STEM CELLS

Tracheal remodelling supports stem cells

The vascular system, plastic and positioned in the vicinity of tissues, is an undervalued regulator of adult stem cells. Two studies now show that the vascular-like *Drosophila* trachea is reshaped after intestinal damage or tumour formation and that this remodelling is required for compensatory intestinal stem cell proliferation and tumour growth.

Louis Gervais and Allison J. Bardin

During homeostasis, adult stem cells of many tissues are quiescent or slowly dividing, compensating for differentiated cell turnover. After damage, these stem cells often offset cell loss by increasing their proliferation rate. Activation of stem cell proliferation involves not only cell intrinsic factors but also extrinsic signals from surrounding cells of the tissue. Inter-tissue communication contributes actively to allow the adaptation of adult stem cells to different tissue contexts such as stress or damage. Because of its role in gas and fluid delivery, the vascular system is in close proximity to many adult stem cells and seems to be involved in crafting their microenvironment[1–3]. Similarly, neovascularization participates in pathological states such as inflammation and tumorigenesis[4]. Despite the recognised importance of the vascular system, specific roles of induced vascular remodelling in tissue regeneration, their mechanisms and how they relate to neo-angiogenesis in tumorigenic contexts remain unclear. Indeed, in vivo experimental systems used to study the interaction between adult stem cells and the vascular system in the context of tissue homeostasis are still rare, and ex vivo organoid cultures do not fully recapitulate the complexity of tissue environments. Two related studies published in *Nature Cell Biology* from Perochon et al. and Tamamouna et al. use *Drosophila* adult intestinal regeneration as a model to investigate the intestinal co-regulation with the tracheal system, an equivalent of the mammalian vascular system (Fig. 1)[5,6].

The adult intestinal epithelium of *Drosophila* is pseudostratified and comprised of differentiated cells, namely enterocytes (EC) and enteroendocrine cells, which are renewed by intestinal stem cells (ISCs)[7]. Damage, infection or oxidative stress induces ISC proliferation to compensate for cell death and return to tissue homeostasis. ISC regulation relies on the concerted action of many signals from dying cells of the tissue, visceral muscles surrounding the epithelium, neurons innervating the tissue and systemic factors[7]. Little is known about the role of the intestinal trachea in the regulation of ISC behaviour, though a possible function in ISC maintenance has been proposed previously[8]. Earlier work also demonstrated that terminal tracheal cells (TTCs) associated with the gut change their organisation under neuronal control in response to nutritional cues[9]. TTCs equivalent to mammalian leading vascular cells called tip cells, found at the periphery of the general tracheal system in target tissues[10]. TTCs have a branched morphology, projecting several cytoplasmic extensions to deliver oxygen. Their branched morphology is known to be plastic during development, allowing adaptation to tissue needs[10]. This tracheal branching phenomenon is reminiscent of angiogenic sprouting in vertebrates. Little is known about TTC plasticity in the adult fly and how it participates in tissue homeostasis and regeneration.

Perochon et al. and Tamamouna et al. now present evidence that local remodelling and extra branching of TTCs is important for the stem cell proliferative response upon intestinal damage (Fig. 1). Tracheal remodelling was found to accompany the well-described increase in ISC proliferation in response to damage by pathogenic bacteria, chemicals or oxidative agents and tumour growth. The authors could further show that increased branching of TTCs is highly dynamic and reversible, does not involve TTC cell division, and precedes changes in ISC proliferation and gut regeneration. Interestingly, the adaptability of the trachea to the microenvironment did not require ISC proliferation, as blocking proliferation through knockdown of *myc* in ISCs did not perturb tracheal branching[5]. In contrast, tracheal remodelling, or at least oxygen delivery, seemed to be essential for gut regeneration as hypoxic conditions or induced apoptosis of TTCs led to a loss of ISC proliferation after damage.

