Current Biology Dispatch

Cell competition: Clonal competition protects against early tumorigenesis

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Summary

Aging tissues accumulate somatic mutations, yet cancer occurrence is relatively rare. A new study provides compelling evidence for why this may be the case and discovers that competition between mutant clones in oesophageal tissues protects against early tumorigenesis.

Main text

Throughout life, cells compete for space and survival in tissues. Cell competition is an evolutionarily conserved process - first described in *Drosophila melanogaster*, it has important functional roles in shaping developing and adult mammalian tissues[1, 2]. Competition eliminates 'loser' cells (e.g., via apoptosis, segregation, extrusion, or loss of stemness) by 'winner' neighbouring cells, which expand to populate the tissue[3]. Research demonstrates that cells carrying cancer-causing mutations are often identified as 'losers' and are outcompeted by normal healthy cells[4-7], preventing early tumorigenesis[4, 8, 9]. In squamous epithelia, which are replenished by progenitor cells located in the basal layers, some genetic mutations confer a 'super-competitor' advantage by increasing the likelihood of generating progenitor cells[10]. Squamous epithelial tissues are devoid of involutions or crypts, which would otherwise limit the expansion of mutant progenitors. Ultimately, expansion of mutant 'super-competitors' leads to the creation of tissues mosaic for mutant clones. Lineage tracing experiments in oesophageal tissues, have revealed that clones of mutant cells continue to compete and expand until they replace the entire epithelium without affecting tissue function[11, 12], suggesting tissue homeostasis is maintained despite the high mutational burden. However, whether tissues harbouring mutant clones are also competitive environments remains unclear. If so, is it plausible to consider that somatic mutations may protect tissues against tumorigenesis? New research by B. Colom et al., and recently published in *Nature*[13] uncovers an unexpected anti-tumorigenic role of clonal competition in histologically normal tissues.

In this new study, the authors used the DEN-mutagenesis model in the mouse oesophagus (DEN: diethylnitrosamine is a mutagen found in tobacco smoke). When administered to wild-type mice, DEN generates an epithelium mosaic for mutant clones with high mutational burdens[12, 13]. Surprisingly, exposure to DEN does not alter tissue structure, which largely remains phenotypically normal, apart from minor cell crowding in the basal layer. DEN treatment also leads to the formation of distinct premalignant tumours, which are positive for keratin stress proteins (KRT6 or KRT17) and visible along the entire epithelium. Using this model, the authors observed hundreds of microscopic tumours, detected as early as 10 days post exposure to DEN, with many (~30%) already showing features of angiogenesis. Remarkably, by scoring the density and size of tumours in entire tissues over time, the authors

found that most micro-tumours are eliminated from tissues soon after they emerge. The discrete lesions that persist, develop with increased malignancy over time (Figure 1A).

Is competition shaping the tumour mutational landscape? To address this question, the authors isolated tumours from DEN-treated tissues at 10-days and 12-months post exposure and compared the mutational signatures. Consistent with their previous study[12], they found that DEN-induced mutagenesis resulted in protein altering mutations, with missense single nucleotide variants (SNVs) being the most common. Interestingly, analysis of the maximum variant allele frequency (VAF) showed that 10-day tumours were polyclonal, whereas 12-month tumours were monoclonal, suggesting a hypothesis whereby clonal competition would drive selection of specific mutant clones. To determine whether specific mutations persist in surviving tumours, the authors calculated the ratio of non-synonymous (dN) to synonymous (dS) mutations (dN/dS) across each sequenced gene. Values of >1.0 would indicate positive selection of a mutated gene, which confers a competitive advantage. Using this method, they showed that mutations in *Notch1* and *TP53* were selected in tumours at 10-days, whereas mutations in *Atp2a2*, *Notch1*, *Notch2*, *Chuk* and *Adam10* were selected in 12-month tumours. Whole exome sequencing of 12-month tumours did not detect additional gene mutations; mutations in *Atp2a2* and *Notch1* were predominant in persisting tumours. Combined with whole genome sequencing data, their analysis also suggests that chromosomal alterations and genome instability do not contribute to DEN-mediated tumorigenesis in vivo.

