Tumour biology

Cancer cells build a bad neighbourhood in the gut

Shi Biao Chia & James DeGregori

Malignant stem cells in the gut secrete factors that promote the differentiation of neighbouring stem cells, thereby aiding the replacement of normal stem cells by those with cancer-promoting mutations. **See p.430, p.436 & p.442**

Decades of research have revealed how mutations contribute to the evolution of malignant cells and to the ultimate characteristics of a given tumour. There is growing recognition that the surrounding tissue environment affects the natural selection of these mutation-driven characteristics. Less appreciated, however, have been the effects of interactions between malignant cells and their neighbouring wild-type cells – and how, through these interactions, malignant cells shape the surrounding environment to their advantage. Writing in *Nature*, Yum *et al.*[1], Van Neerven *et al.*[2] and Flanagan *et al.*[3] provide crucial insights into the competitive dynamics of cancer cells and their neighbouring cells in the intestine.

To study interactions between cells with cancer-promoting mutations and neighbouring cells in their native environment, Yum *et al.*[1] (page 442) developed a microscopy-based approach that uses a multicolour system to monitor cellular lineages (clones) in mice. This enabled the authors to track intestinal stem cells that express cancer-associated mutations in two key genes, *Kras* and *Pik3ca*, and also to assess their wild-type neighbouring cells. The authors report that the presence of intestinal stem cells harbouring these mutated genes increased the rate of differentiation of the surrounding wild-type cells. This outcome was driven by the mutant stem cells secreting specific factors – molecules that activate the BMP signalling pathway, and others that inhibit the WNT signalling pathway (Fig. 1).

Moreover, Yum *et al.* found that structural cells (termed stromal cells) that surround stem cells – and that normally promote stem-cell maintenance – instead enhanced their secretion of pro-differentiation factors if mutant stem cells were present. This results in a less-supportive environment for the maintenance of intestinal stem cells. These secreted factors promote differentiation regardless of whether the stem cells have cancer-promoting mutations. Crucially, the cells with mutations are less affected than are those without, which gives the mutant stem cells a competitive edge. The intestinal regions (units called crypts) adjacent to the clones of cells produced through mutation showed a rise in cellular turnover, further promoting the establishment of the clones of mutant cells.

For a deep dive into mechanisms underlying the interactions between normal and malignant stem cells, Van Neerven *et al.*[2] (page 436) and Flanagan *et al.*[3] (page 430) used mouse and human models of intestinal cancer mediated by inactivation of the *Apc* gene. Mutation of *Apc* initiates most colon cancers[4,5], and APC protein is a key negative regulator of the WNT signalling pathway. APC mediates the destruction of the protein β-catenin, a gene-expression regulator that helps to maintain the intestinal stem-cell state.

Van Neerven *et al.* and Flanagan *et al.* found that several genes associated with inhibition of the WNT pathway, particularly the gene *Notum*, showed higher expression in cells with an *Apc* mutation than in wild-type cells. The authors of these two papers used an *in vitro* system to culture cells together; in this system, individual stem cells developed into miniature intestine-like 'organoid' structures, providing a measure of stem-cell potential. Wild-type stem cells grown with cells that had mutant *Apc*, or exposed to liquid taken from the medium in which such cells had been grown, had a slower rate of organoid formation and growth than did wild-type stem cells that were exposed to other wild-type stem cells or their media. This result supports the hypothesis that cells with an *Apc* mutation adversely affect the potential of wild-type stem cells through the secretion of a diffusible factor. NOTUM protein, a secreted inhibitor of WNT signalling, was found by Van Neerven *et al.* and Flanagan *et al.* to be crucial for this inhibition of stem-cell potential.

In the fruit fly *Drosophila melanogaster*, cells with an *Apc* mutation can outcompete their surrounding wild-type cells by inducing the death of those cells[6]. By contrast, none of the three papers highlighted here reported an increase in cell death in wild-type cells adjacent to cells with *Apc*, *Kras* or *Pik3ca* mutations.

