CANCER

Tumour stem-cell surprises

Stem-cell divisions are thought to be essential to tumour growth. Targeted removal of a specific stem-cell population reveals its role in tumour development and in the growth of tumours formed by cell migration to distant sites. SEE ARTICLE P.676

FLORIAN R. GRETEN

Elimination of cancer stem cells has been proposed as a therapeutic strategy because such cells are considered to be essential to tumour initiation and maintenance[1]. In the epithelial cells that form the surface layer of the intestine, cells known as crypt base columnar cells that express the receptor protein Lgr5 (Lgr5$^+$ cells) can act as stem cells during intestinal homeostasis[2]; these are also the cells from which colorectal cancer originates[3]. On page 676, de Sousa e Melo *et al.*[4] report their analysis of the role of these cells in established tumours.

The intestinal epithelium has a remarkable capacity for self-renewal, and actively proliferating Lgr5$^+$ cells are usually responsible for the daily generation of all epithelial cell types in the intestine[2], although several other more-differentiated cell types can be reprogrammed to replenish Lgr5$^+$ cells in response to tissue damage and cell stress[4–6]. De Sousa e Melo and colleagues investigated whether loss of the Lgr5$^+$ population of stem cells can also be compensated for in cancer.

Previous work from the same laboratory generated genetically engineered mice[7] that express the diphtheria toxin receptor in Lgr5$^+$ stem cells, along with a fluorescent protein that aids microscope observation of the cells. When diphtheria toxin is administered to these animals, it specifically destroys the Lgr5$^+$ cells. Using cells from these mice, the authors established *in vitro* populations of intestinal cells known as organoids, and applied genome-editing techniques to introduce several mutations required for the development of colorectal cancer into the cells.

The authors subcutaneously transplanted the mutated organoids into mice, where the cells formed tumours. The animals were treated with diphtheria toxin to cause long-term depletion of the tumour's Lgr5$^+$ cells. Destruction of the Lgr5$^+$ cells did not shrink the tumours, which remained at a constant size. This indicates that some non-Lgr5-expressing cells might compensate for the loss of Lgr5$^+$ cells to maintain tumour size. Moreover, once administration of the diphtheria toxin was halted, the Lgr5$^+$ cells rapidly reappeared and the tumour grew at a rate comparable to that seen in the control tumour transplants that had not been treated with diphtheria toxin (Fig. 1). Transcriptional profiles of tumour cells derived from Lgr5$^+$-depleted tumours, compared with non-depleted controls, indicated upregulation of downstream targets of Myc — a protein that can drive cell-cycle progression. This might account for the compensatory growth of tumour cells in the absence of Lgr5$^+$ cells.

The demonstration that Lgr5$^+$ cells are dispensable for tumour maintenance is undoubtedly surprising. However, it adds to the compelling evidence for cell plasticity — the ability of cells to change from one type to another — in the intestine, and the ability of intestinal epithelial cells to revert to stem cells through dedifferentiation (the process of changing their differentiated state) during regeneration[8] and tumour initiation[9,10].

Evidence suggests[1] that stemness (having the stem-cell properties of being able to self-renew and differentiate) is a prerequisite for metastasis, the process whereby tumour cells migrate from the primary site of tumour formation to form tumours at distant sites. When the authors transplanted the tumour-forming organoids into the mouse colon, metastasis occurred, and tumours formed in the liver. However, if mice were treated with diphtheria toxin, the metastatic liver tumours were substantially reduced, indicating that Lgr5$^+$ cells are required to initiate metastasis. Perhaps even more strikingly, the authors observe that loss of Lgr5$^+$ cells from already-established liver metastases caused those tumours to shrink, and that when diphtheria toxin administration was stopped these metastases did not start to grow again — in stark contrast to the regrowth observed in primary tumours. Although it remains to be determined