How are micro-tumours cleared from tissues at early time points? Taking several approaches, Colom et al., comprehensively showed that tumour cells are not dying by apoptosis or by decreasing cell division rates. Moreover, DEN-induced tumours form, and are lost to the same extent in immuno-deficient mice as wild-type controls, suggesting that the immune system is not required for tumour initiation or tumour loss. In a previous study[12], the authors elegantly showed that clonal competition shapes the oesophageal epithelium, with specific mutations being positively selected over time. Within the basal layer, mutant progenitor cells compete for space and survival whereby only cells carrying 'winner' mutations expand laterally in the tissue, outcompeting the less fit 'loser' cells (Figure 1B). Collision between clones of expanding mutant cells, which carry other mutations but of similar 'fitness', will halt expansion of mutant cells, eventually leading to equilibrium and homeostasis. Thus, the expansion of mutant clones is dependent on the local neighbourhood. Based on this knowledge, the authors speculated that competition may underpin early elimination of 'loser' micro-tumours; only tumours carrying additional 'winner' mutations would persist and expand. This hypothesis assumes that the surrounding epithelium is composed of mutant clones. Indeed, ultradeep targeted sequencing of normal epithelium surrounding 10-day tumours identified hundreds of small mutant clones per mm^2 of histologically normal epithelium. dN/dS ratios identified positive selection of mutations in *Notch1*, *Trp53* and *Fat1*, with *Notch1* mutations being the most common. Remarkably, the mutational pattern of the normal mouse epithelium resembled the mutational landscape of aging human oesophagus. At 10-day time points, *Notch1* mutations were predominant in normal tissues, whereas *Atp2a2* mutations were enriched in tumours, indicating 'winner' clones emerge very early in tumorigenesis. Importantly, the data suggest that loss of functional Notch in the normal epithelium protects against early tumorigenesis following exposure to DEN.

To experimentally test the requirement for Notch signalling, the authors used the genetically engineered AhCre^{ERT} R26$^{DNM\text{-}GFP/wt}$ mouse, which carries an inducible highly competitive dominant negative allele of *Maml-1* (DN-MAML1 and fused to GFP) and inhibits Notch signalling[11]. DN-MAML1 expression was induced in mice 10 days after DEN exposure and the number of tumours/mm^2 was scored. Tumour clearance was significantly increased in regions of the tissue where Notch signalling was inhibited. Moreover, 3D confocal imaging of GFP-tagged DN-MAML1 cells surrounding tumours added information on a possible mechanism underlying tumour elimination. Here, KRT6-labelled tumours were often encircled or enclosed by GFP-labelled neighbouring cells, suggesting tumours are eliminated by expanding Notch inhibited cells. These phenotypes were also observed in DEN-treated tissues labelled with Confetti markers (using Confetti mouse). Together the data show that outcompeted tumour cells are displaced and shed from tissues by expanding mutant clones (Figure 1C). Finally, the authors addressed whether clonal competition is required for tumour elimination. DEN-treated mice were treated with dibenzazepine (DBZ) an inhibitor of Notch signalling, thereby increasing relative fitness of all cells, and effectively removing competition between emerging tumours and the surrounding epithelium. As predicted, DBZ treatment significantly decreased tumour loss, leading to higher tumour survival. Together with mathematical modelling predictions, the data elegantly show that expanding mutant clones outcompete newly formed tumours in DEN-exposed tissues. Competition is tumour protective, suggesting persisting tumours carry genetic mutations that give cells a competitive advantage over their neighbours.

Large-scale genome sequencing studies of human tissues reveal that as we age, many epithelial tissues (e.g., skin, colon, bladder, oesophagus, endometrium) accumulate somatic mutations, without leading to cancer[14]. Interestingly, *NOTCH* mutations are positively selected in human oesophagus, skin, and bladder[14], with *NOTCH1* mutations being the most frequently occurring mutation in human oesophagus[14, 15]. Whether clonal competition protects against tumorigenesis in human tissues remains to be elucidated. Nonetheless, results from this study by Colom et al., challenge our current understanding of early tumorigenesis and may explain why cancer incidence is rare, despite the high frequency at which tissues accumulate harmful genetic mutations. The molecular mechanisms underlying clonal competition are less clear; however, local cell-cell interactions (and Notch signalling) may play an important role.

In conclusion, this ground-breaking study demonstrates that emergence and development of a tumour not only depends on the tumour genotype but also on the mutational signature of the surrounding neighbouring cells. Cell competition is a complex and context-dependent process, controlled by multiple mechanisms. While competitive interactions are triggered by changes in relative 'fitness' between neighbouring cells, external factors also impact on competition outcomes. For example, proinflammatory tissue microenvironments abolish competition and subsequent elimination of mutant cells, whereas anti-inflammatory treatments restore competition in favour of normal healthy cells[16-18]. A better understanding of tissue homeostasis in aging and how competition favours tumour cells may lead to new methods to detect and treat cancer early.