NOTUM-mediated inhibition of the WNT pathway was observed only in the wild-type cells examined by Van Neerven, Flanagan and their respective colleagues, not in the mutant cells. This is because the *Apc* mutation

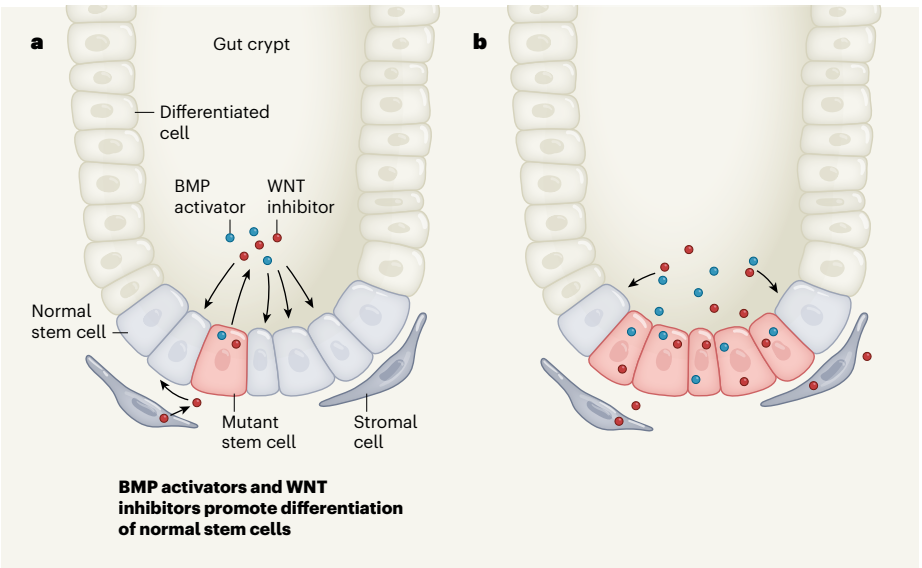

Figure 1 | Cellular competition in the mammalian gut that promotes tumour formation. Yum *et al.*[1], Van Neerven *et al.*[2] and Flanagan *et al.*[3] present evidence that reveals how stem cells in the gut that carry cancer-promoting mutations can affect neighbouring normal stem cells in crypt structures. **a**, Yum *et al.* report that mutant stem cells secrete molecules that activate the BMP signalling pathway and inhibit the WNT signalling pathway. These molecules promote stem-cell differentiation and predominantly affect normal stem cells. Van Neerven *et al.*[2] and Flanagan *et al.*[3] also report such secretion of WNT inhibitors. Moreover, Yum *et al.* found that mutant stem cells drive cells called stromal cells in their vicinity to secrete WNT inhibitors. **b**, After the normal stem cells differentiate, the mutant stem cells dominate the stem-cell pool.

activates the WNT pathway downstream of the site of NOTUM action[7] – NOTUM secretion by cells with an *Apc* mutation does not affect the mutant cells themselves, but has a negative effect on wild-type cells. Thus, cells with *Apc* mutations not only outcompete their neighbours by driving them to differentiate, but they are also shielded from NOTUM-mediated adverse effects. Activating the WNT pathway using molecules such as lithium chloride (as done by Van Neerven and colleagues) or a NOTUM inhibitor (used by Flanagan *et al.*), thus levelling the playing field between wild-type intestinal cells and those with mutant *Apc*, reduced cancer formation in the mouse intestine mediated by mutant *Apc*; this suggests a possible approach in developing innovative anticancer therapies.

Together, these three studies reveal how malignant intestinal stem cells can win competitive battles in the gut by promoting neighbouring stem cells to differentiate into specialized and less-proliferative cell types (Fig. 1). Other studies using models for the formation of leukaemia have similarly shown that malignant cells secrete factors, such as pro-inflammatory cytokine molecules, that impair the fitness of competing normal cells and boost the fitness of the malignant cells[8,9].

Although cell competition is a process that is clearly involved in the progression of malignancy, it also serves as a quality-control mechanism for maintaining tissue health. For example, during embryonic development, defective cells are eliminated to ensure healthy growth through a process that involves factors secreted from normal cells[10]; this phenomenon has echoes of the mechanism described in these three studies.

There are numerous other examples of cellular competition processes affecting health and disease. One study[11] reported that the expression of the protein COL17A1 regulates basal (stem) cell divisions in the skin. Damage to a cell results in the downregulation of COL17A1 expression, which drives the differentiation of these stem cells into mature cells of the outer skin layer. This results in healthy basal cells replacing damaged ones. Moreover, normal cells can 'evict' mutant cells; for example, normal epithelial cells force the extrusion of cells with a cancer-promoting mutation from the skin layer, thereby preventing cancer initiation[12]. Beyond tumour-suppressive roles, the efficient recognition and elimination of 'loser' cells during cell competition is also crucial for the longevity of *D. melanogaster*[13].

