CANCER STEM CELLS

Can mutated stem cells produce tumours?

Which cells need to be mutated in order for solid tumours to fully develop? Many have postulated that mutation of a normal tissue stem cell might be required for both tumour initiation and progression, whereas others have argued that mutations in progenitor cells can induce stem cell-like qualities, giving rise to long-lived and clinically manifest disease. Two papers recently published in *Nature* add thought-provoking data to this debate.

Hans Clevers and colleagues have recently identified cells within the crypts of the small intestine and colon expressing *LGR5* to be long-lived stem cells. Having established that tumours form only when the tumour suppressor *Apc* is deleted in a rare population of cells in the crypt, the authors crossed transgenic mice expressing floxed *Apc* alleles with knock-in mice expressing enhanced green fluorescent protein (EGFP) and *tamoxifen*-regulated Cre recombinase under the control of the *Lgr5* promoter. Activation of the Cre recombinase resulted in EGFP-positive cells expressing increased levels of β -catenin (which is regulated by APC) in a few cells in the base of the crypt. These cells continued to

proliferate and, after 5 days, cells expressing high levels of β -catenin were evident in the progenitor population. By 8 days microadenomas were seen and these progressed to large multi-villus adenomas by 14 days. Interestingly, although the transformed stem cells continued to express EGFP (and therefore *LGR5*), the transformed progenitor cell population did not. Moreover, use of a different Cre mouse to delete *Apc* in the progenitor cell population, and not the stem cell population, rarely produced adenomas. These findings imply that loss of APC in *LGR5*-expressing stem cells in the intestine is necessary and sufficient to induce tumour formation and that the stem cell hierarchy is maintained early in the transformation process.

In order to examine whether *PROM1* (also known as CD133) is a stem cell marker, Richard Gilbertson and colleagues have generated a knock-in mouse that expresses tamoxifen-regulatable Cre recombinase and a nuclear LacZ marker from the *Prom1* locus (*Prom1*^{C-L}). *Prom1* is null in *Prom1*^{C-L} mice and these animals develop normally, indicating that the gene is not required for development.

Unlike other tissues in the *Prom1*^{C-L}-heterozygous mice, *PROM1* expression was restricted to the stem cell compartment in the small intestine. The distribution of *Prom1* and *Lgr5* overlapped: 75% of *Lgr5* mRNA-positive cells in *Prom1*^{+/C-L} mice also expressed nuclear LacZ. Crossing *Prom1*^{+/C-L} mice with Cre-dependent Rosa26 yellow fluorescent protein (YFP)-expressing mice, thereby constitutively activating YFP in *PROM1*-expressing cells and their progeny after injection of tamoxifen, showed that *PROM1* cells can give rise to all cells of the small intestinal epithelium. Crossing these mice with mice expressing an inducible oncogenic form of β -catenin resulted in tumour formation in these animals. Interestingly, only 7% of the cells within these tumours remained *PROM1*-positive; the authors suggest that these might be a subpopulation of transformed stem cells.

Both papers indicate that a single mutation in normal intestinal stem cells can give rise to tumours, as has been suggested. It is interesting that, although *LGR5* and *PROM1* seem to mark similar stem cells in the small intestine, *PROM1* does not mark colonic stem cells, whereas *LGR5* does. This illustrates the need to clearly define markers and their limitations if we are to begin to understand the contribution of normal tissue stem cells and cancer stem cells to tumorigenesis.

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ORIGINAL RESEARCH PAPER Barker, N. et al. Crypt stem cells as the cells of origin of intestinal cancer. *Nature* 17 Dec 2008 (doi 10.1038/nature07602) | Zhu, L. et al. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* 17 Dec 2008 (doi 10.1038/nature07589)

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