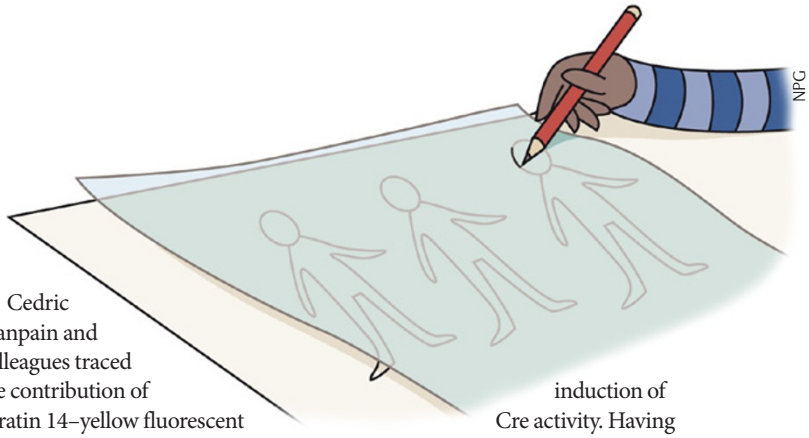
Nature Reviews Cancer | AOP, published online 17 August 2012; doi:10.1038/nrc3354



CANCER STEM CELLS

Tracing clones

The cancer stem cell (CSC) theory of tumour propagation has experienced a relative roller-coaster ride over the past few years. Now, three papers that use lineage tracing to look at the contribution of individual cells to the tumour mass indicate that the CSC theory is back on the up.

All three papers used tamoxifen-inducible Cre–Lox technologies to express fluorescent proteins in different tissues. Luis F. Parada and colleagues crossed mice with green fluorescent protein (GFP)-labelled cells in the subventricular zone of the brain — a known residence of neural stem cells — with glioma-prone mice and found that the GFP$^+$ tumour cell subset was quiescent compared with GFP$^-$ progenitor cells. Treatment of these mice with temozolomide (TMZ) , which is used to treat patients with glioma, mostly eradicated the proliferating progenitor cell population, but the quiescent GFP$^+$ population remained. Pulse chase labelling with two different analogues of bromodeoxyuridine showed that GFP$^+$ cells began proliferating after TMZ treatment, and 7 days after treatment the majority of proliferating cells no longer expressed GFP. This indicates that the hierarchy of stem cells giving rise to proliferative progenitor cells seems to exist in gliomas before treatment with TMZ and is re-established after treatment. The inclusion of a herpes simplex thymidine kinase 'suicide gene' in the GFP construct enabled these authors to eliminate the GFP$^+$ population using ganciclovir (GCV). This approach improved the survival of the mice with established gliomas, and the sequential administration of TMZ and GCV mostly eradicated these tumours.

> “a fairly small number of cells can generate a tumour, and … these might be the cells that we need to target more effectively in the clinic”

Cedric Blanpain and colleagues traced the contribution of keratin 14–yellow fluorescent protein (YFP)-expressing basal-layer epidermal cells to benign papillomas and malignant squamous cell carcinomas (SCCs) that arise in the skin of mice treated with a chemical protocol. In the 1% of basal tumour epithelial cells expressing YFP, the proliferative capacity was heterogeneous, with some cells contributing thousands of cells to the papilloma and others generating 100 cells or fewer. Seven weeks after the activation of the YFP-expressing transgene, the numbers of YFP$^+$ basal cells had dropped substantially, indicating that only a few cells that initially expressed YFP are capable of prolonged self-renewal. The examination of serial sections of the papillomas and the construction of a mathematical model of cell fate indicated that a sustained cellular hierarchy, similar to that seen in normal epidermis, persists in the papillomas: a few cells with stem-cell-like properties give rise to a transient progenitor cell pool. Lineage tracing in SCCs that spontaneously develop from papillomas indicated that the cellular hierarchy that is evident in the papillomas is lost in the SCCs, with more YFP$^+$ cells generating large clones and having a reduced capacity for differentiation.

Hans Clevers and colleagues carried out lineage tracing of LGR5$^+$ intestinal crypt stem cells in *Apc*-mutant mice that develop intestinal adenomas. They used an R26R-confetti allele to label the LGR5$^+$ cells. This allele allows the fluorescent protein that is initially expressed by the traced cells to be changed to a fluorescent protein of a different colour following a second induction of Cre activity. Having established that LGR5$^+$ cells clearly contribute to the initial stages of adenoma growth, the authors then switched confetti colours in LGR5$^+$ adenoma cells to examine their function in established lesions. Very few cells switched colour, allowing the authors to be sure that they were following clonal populations. Interestingly, these retracings indicated that the hierarchy of crypt stem cells persisted, with retraced LGR5$^+$ cells at the bottom of the crypt giving rise to a ribbon of cells that progressed towards the lumen. The number of LGR5$^+$ cells remained relatively consistent at 5–10% of the total tumour cell population, a similar proportion to that of LGR5$^+$ cells present in a normal crypt. Furthermore, these LGR5$^+$ tumour cells were able to generate all of the other cell types present in the adenoma, indicating that these cells are multipotent stem cells of the adenoma.

Thus, tracing the contribution of individual tumour cells to tumour formation lends weight to the concept that a fairly small number of cells can generate a tumour, and that these might be the cells that we need to target more effectively in the clinic.

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