The incidence of colon cancer rises exponentially in old age, and the expression of NOTUM increases with ageing, too[14]. This raises the question of whether the processes reported in these studies might be some of the ways in which ageing creates an environment that promotes cancer initiation. Numerous studies over the past decade have described how cells with cancer-promoting mutations become increasingly abundant in our tissues as we age[15]. Given that the papers by Yum *et al.*, Van Neerven *et al.* and Flanagan *et al.* indicate that such cells can impair the maintenance of neighbouring stem cells, such pre-malignant clones could hypothetically contribute to both tissue ageing (by reducing tissue maintenance) and ageing-associated cancers (through selection for cancer-promoting mutations that might resist, and perhaps even reinforce, pro-differentiation forces).

Current developments in cancer therapeutics often focus on exploiting our own natural systems of defence against the disease. The focus so far has been on the promotion of defences mediated by the immune system. However, we are now gaining an appreciation of how stem cells and aspects of the tissue environment are important for tumour progression. Hence, interventions might be developed that promote stem cells and tissue contexts that are unfavourable to the evolution of malignancy. Given the ability of healthy tissues to eliminate malfunctional or malignant cells[12], these three studies should further encourage the development of therapeutic strategies in which cancer is halted by counteracting the pro-differentiative influences of malignant cells, thus boosting the fitness of the competing normal cells.

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1. Yum, M. K. *et al. Nature* **594**, 442–447 (2021).
2. van Neerven, S. M. *et al. Nature* **594**, 436–441 (2021).
3. Flanagan, D. J. *et al. Nature* **594**, 430–435 (2021).
4. Kinzler, K. W. & Vogelstein, B. *Cell* **87**, 159–170 (1996).
5. Kwong, L. N. & Dove, W. F. *Adv. Exp. Med. Biol.* **656**, 85–106 (2009).
6. Suijkerbuijk, S. J. E., Kolahgar, G., Kucinski, I. & Piddini, E. *Curr. Biol.* **26**, 428–438 (2016).
7. Galluzzi, L., Spranger, S., Fuchs, E. & López-Soto, A. *Trends Cell Biol.* **29**, 44–65 (2019).
8. Welner, R. S. *et al. Cancer Cell* **27**, 671–681 (2015).
9. Reynaud, D. *et al. Cancer Cell* **20**, 661–673 (2011).
10. Sancho, M. *et al. Dev. Cell* **26**, 19–30 (2013).
11. Liu, N. *et al. Nature* **568**, 344–350 (2019).
12. Tanimura, N. & Fujita, Y. *Semin. Cancer Biol.* **63**, 44–48 (2020).
13. Merino, M. M. *et al. Cell* **160**, 461–476 (2015).
14. Pentinmikko, N. *et al. Nature* **571**, 398–402 (2019).
15. Kakiuchi, N. & Ogawa, S. *Nature Rev. Cancer* **21**, 239–256 (2021).

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Neuroscience

Attraction and repulsion in brain-circuit wiring

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Examination of the molecular interactions that govern the assembly of neural circuits in a brain region called the hippocampus reveals that neuronal projections are guided to their targets by both attractive and repulsive cues.

Our ability to sense and navigate the world requires the precise assembly and function of neural circuits in the brain. During development, neuronal-cell projections called axons are guided by molecular cues to extend away from non-target regions of the brain and towards their target regions[1], where axons make synaptic connections with partner neurons. Over the past few decades, several candidate molecular cues have been identified[2]; however, questions remain as to whether distinct sets of cell-surface molecules mediate attraction to targets and avoidance of non-target regions. Writing in *Science*, Pederick *et al.*[3] show in mice that axon attraction and repulsion are guided by the same cell-surface molecule during circuit assembly in the hippocampus, a brain region involved in spatial memory and navigation[4].

The hippocampus contains the subfields CA1, CA2 and CA3, and neurons in the CA1 subfield project to a target region in an adjacent brain structure called the subiculum. CA1 projections to the subiculum are organized along a medial-to-lateral anatomical axis. In this way, in the medial part of the network, neurons in the proximal CA1 (located near the border with the CA2 region) project to the distal subiculum (the part farthest from the CA1 border), whereas, in the lateral part of the network, distal CA1 neurons project to the proximal subiculum (Fig. 1).

A previous study by Pederick and colleagues' laboratory showed[5] that a cell-surface molecule called teneurin-3 (Ten3) is expressed both by proximal CA1 neurons and by neurons in their target region, the distal subiculum. The study showed that molecules of Ten-3