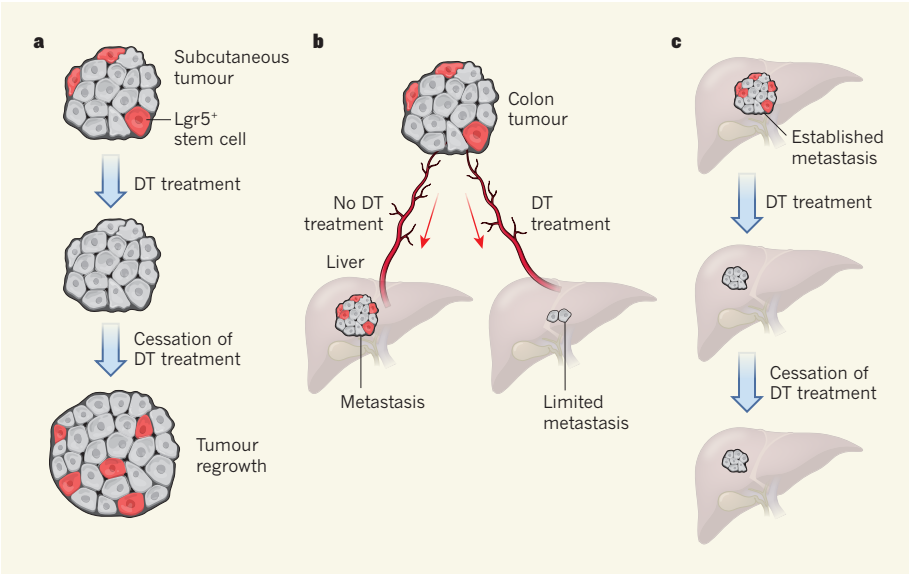

Figure 1 | Tumour stem cells. De Sousa e Melo *et al.*[4] investigated the role of a stem-cell population in the growth of primary tumours and of tumour cells that had migrated through the bloodstream to form tumours at a distant site in a process known as metastasis. The authors used a mouse model of colorectal cancer in which administration of diphtheria toxin (DT) selectively destroys any cancer stem cells that express the protein Lgr5 (Lgr5$^+$ stem cells). **a**, When a subcutaneous primary tumour was treated with DT, the Lgr5$^+$ stem cells were destroyed, but the tumour remained the same size. This suggests that other cells fulfilled stem-cell functions to replace the cells that died after DT treatment. When treatment with DT ceased, the tumour increased in size and Lgr5$^+$ stem cells reappeared. **b**, If Lgr5$^+$ stem cells in a colon tumour were destroyed, this resulted in a substantial reduction in metastatic liver tumours. **c**, In an already-established liver metastasis, Lgr5$^+$-stem-cell destruction caused the metastasis to shrink. When DT treatment ceased, the tumour did not grow.

whether this finding will also hold for metastases that develop in tissues other than the liver, these results underscore the general importance of stemness for metastasis in colorectal cancer.

The findings of de Sousa e Melo *et al.* call into question the idea, known as the unidirectional hierarchy model, that there is a dedicated stem-cell population in cancer. Stem cells are usually thought to comprise only a small fraction of a cell population. However, considering that the number of $Lgr5^+$ cells in the tumours examined here was relatively high (15–25% of all tumour cells before $Lgr5^+$ cell depletion), another interpretation might be that colorectal cancer $Lgr5^+$ cells do not have the same stem-cell characteristics as the $Lgr5^+$ cells that support tissue homeostasis. But, given that previous cell-lineage-tracing experiments to track the fate of cells in early benign intestinal tumours confirmed the stem-cell properties of $Lgr5^+$ cells[11], and that tumours rapidly replenish lost $Lgr5^+$ cells, the possibility that different types of stem cell are involved in cancer and homeostasis seems unlikely. The lack of a dedicated stem-cell population thus seems a more reasonable explanation, and would support the idea that stemness should be seen as a property that can be acquired at any time during the life of a cell, independently of its differentiation status, rather than as a cell-intrinsic property acquired only on a cell's formation.

De Sousa e Melo and colleagues' work raises some interesting questions. Is there a distinct cell type that acts as a 'reserve' stem cell, compensating for the loss of $Lgr5^+$ cells in tumours, or does any epithelial cell type in the tumour have the capacity to adapt and dedifferentiate to form stem cells? Perhaps even the neighbouring non-epithelial stromal cells have the ability to form stem cells. The contribution of the tumour microenvironment and the location of the adapting cells should be examined. The authors report an increase in the expression of the immune-system signalling protein interferon upon the loss of $Lgr5^+$ cells in tumours, although this expression is probably not required for reserve stem-cell action and merely reflects an inflammatory response to $Lgr5^+$-cell death.

In a paper online in *Nature*, Shimokawa *et al.*[12] report that in human colorectal cancer, the selective destruction of $LGR5^+$ cells led to temporary tumour regression and other cells exhibited compensatory proliferation. Thus, it will be essential to determine the signalling pathways that are responsible for the reappearance of $Lgr5^+$ cells. The preliminary evidence reported by de Sousa e Melo *et al.* points to a role for Myc signalling, but how $Lgr5^+$ cell loss is sensed by the remaining cells in the tumour is not known, and which signalling cascades might lead to Myc activation in the absence of $Lgr5^+$ cells is also unknown. Furthermore, the authors did not test whether inhibition or loss of Myc prevents the proposed reserve stem-cell pool from functioning, nor whether Myc inhibition can cause primary-tumour regression in the absence of $Lgr5^+$ cells.

