mice was *Notch1*, with 1,601 codon-altering mutations, and the clones bearing these *Notch1* mutations occupied over 80% of the mutagenized epithelium. By contrast, only a small number of coding mutations were found in control mice, and the eight positively selected genes colonized only 1.6–3.2% of the normal epithelium consistent with random genetic drift of mutant genes that are selectively neutral.

The genetic selection observed in oesophageal epithelium from DEN-treated mice suggested that the clonal behaviour might change over time. To assess this, the authors carried out lineage tracing in transgenic mice whereby individual progenitor cells were randomly labelled with heritable yellow fluorescent protein (YFP). 3D confocal imaging showed that the number of labelled clones was reduced over time in control oesophageal epithelium — an expected result for tissues exhibiting neutral competition as clones are lost through differentiation. Dissimilarly, the removal of clones in DEN-exposed oesophageal epithelium was far higher than under control conditions consistent with ‘winner’ mutant clones outcompeting their ‘loser’ neighbouring clones.

Selection can occur through a number of different mechanisms. These include increasing the rate of cell proliferation or decreasing the rate of differentiation; evading apoptosis; or colonizing nearby areas through cell competition with neighbouring wild-type cells. The first two possibilities could be excluded by further lineage tracing experiments — the rate of cell division was unchanged between control and mutagen-treated mice, and cell death was minimal in DEN-treated epithelium. Testing the third possibility through whole-genome sequencing of individual YFP-labelled, microdissected clones at 9 or 18 months after mutagen treatment showed that there was no correlation between clonal size and the presence of specific driver mutations. This contradicted the assumption that the rate of clonal growth was determined by the fitness of the mutation it bears. Last, the authors ruled out clonal copy number alterations as an explanation for the clonal expansion seen in DEN-treated epithelium.

To account for these observations, Colom et al. surmised that the fate of mutagen-exposed cells is governed by the genotype of their neighbours whereby following clonal expansion, mutant clones with similar competitive fitness end up colliding with each other in a confined space. This in turn results in the fate of mutant cells returning to homeostasis. The authors stimulated this prediction with a mathematical ‘neighbour-constrained fitness’ model and then subsequently tested their model by artificially introducing a dominant negative mutant of MAML1 (DN-MAML1), which inhibits Notch signalling, in single progenitors on a background of DEN-treated epithelium. In this setting, the growth of DN-MAML1 clones was limited, implying that these clones collided with others bearing mutations of an equal fitness, for example *Notch1* mutations, causing them to return to neutrality.

The hope is that interrogating clonal dynamics in normal tissues, like in this study, will eventually inform our understanding of cancer development.



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ORIGINAL ARTICLE Colom, B. et al. Spatial competition shapes the dynamic mutational landscape of normal esophageal epithelium. *Nat. Genet.* https://doi.org/10.1038/s41588-020-0624-3 (2020)