temporal lobe. The results also prompt many further lines of investigation. Why do humans have a limited working-memory capacity, and why do certain individuals have a better working memory than others do? How might different forms of information, such as names, faces and pictures, be optimally stored? Insights into how the frontal lobe exerts control over working memory could also indicate how memory failure originates, and could make way for new avenues of research aimed at developing treatments for conditions such as Alzheimer's disease, in which working memory is often affected[8,9].

This understanding of working memory could open up many exciting prospects in the field of human neurophysiology. These might include the development of closed-loop neural prosthetics (self-modulating devices designed to replace a lost neurological function) that could modulate or enhance working memory. For example, it would be interesting to explore whether theta–gamma phase–amplitude coupling can be enhanced using precisely aligned deep-brain stimulations, or whether working memory can be improved in individuals with neurodegenerative disorders or conditions associated with traumatic brain injury.

Future research could also examine whether it is possible to limit the effects of distractors, or enhance working-memory capacity, through biofeedback — a therapeutic technique in which sensors are used to enable a person to monitor physiological changes, including patterns of neurological activity, in their own body. Daume and colleagues' study provides fertile ground on which to begin investigating these possibilities — including how to remember the name of that person you just met.

Ziv Williams is in the Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, USA.
e-mail: zwilliams@mgh.harvard.edu

1. Daume, J. *et al. Nature* **629**, 393–401 (2024).
2. Kornblith, S., Quian Quiroga, R., Koch, C., Fried, I. & Mormann, F. *Curr. Biol.* **27**, 1026–1032 (2017).
3. Jeneson, A. & Squire, L. R. *Learn. Mem.* **19**, 15–25 (2012).
4. Kamiński, J. *et al. Nature Neurosci.* **20**, 590–601 (2017).
5. D'Esposito, M. & Postle, B. R. *Annu. Rev. Psychol.* **66**, 115–142 (2015).
6. Lara, A. H. & Wallis, J. D. *Nature Neurosci.* **17**, 876–883 (2014).
7. Daume, J., Gruber, T., Engel, A. K. & Friese, U. *J. Neurosci.* **37**, 313–322 (2017).
8. Baddeley, A. D., Bressi, S., Della Sala, S., Logie, R. & Spinnler, H. *Brain* **114**, 2521–2542 (1991).
9. Huntley, J. D. & Howard, R. J. *Int. J. Geriatr. Psychiatry* **25**, 121–132 (2010).

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Tumour biology

'Mini-colons' shed light on cancer progression

Nicolò Riggi & Felipe de Sousa e Melo

Cells grown on a 3D scaffold have generated a 'mini-colon' that mimics key features of the organ. Controlled expression of cancer-associated genes in the system offers a way to examine tumour formation over space and time. **See p.450**

Improvements in the technologies and models available to investigate and monitor tumour growth should lead to biological insights and might pave the way for the development of more-effective clinical treatments. On page 450, Lorenzo-Martín *et al.*[1] present a system for examining colon cancer.

Tumours progress in a stepwise process, often transitioning through a series of stages defined by visible (histological) and molecularly hallmarks that are accompanied by the accumulation of genetic changes and modifications of DNA and its associated proteins (termed epigenetic alterations)[2]. Although the molecular basis of tumour formation has been studied extensively and is quite well understood, the precise cellular-adaptation mechanisms associated with the transitions

"This system provides notable advances in the opportunities available for studying how tumours develop."

between tumour stages remain elusive. For example, how do newly arising tumour cells adapt to having cancer-causing mutations in a seemingly normal tissue? How are the tumour and its surrounding microenvironment remodelled over the course of tumour progression? How are communications between tumour cells and normal cells rewired to provide a suitable home (spatial niche) in which the tumour can survive?

A key barrier to understanding these crucial steps is often attributed to the paucity of tumour models that recapitulate the complex spatiotemporal trajectories of human cancers. Over the past few decades, many model systems have been developed, each offering unique insights into cancer biology. These models range from conventional 2D cell lines grown *in vitro* in Petri dishes to sophisticated *in vivo* animal models and patient-derived 3D 'mini-tumours' (groups of cells called tumour organoids)[3].