Because pharmacological inhibition of Myc is currently a challenge, identification of its upstream signalling pathways or other essential pathways might prove useful and potentially enable the eradication of primary colorectal tumours when combined with, for example, a targeted antibody treatment to deplete $Lgr5^+$ cells[13]. The authors' work indicates that the elimination of $Lgr5^+$ cells might be an approach worth testing for the treatment of liver metastases, suggesting an exciting avenue for future investigation. ■

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1. Nassar, D. & Blanpain, C. *Annu. Rev. Pathol.* **11,** 47–76 (2016).
2. Visvader, J. E. & Clevers, H. *Nature Cell Biol.* **18,** 349–355 (2016).
3. Barker, N. *et al. Nature* **457,** 608–611 (2009).
4. de Sousa e Melo, F. *et al. Nature* **543,** 676–680 (2017).
5. Buczacki, S. J. A. *et al. Nature* **495,** 65–69 (2013).
6. Tetteh, P. W. *et al. Cell Stem Cell* **18,** 203–213 (2016).
7. Tian, H. *et al. Nature* **478,** 255–259 (2011).
8. Tetteh, P. W., Farin, H. F. & Clevers, H. *Trends Cell Biol.* **25,** 100–108 (2015).
9. Schwitalla, S. *et al. Cell* **152,** 25–38 (2013).
10. Davis, H. *et al. Nature Med.* **21,** 62–70 (2015).
11. Schepers, A. G. *et al. Science* **337,** 730–735 (2012).
12. Shimokawa, M. *et al. Nature* http://dx.doi.org/10.1038/nature22081 (2017).
13. Junttila, M. R. *et al. Sci. Transl. Med.* **7,** 314ra186 (2015).

INORGANIC CHEMISTRY

Making iron glow

An iron complex has been made that has a long-lived excited state and emits light at room temperature as a result of a charge-transfer process. This breakthrough might allow the production of cheap solar cells. SEE LETTER P.695

FELIX N. CASTELLANO

The incorporation of iron into light-absorbing compounds known as photosensitizers offers great promise for solar-energy applications. To realize these applications, the light-activated excited state of an iron-containing photosensitizer needs to persist long enough for it to react with other compounds or materials. Numerous iron(II)-based complexes have been prepared for this purpose, but these suffer from short excited-state lifetimes that limit their solar-energy applications. On page 695, Chábera *et al.*[1] report a strategy for realizing long-lived excited states in iron-based compounds. Notably, the direction in which electronic charge is transferred to form the excited state is the reverse of that seen in most previously reported iron complexes.

Transition-metal photosensitizers typically feature low-energy excited states in which a complete unit of electron charge has been transferred between the metal centre and one of the bound species, called ligands, upon exposure to light. The field has been dominated by complexes that incorporate ruthenium(II) and iridium(III), and in which charge is transferred from the metal to the ligands. Such metal-to-ligand charge-transfer (MLCT) compounds are used in applications ranging from solar cells[2] to organic light-emitting diodes[3]. But because ruthenium and iridium are scarce, expensive elements, it is desirable to find photosensitizers based on cheaper and more-abundant metals such as iron.

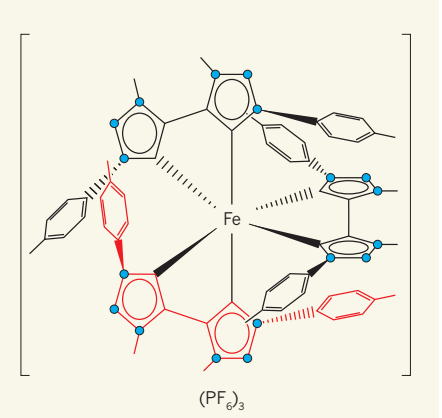

Figure 1 | Structure of a photoluminescent iron-containing complex. Chábera *et al.*[1] report that the $[Fe(btz)_3](PF_6)_3$ complex has a long-lived excited state (duration 100 picoseconds; 1 ps is 10^{-12} s) that relaxes to its ground state at room temperature by emitting light. This opens the door to the development of cheap, inorganic photosensitizer compounds for solar cells. One of the three btz ligands is shown in red. Blue dots represent nitrogen atoms.

Iron(II) has the same number and configuration of electrons in its outermost *d* orbitals as ruthenium(II) and iridium(III), so similar principles generally apply for manipulating the excited-state behaviour of all three metals[4]. However, iron has smaller 3*d* orbitals than the 4*d* and 5*d* orbitals found, respectively, in ruthenium and iridium. This results in much