

FORUM: Cancer

Resolving the stem-cell debate

New research backs the contentious idea that solid tumours are not masses of equivalent cells, but instead contain cancer stem cells that support tumour maintenance. Here, two experts provide complementary views on the findings and on the implications for potential therapies. **SEE LETTERS P.522 & P.527**

THE PAPERS IN BRIEF

- Evidence that cancer stem cells spawn more highly differentiated (non-stem) cells in solid tumours has relied almost entirely on analyses of tumours formed by human cancer cells injected into mice that have compromised immune systems.
- Chen *et al.*¹ (page 522) and Driessens *et al.*² (page 527), together with Schepers *et al.*³ (writing in *Science*), traced individual cells in intact tumours as the tumours developed

from non-cancerous cells in mice*.

- The studies identify specific cell subsets that act as cancer stem cells in brain, skin and intestinal tumours, with one of the reports¹ indicating that targeting such cells may improve therapeutic outcome.
- The papers also describe how different cell subpopulations emerge and evolve as a tumour grows and in response to anticancer treatment.

Meet the parents

RICHARD J. GILBERTSON

Despite decades of research, one-third of patients with cancer die within five years of diagnosis. Therefore, it is not surprising that any concepts offering a sea change in the way we think about and treat cancer garner enormous attention and resources. One such concept is the cancer stem cell (CSC) hypothesis, which suggests that cancers are organized into aberrant cell hierarchies in which 'differentiated' daughter cells that have limited capacity to proliferate are produced by a subset of parent CSCs that replicate indefinitely (Fig. 1).

Until now, evidence for the existence of CSCs has been controversial, but the hypothesis is extremely attractive because it provides a conceptual framework on which new therapeutic approaches could be built: any drug capable of killing CSCs would, in theory, be curative. Now, three independent studies of mouse models of brain¹, skin² and intestinal³ tumours provide the first evidence that CSCs do exist and arise *de novo* during tumour formation in intact organs.

Lineage tracing is a technique that allows permanent *in vivo* fluorescent marking of stem cells and their progeny. This method has been used previously⁴ to identify intestinal stem

cells, which give rise to the various cell types that make up intestinal epithelial tissue in mice. The same study also revealed that when a gene encoding the protein APC is deleted, these stem cells generate benign tumours (intestinal adenomas).

To test whether the tumours were maintained by CSCs, Schepers *et al.*³ used a lineage-tracing strategy involving intestinal stem cells in which APC had been knocked out, so that cells randomly adopted one of four fluorescent tagging colours when the mice were given a low dose of the drug tamoxifen. Initial tamoxifen dosing often generated single-colour 'clonal' adenomas, indicating that they typically originated from single intestinal stem cells. Remarkably, a subsequent dose of tamoxifen switched the colour of individual cells in the adenomas, and the progeny of these newly coloured cells (which included differentiated tumour cells) went on to populate the tumour, pinpointing their parents as CSCs.

Similar observations were made by Driessens *et al.*² in a mouse model of a benign skin tumour (papilloma). Using lineage tracing of individual papilloma cells, the authors observed great variability in the cells' proliferative potential, with only 20% of them being able to generate daughters that populated large swathes of tumour.

The studies by Schepers *et al.* and Driessens

et al. provide elegant demonstrations of stem-cell activity in intact tumours. But adenomas and papillomas are benign tumours, not cancers. The cells in these tumours are organized in much the same way as the corresponding normal tissue, and so it is not surprising that these benign tumours contain cell hierarchies that approximate to normality.

A key question, therefore, is whether cell hierarchies driven by CSCs exist in the invasive malignant tumours that kill patients. With this in mind, Driessens *et al.* also analysed a mouse model of squamous skin cancer. The researchers found that, in comparison with papillomas, the malignant tumours contained much higher numbers of long-term replicating cancer cells that showed little evidence of cell differentiation. This raises the possibility that cancers slip from hierarchical organization into relative anarchy as they progress from the benign to the malignant state.

So what is the evidence that malignant tumours contain CSCs? Chen *et al.*¹ provide compelling data that glioblastomas (the deadliest brain tumours) are organized hierarchically. Using a clever combination of 'suicide-gene' technology that selectively killed glioblastoma CSCs, and antitumour drugs that eliminate the bulk of dividing cancer cells, they show that CSCs repopulate the cancer when the bulk of the tumour is wiped out by anticancer drugs (Fig. 1a). Targeting both CSCs and their daughter cells with a combination of suicide-gene targeting and anticancer drugs, the authors dramatically impeded the growth of glioblastomas *in vivo*.

The three papers represent an important new chapter in the debate over CSCs. They introduce us for the first time to these cells in their native habitats and provide the first hard evidence that such cells are a legitimate therapeutic target. The next steps will include determining how well mouse CSCs recapitulate their human counterparts, and how best to destroy these for the benefit of patients.

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Stemming tumour evolution

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Cancer formation is an evolutionary process: repeated rounds of mutation and selection lead to outgrowth of the fittest mutant clones and transform normal healthy cells into cancer cells⁵. The identification of CSCs as a restricted population of cells responsible for the maintenance of tumours suggests that these are the cells that have the inherent ability to propagate mutations throughout a tumour and to drive cancer evolution: CSCs are the 'movers and shakers' of carcinogenesis. Selective killing of such cells is therefore an appealing therapeutic prospect. Indeed, Chen *et al.*¹ observed a near-twofold reduction in the density of brain tumours in mice when they combined standard anticancer drugs with selective killing of CSCs, compared with standard agents alone.