Each of these systems has inherent limitations. For instance, *in vitro* models often lack the cellular diversity (heterogeneity), cellular adaptability (plasticity) and spatial organization found in *in vivo* models, whereas *in vivo* models provide limited opportunities for experimentally controlled perturbations, preclude highly detailed exploration of transition stages and are often expensive. Gaining a concise and comprehensive understanding of the various steps of tumour formation will therefore require model systems capable of offering the high throughput and practicality of *in vitro* models together with the cellular heterogeneity and spatial organization of *in vivo* systems.

Lorenzo-Martín and colleagues present a strategy for solving this conundrum. The authors have developed a state-of-the-art biological system that harnesses organoids and the spatiotemporal controlled activation of cancer-associated genetic alterations, together with a bioprinting engineering approach, to collectively recapitulate the crucial steps of tumour initiation and growth in a system that closely mimics what happens in real organs.

As a first step, the authors used a previously developed bioengineered scaffold[4] that provides a framework for colon cells that are being used to form a 3D organoid, so that the cells can grow into an organized pattern, called a mini-colon (Fig. 1). This system closely mirrors the architecture of the colon. Although it uses only epithelial cells, which line the colon, the approach enables the cells to self-assemble into key structures of the organ — a rudimentary version of a gland called a crypt and the internal lining of the lumen (Fig. 1).

To investigate the effect of frequently occurring mutations associated with colorectal cancer, the authors built such mini-colons out of mouse intestinal organoids engineered to express a light-sensitive drug-inducible version of an enzyme that can be used to selectively manipulate gene expression through

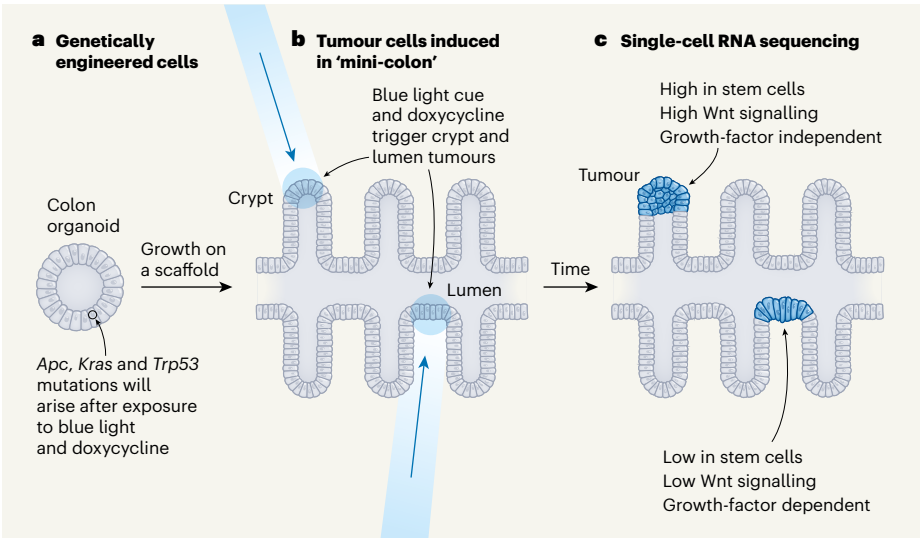

Figure 1 | A system for investigating colon tumours. Lorenzo-Martín *et al.*[1] present an approach for examining the formation and progression of colon tumours. **a**, Mouse colon cells were engineered so that they would express three mutated genes (*Apc*, *Kras* and *Trp53*) associated with colon cancer after application of the drug doxycycline and exposure to blue light. They were grown as a group of cells called an organoid. **b**, Growing this organoid on a scaffold provided a way to generate a 'mini-colon' with features characteristic of this organ, such as regions called the crypt and the lumen. The authors initiated the formation of tumours in regions of interest. **c**, Tumours arising in different regions of the mini-colon had differing characteristics, as revealed by single-cell RNA sequencing, in terms of the prevalence of stem cells, the level of signalling mediated by the protein Wnt, and the need for proteins called growth factors to drive tumour growth.

an approach called optogenetics. They then exposed the mini-colons to the drug doxycycline and the light cue needed, thus enabling temporally and spatially controlled expression of the colorectal-cancer-associated[5] mutated genes *Apc*, *Kras* and *Trp53*. This method provides a precise and powerful tool with which to track tumour initiation and progression over time.