In *Drosophila* larvae, tracheal branching is controlled by hypoxia-driven induction of HIF-1α/Sima, which induces expression of a Bnl/FGF ligand in the targeted tissue and then activates Btl/FGFR in the trachea, leading to its remodelling[11]. Using classical genetic approaches, the authors found that similar participants are involved in back-and-forth communication between the adult gut and trachea. Both studies found that HIF-1α/Sima expression was induced upon damage in TTCs, whereas Tamamouna et al. noted an additional induction of HIF1α/Sima in the gut epithelium. Similarly, *btl/FGFR* was upregulated in the trachea after damage, whereas *bnl/FGF* was induced in the midgut cells and trachea. However, the genetic data indicate that *btl/FGFR* and *bnl/FGF* function in both gut epithelial cells and TTCs. Using loss- and gain-of-function approaches in a cell-type-specific manner (in TTCs, ISCs/enteroblasts (EBs), and enterocytes (ECs)), *sima* and *btl* were demonstrated to be necessary in TTCs for their remodelling and induction of ISC proliferation after damage. Despite the lack of an obvious increase of expression in gut epithelial cells, *btl* knockdown using *esg-Gal4* (ISCs/EBs) or *Myo1-Gal*4 (ECs) also impaired branching and ISC proliferation. Nevertheless, the exact roles of *bnl* and *btl* in the midgut cells, as well as potential differences in paracrine and autocrine signalling, remain to be elucidated. Indeed, even though Tamamouna et al. showed that *btl* and *bnl* knockdown in ISCs and ECs affected both TTC branching and ISC proliferation, Perochon et al. observed that these treatments reduced ISC proliferation, but did not detect changes in tracheal branching. These differences may be due to the strength of the knockdown approach, perhaps suggesting that subtle differences in protein levels may have important physiological consequences. Altogether, both studies illustrate the

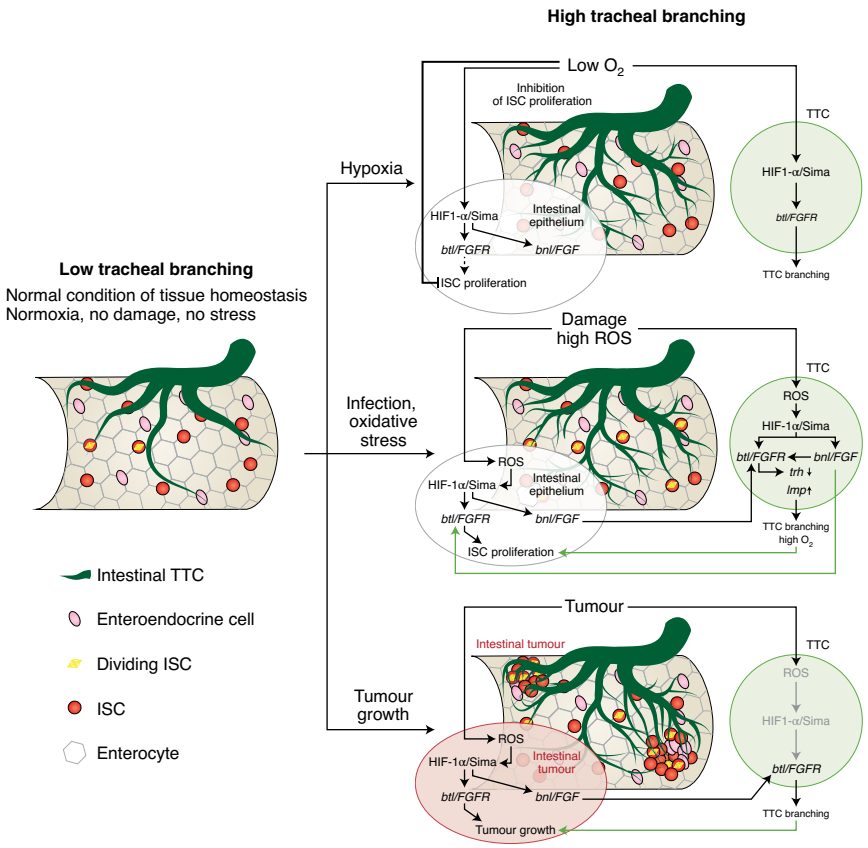

Fig. 1 | Schematic of the dialogue between the intestinal epithelium and the tracheal system in homeostasis, infection, cancer and hypoxia. In tissue homeostasis, intestinal stem cells (ISCs) are slowly dividing, balancing turnover of differentiated cells while the terminal tracheal cells (TTCs) provide oxygen (O_2) to the intestine. During hypoxia, the lack of oxygen induces HIF1α/Sima activation and *branchless/Fibroblast Growth Factor* (*bnl/FGF*) expression in the midgut, allowing increased TTC coverage after Breathless/Fibroblast Growth Factor Receptor (Btl/FGFR) pathway activation. In the epithelium, the absence of sufficient oxygen distribution in hypoxic conditions blocks ISC proliferation and tissue regeneration after damage. After bacterial infection or oxidative stress, accumulation of reactive oxygen species (ROS) induces the communication between the trachea and intestinal epithelium, ultimately resulting in ISC proliferation and tracheal branching to provide extra oxygen to the tissue. Both processes involve ROS-induced HIF-1α/Sima and Btl/FGFR signalling and are necessary for gut regeneration. Strikingly, intestinal tumour development involves the same factors active during the damage response. ROS production likely induces HIF1α/Sima and Btl/FGFR pathway activation, allowing TTC extra-branching and tumour growth. Note, interactions in gray were not experimentally demonstrated but are hypothesized. *Imp, IGF-II mRNA-binding protein*.

importance of communication between the trachea and the gut epithelia to reshape the trachea and adapt ISC proliferation during regeneration (Fig. 1).