But should the primary goal of cancer therapy now be to kill the CSC 'root' of the tumour? This is akin to asking if we may safely ignore the non-stem-cell population of tumour cells. It is conceivable that selected mutations in non-stem cells will cause them to revert to a stem-cell-like state and so contribute to tumour evolution. Furthermore, non-stem cells in tumours may revert to such a stem-cell-like state even in the absence of mutation⁶. If that is the case, then selectively killing a

"The results place competition between tumour cells at the centre of cancer evolution."

CSC population may vacate a niche within the tumour, opening it up to occupancy by a rival population of cells. Trying instead to limit 'stemness' — perhaps by modifying the microenvironment that supports

stem cells in tumours⁷ — may prove a more effective therapeutic strategy than simply eradicating CSCs.

Driessens *et al.* and Chen *et al.* show that the cellular organization of the early (pre-cancer) skin and intestinal tumours are caricatures of their normal organs, and are composed of both stem cells and non-stem cells. The presence of non-stem cells may represent a brake on tumour evolution: such cells not only consume the limited resources available, but also may be evolutionary dead ends, in the sense that (unlike the stem cells) they have only limited potential for growth. Interestingly, Driessens *et al.* observed that progression to cancer in benign skin tumours was associated with an expansion of the CSC population and a decrease in the production of non-stem cells. This suggests that tumour evolution enriches the CSC population.

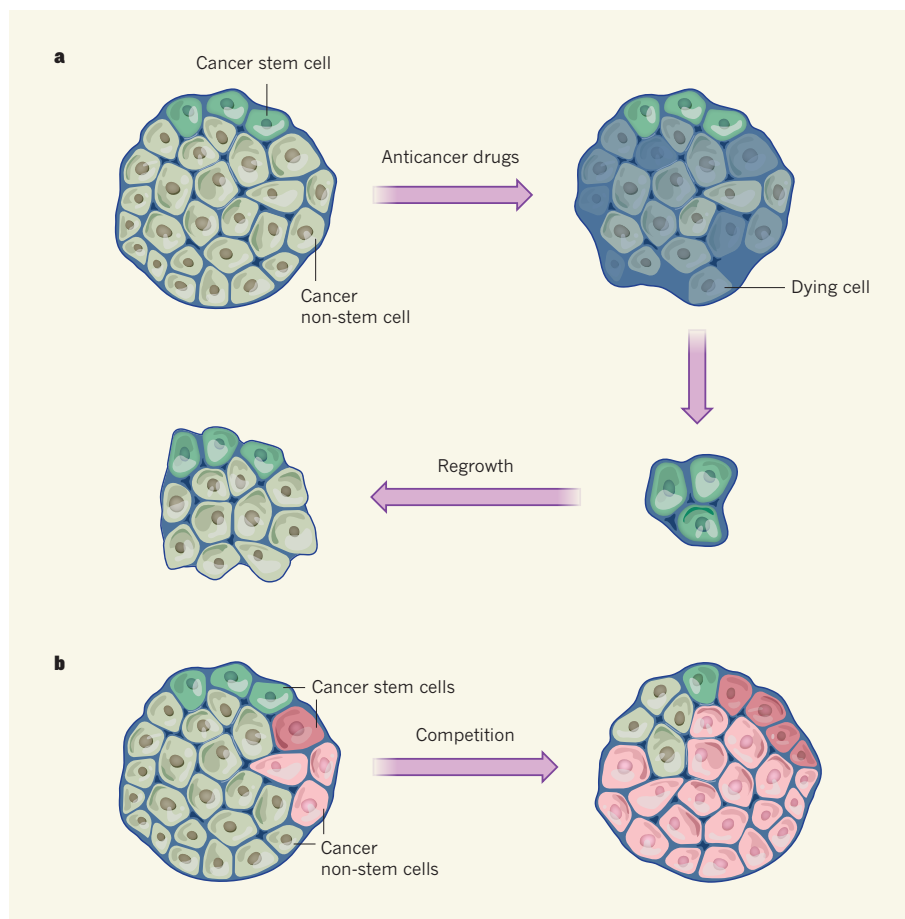


Figure 1 | Not all cells in a tumour are equal. Chen *et al.*¹, Driessens *et al.*² and Schepers *et al.*³ show that brain, skin and intestinal tumours include cancer stem cells (CSCs) that self-renew and that produce other, more-differentiated (non-stem) cells that constitute the bulk of the tumour-cell population. **a**, Chen and colleagues' results indicate that, although current anticancer drugs can wipe out most of the dividing non-stem cells, surviving CSCs can repopulate the tumour. Therefore, targeting both CSCs and the dividing cells would be required for complete tumour eradication. **b**, Driessens *et al.* report that CSCs continuously compete with each other for a place in the tumour, and that the winners' daughter cells predominate. Red and green colours indicate different clonal populations, each one originally derived from an individual CSC.

Thus, designing therapies that prevent increases in stemness may be a means to restrict tumour progression to cancer.

A common model of cancer evolution involves sequential waves of clonal expansion, each triggered by a new mutation⁵. Remarkably, Driessens and colleagues' results are at odds with this model. The authors found neutral competition between CSCs; that is, every CSC within a tumour is equally likely to clonally expand or die off, probably even in the absence of new mutations (Fig. 1b). Their observations suggest that clonal expansion is a continuous process in tumours, not a rarity driven by a new, selectively advantageous mutation. And the results place competition between tumour cells at the centre of cancer evolution. In this context, mutations that simply drive proliferation may be of less importance than previously thought, whereas mutations that slightly tip the balance of competition to favour one CSC over another — perhaps by improving survival, promoting self-renewal or monopolizing limited

resources — might be the ones that are highly selected in tumours.

The main take-home message from the three studies is that cells are organized hierarchically within tumours; all tumour cells are not equal. Understanding how these cellular hierarchies shape carcinogenesis, and exploiting them to change the course of tumour evolution, holds promise for effective treatment. ■

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