The initial characterization of the tumours revealed some of the expected hallmarks of the formation of a colon tumour, including loss of differentiation and a rise in cellular proliferation. Yet, surprisingly, cell death emerged as the primary response of the tissue after cancer arises. This highlights the intense pressure for the tumour to select for tumour cells that can survive stress and avoid cell death, and which would then be endowed with better potential to grow. Importantly, tumours arising from the 'mini-colon' were molecularly more closely related to tumours initiated *in vivo* than were those of more-standard 3D organoids, confirming the biological relevance of the system developed by Lorenzo-Martín and colleagues.

The authors examined the effect of engineering the expression of mutations in precisely defined regions of the colon. This strategy unveiled distinct location-dependent trajectories of tumour growth. Tumours originating from the crypt were dense and had populations of stem cells and immature cells, whereas tumours with swollen (cystic) architectures were associated with the lumen. This result recapitulates similar observations in the mouse intestine, corroborating the relevance of the model for studying tumour-initiation events as well as for examining the cell of origin of this cancer[6]. Together, these findings strongly support the idea that identical genetic alterations can generate distinct types of malignancy depending on the specific cellular context, and might therefore participate in the emergence of the tumour heterogeneity (from person to person) that is observed in colorectal cancer.

This system provides notable advances in the opportunities available for studying how tumours develop into complex and heterogeneous subtypes. Moreover, given the high versatility of this technique, it should provide insights into tumour biology across multiple settings. For instance, the observation that certain kinds of tumour derived from mini-colon cultures, in contrast to those derived from organoids, continue to depend on growth-promoting (mitogenic) signals, suggests that other cancer vulnerabilities could be revealed through this innovative approach.

In addition, the modularity of the mini-colon system will undoubtedly accommodate the incorporation of other types of cell, including stromal cells and immune cells, to further recapitulate interactions between the tumour and its neighbouring cells. This, coupled with the fact that the 'mini-colon' is amenable to a variety of analytical approaches, such as single-cell analysis of gene expression, could prove fundamental in providing a way to model the initiation and progression of human tumours.

The formation of colon cancer is widely thought to follow the pattern of a stepwise accumulation of mutations, each of which contributes to selection of tumour cells (clones) and tissue remodelling. Although the work of Lorenzo-Martín and colleagues provides a big step forward, a limitation of this study was the requirement for simultaneous introduction of all the mutations, which precluded the investigation of the sequential effect of individual mutations on tissue remodelling. Future studies should also try to ensure that the longevity of the 'mini-colon' can be extended to explore the role of mutations that come to prominence after a longer time span, and it would also be worth examining normal and malignant human organoids, which have distinct and much longer self-renewal dynamics for the organoid tissue than do their mouse counterparts[7,8].

We anticipate that these engineered complex models will be adopted by scientists when they become more widely available. Although animal models will remain crucial for basic research and drug development, in particular for modelling the complex pharmacology of how drugs interact with the body, Lorenzo-Martín and colleagues' work is highly innovative and will lead towards more ethically responsible research practices.

Nicolò Riggi is in the Department of Cell and Tissue Genomics, Genentech, South San Francisco, California, 94080, USA. **Felipe de Sousa e Melo** is in the Induced Proximity Platform, Amgen Research, Thousand Oaks, California 91320, USA.
e-mails: riggin@gene.com; felipedesousamello@gmail.com

1. Lorenzo-Martín, L. F. *et al. Nature* **629**, 450–457 (2024).
2. Hanahan, D. & Weinberg, R. A. *Cell* **144**, 646–674 (2011).
3. Clevers, H. *Cell* **165**, 1586–1597 (2016).
4. Nikolaev, M. *et al. Nature* **585**, 574–578 (2020).
5. Drost, J. *et al. Nature* **521**, 43–47 (2015).
6. Barker, N. *et al. Nature* **457**, 608–611 (2009).
7. Kozar, S. *et al. Cell Stem Cell* **13**, 626–633 (2013).
8. Nicholson, A. M. *et al. Cell Stem Cell* **22**, 909–918 (2018).

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