Interestingly, although hypoxia was shown to induce tracheal branching in the adult gut, it concomitantly blocked ISC mitosis, probably because oxygen is required for ISC proliferation (Fig. 1). As reactive oxygen species (ROS) have been implicated in the activation of hypoxia-inducible factor-1α/Similar (HIF-1α/Sima), the authors tested whether ROS production might be involved in tracheal remodelling and ISC proliferation upon stress. Feeding flies with the antioxidant *N*-acetyl cysteine (NAC) reduced *HIF-1α/sima* and *btl/FGF* expression after damage, arguing that expression is ROS dependent[5]. Furthermore, infecting flies with bacteria mutants that could not produce pyocyanin, a ROS-generating secreted virulence factor, prevented TTC branching and ISC proliferation[6], suggesting ROS involvement in both processes. Through genetic manipulation of ROS levels, the authors demonstrated that ROS production in both tissues is sufficient to induce tracheal branching and ISC proliferation. Again, this finding illustrates the cooperation between tracheal and gut tissues, which facilitates the adaption to tissue requirements, delivering increased oxygen and compensating for cell death during regeneration. It is worth noting how similar mechanisms of TTC branching are involved in developing and adult *Drosophila* after hypoxia[11] and in response to ROS production. The conservation of angiogenic factors HIF-1α, FGF, FGFR and ROS in vascularization in vertebrates[12] and described here in *Drosophila* is striking.

Perochon et al.[5] identified genes with changed expression in the TTCs during remodelling using DNA adenine methyltransferase identification sequencing (DamID-seq) to evaluate genes differentially bound by RNA Pol II after bacterial infection. The conserved mRNA-binding protein Imp/IGF2BP, known to be involved in axonal remodelling in flies, was upregulated in TTCs in a ROS-dependent manner. Furthermore, Perochon et al. demonstrated that *Imp/IGF2BP* expression is required for both TTC remodelling and ISC proliferation upon damage. Surprisingly, TTC branching after damage also involved the downregulation of *trachealess* (*trh*), a transcription factor essential for the specification of tracheal fate during development. *trh* is believed to initiate or to act very early in the specification of tracheal cells during development[10] Therefore, it is somewhat counterintuitive that *trh* should be downregulated in damaged TTCs to allow for proper branching and ISC proliferation. The authors propose that TTCs might dedifferentiate due to the downregulation of *trh*, which may be important for allowing more plasticity and reshaping. Such a hypothesis is intriguing and merits further investigation into the mechanism behind *trh* downregulation, its role in TTC plasticity, and mammalian conservation during sprouting angiogenesis.

Neo-angiogenesis participates actively in tumour development and metastasis, providing oxygen and nutrients required for tumour growth and invasion. Counteracting angiogenesis has become a therapeutic strategy against cancer. Tamamouna et al.[6] investigated the role of neo-tracheogenesis in intestinal tumour growth. Inducing ISC-derived tumours expressing an activated form of Ras (Ras^{V12}) or inactivating *Notch* in ISCs, they observed increased tracheation locally. In accordance with previous studies[13], transplantation of tumour cells induced the recruitment of tracheal cells around the transplant. The excess tracheal branches seemed to be important for tumour growth and survival, as both were strongly reduced in hosts in which *btl* was knocked down. Mechanistically, Ras^{V12} tumours induced *btl*, *bnl* and *sima*

expression and knockdown of these factors in the tumour reduced tracheal branches and tumour size and increased fly survival. Prevention of ROS production in the tumour reduced tracheal coverage, ISC mitosis, and tumour growth. However, it remains difficult to dissociate a ROS function on vascularization of the tumour from previously described roles of ROS directly on stem cell proliferation[14,15]. Nevertheless, the conservation of mechanisms and factors involved in damage response and upon tumorigenesis in the fly intestine and neo-angiogenesis is evident.

An interesting finding reported by both groups is the reversibility of tracheogenesis after epithelial damage[5] or upon tumorigenesis[6] (after switching off Ras^{V12} expression). Thus, a future aim for the field will be to better understand mechanisms and factors regulating trachea plasticity during this recovery phase concomitant with the slowdown of stem cell proliferation.