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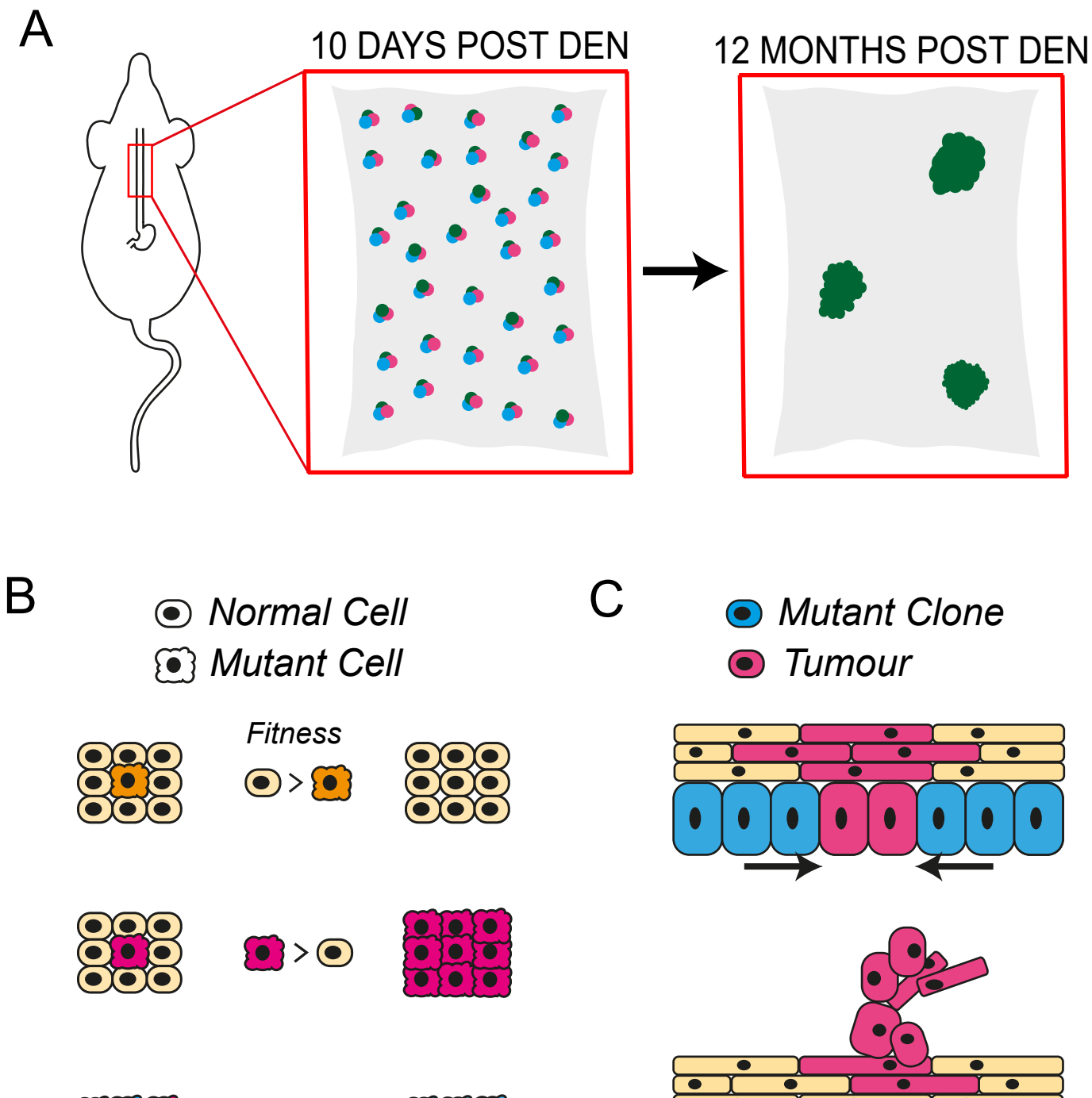
A
10 DAYS POST DEN
12 MONTHS POST DEN
B
Normal Cell
Mutant Cell
Fitness
C
Mutant Clone
Tumour

Figure 1: In a Diethylnitrosamine (DEN) mutagenesis mouse model, mutant clones outcompete and eliminate emerging tumours from the oesophageal epithelium. (**A**) 10-days post exposure to DEN generates an epithelium mosaic for mutant clones and polyclonal microscopic tumours. By 12 months post exposure, micro-tumours are lost, and monoclonal tumours persist in the tissue. (**B**) Cell competition monitors relative 'fitness' between neighbouring cells. Genetically mutated cells (orange) are often eliminated as 'loser' cells by wild-type normal cells. Some genetic mutations confer a competitive advantage (pink) over wild-type cells. Less fit mutant cells (pink) are outcompeted in a neighbourhood of 'winner' mutant cells (blue). (**C**) Micro-tumours (pink) are displaced by expanding mutant clones (blue) and eliminated from the epithelium. Black arrows denote cell expansion.