In summary, both studies establish the *Drosophila* intestine as an excellent example of tissue collaboration regulating adult stem cells, tissue regeneration or tumour growth. Future studies using this model will provide a better understanding of the mechanisms required for co-regulation and dynamic plasticity of vascular tissues and stem cell responses. ❐

Louis Gervais ✉ and Allison J. Bardin ✉
Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France.
✉*e-mail: louis.gervais@curie.fr; allison.bardin@curie.fr*

Published online: 26 May 2021
https://doi.org/10.1038/s41556-021-00695-w

References
1. Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. J. *Nature* **481**, 457–462 (2012).
2. Kiel, M. J. et al. *Cell* **121**, 1109–1121 (2005).
3. Shen, Q. et al. *Cell Stem Cell* **3**, 289–300 (2008).
4. De Palma, M., Biziato, D. & Petrova, T. V. *Nat. Rev. Cancer* **17**, 457–474 (2017).
5. Perochon, J. et al. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-021-00676-z (2021).
6. Tamamouna, V. et al. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-021-00674-1 (2021).
7. Gervais, L. & Bardin, A. J. *Curr. Opin. Cell Biol.* **48**, 97–105 (2017).
8. Li, Z., Zhang, Y., Han, L., Shi, L. & Lin, X. *Dev. Cell* **24**, 133–143 (2013).
9. Linneweber, G. A. et al. *Cell* **156**, 69–83 (2014).
10. Hayashi, S. & Kondo, T. *Genetics* **209**, 367–380 (2018).
11. Centanin, L. et al. *Dev. Cell* **14**, 547–558 (2008).
12. Fraisl, P., Mazzone, M., Schmidt, T. & Carmeliet, P. *Dev. Cell* **16**, 167–179 (2009).
13. Campbell, K. et al. *Nat. Commun.* **10**, 2311 (2019).
14. Hochmuth, C. E., Biteau, B., Bohmann, D. & Jasper, H. *Cell Stem Cell* **8**, 188–199 (2011).
15. Patel, P. H. et al. *Nat. Commun.* **10**, 4365 (2019).

Competing interests
The authors declare no competing interests.

IMMUNOLOGY

Immune memory in individuals with COVID-19

COVID-19 has led to a global pandemic, but the long-term immunological effects of the infection are only partially understood. A new study now provides important new clues by describing the transcriptional and epigenetic processes behind the immune memory of both adaptive and innate immune cells in individuals who have recovered from COVID-19.

Mihai G. Netea and Yang Li

Initially emerging in China in December 2019, the coronavirus disease COVID-19 caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has become a major health crisis, with high morbidity and mortality, especially among the elderly and people with various comorbidities[1]. Much has been learned in the past year regarding pathophysiology of the disease, in which immune-based mechanisms play a major role not only for protection, but also for immunopathology. While novel therapies have been developed based on immunomodulatory drugs (such as dexamethasone or IL-6 blockers), vaccination remains the most effective approach to blocking the spread of the virus and alleviate the consequences of the pandemic.

Several successful vaccines have been already developed based on various technological platforms, including mRNA technology, adenovirus platforms, recombinant proteins or inactivated viruses. While several of these have proved to be effective in short-term phase 3 trials[2], little is known regarding the duration of their effects and the precise immune correlates of protection mediating the protection. It is therefore crucial to understand in detail the immunological memory processes inducing protection against the virus after natural COVID-19 infection. We would be thus able to employ more rationally the available vaccines and even design and develop the next generation of vaccines with improved and longer protection against infection. In this issue of *Nature Cell Biology*, You et al. now make one important step in that direction by describing the transcriptional and epigenetic processes behind the long-term memory of immune cells in individuals who have recovered from COVID-19[3]. By using cutting-edge single-cell sequencing technology, the authors describe the transcriptional modules, the regulatory nodes at the level of transcription factors, and the chromatin accessibility in various immune cell types after COVID-19 recovery.

The first line of evidence provided by You and colleagues addresses the mechanisms of adaptive immune memory at the level of B cells and T cells (Fig. 1). Lymphocytes are crucial components of the long-term protection induced either by natural infection or by vaccination; while their role as correlates of protection against COVID-19 has not been formally demonstrated, it is widely assumed that they mediate the protection induced by natural infection or vaccines[4,5]. First, You et al. identified important differences in the developmental processes in B lymphocytes from COVID-19 convalescent individuals compared to healthy volunteers, based on differential B-cell lineage trajectories in chromatin accessibility. Second, the authors also identified crucial differences in transcription factor (TF) regulators, with the NF-κB subunits RELA and RELB being enriched in B cells of healthy volunteers, while