



Cancer and Intestinal Stem Cells

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To address how stem cell biology is exploited to maintain intestinal cancers we are developing new functional approaches to assaying stem cells *in situ*. After validation in normal intestine these end-points will be applied to assess stem-like cells in cancer models where they can be used to determine the efficacy of therapies.

Renewing tissues and many cancers are maintained by a small number of long-lived stem cells and this explains the interest in trying to define the distinguishing properties of stem cells. Most models of stem cell organisation take account of their longevity and the fact that they self-renew, and also assume that they are stable populations carrying unique identifying characteristics. The results of assays used to test different cell populations for their 'stemness' have appeared consistent with such deterministic models. These assays commonly challenge the ability of cells, separated into discrete populations based on the expression of cell surface antigens, to undergo growth when cultured or engrafted. Cells that are able to support long-term growth are taken as being synonymous with stem cells, and it is assumed that the differential expression of transcription factors underpins the fate of stem cell populations.

However, this interpretation of stem cell organisation now seems too simplistic. For example: cell fate is likely determined by small changes in the expression of regulatory transcription factors in the context of transcriptional

networks; the cell surface signatures of stem cells may not be as stable over time as previously thought; the success of stem cell engraftment may be partly determined by properties of the recipient rather than the transplanted cells (Chang et al., *Nature* 2008; 453:544; Quintana et al., *Nature* 2008; 456:593). Stem cell biology may be driven by stochastic switching between different states in response to variations in the balance of signals coming from complex transcriptional networks.

Given the above our approach is pragmatic: to identify novel ways of assaying stem cells *in situ* with respect to the functional endpoints that are integral to their biology.

What is the multi-potentiality of stem-like cells in intestinal cancers?

Our long-term objective is to determine the repertoire of differentiation options available to cancer stem cells and how this differs from their normal counterparts, and thereby identify unique opportunities for therapies. To measure potentiality we are exploiting the known differences between cell types in the timing of DNA replication during the cell cycle. In undifferentiated embryonic stem cells the key master regulator genes are reproducibly and differentially replicated during S-phase. Genes associated with maintaining pluripotency are replicated early in S-phase, while those associated with neural lineages are replicated late in S-phase (Azura et al., *Nat. Cell Biol.* 2006; 8:532). Promoting neural fate causes switches in replication timing at some loci. The pattern of replication timing for key transcription factors has been described as a barcode of potentiality, indicative of the accessibility of the chromatin for subsequent expression.

We are attempting to devise such a barcode for intestinal stem cells to identify changes in potentiality during carcinogenesis. S-phase cells can be isolated and sorted by

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DNA content into four fractions. Immunoprecipitation for bromodeoxyuridine (brdU) allows newly synthesised DNA to be analysed. To date we have shown reproducible differences in replication timing between different loci (Figure 1). For example, the neural transcription factor Mash1 is replicated late, while the transcription factor Ngn3, expressed in the intestine, is replicated early. Intriguingly, some loci appear to be replicated both early and late suggesting heterogeneity that may be cellular in origin and relate to discrete classes of stem and progenitor cells. Currently, the amount of material obtained on pull-down is restrictive. We aim to increase genomic coverage by amplification to generate a comprehensive characterisation of replication timing. The effect of deleting the APC tumour suppressor gene on replication timing patterns will be determined — deleting this gene also results in dramatic changes in cell type (loss of secretory cell lineages) and differentiation.

Composition and responsiveness of the intestinal niche

Stem cells are thought to be highly dependent on interactions with their immediate microenvironment or niche (Kosinski et al., *PNAS* 2007; 104:15418). The details of this interaction for normal stem cells are poorly understood as is the extent to which stem cells in cancers may also retain such dependency. To devise reporter systems that can recognise an active niche, we are determining the composition of the pericryptal environment and its transcriptional responsiveness to perturbation within the adjacent epithelium. Epithelial specific, Cre-mediated deletion of APC causes profound epithelial dysplasia and hyperproliferation, such that normal intestinal crypts become enlarged and populated with undifferentiated cells. Using laser capture microscopy we have collected pericryptal tissue next to both normal and APC-deficient crypts with the aim of identifying transcriptional changes. RNA has been isolated, amplified and analysed on a transcriptomic microarray. Over 900 genes show changes, with the majority (85%) being downregulated following APC deletion. Twenty-five, with potential roles in tissue remodelling or cell-cell signalling, have been selected for validation by *in situ* hybridisation, in collaboration with Richard Poulson at the Cancer Research UK London Research Institute. Subsequent investigation will use immunohistochemistry and/or the use of reporter transgenic animals to identify the role of the niche in tumours.

Cancer models and tumour progression

At a molecular level the development of intestinal cancers is well characterised, with the most common genetic changes incorporated into a paradigm of progression for colorectal cancers in which loss of APC

is a central early event (as described by Bert Vogelstein's lab at Johns Hopkins University). Despite this it has been shown that many other gene specific mutations can also be associated with the disease (Sjoblom et al., *Science* 2006; 314:268). APC has been deleted in animal models by a variety of strategies that usually lead to the development of benign adenomas. Introduction of additional mutational events in candidate genes has only been partly successful in creating the full carcinomatous (cancer-like) disease. Our ability to induce deletion of APC in the intestinal epithelium lends itself to investigating the nature of other gene mutations that might interact with APC and contribute to the formation of malignant disease. Therefore as an alternative unbiased approach to identifying such genes we are using our Cre models to mobilise a Sleeping-Beauty activated transposable element in mice predisposed to intestinal tumorigenesis by virtue of APC deficiency (Collier et al., *Nature* 2005; 436:272). Cloning and sequencing of the insertion sites in tumours allows affected genes to be identified and associated with tumour pathology. To date we have confirmed, in APC deficient animals, that tumours are increased in both size and number in those animals with a mobilisable transposon. Molecular analysis of insertion sites will begin shortly.

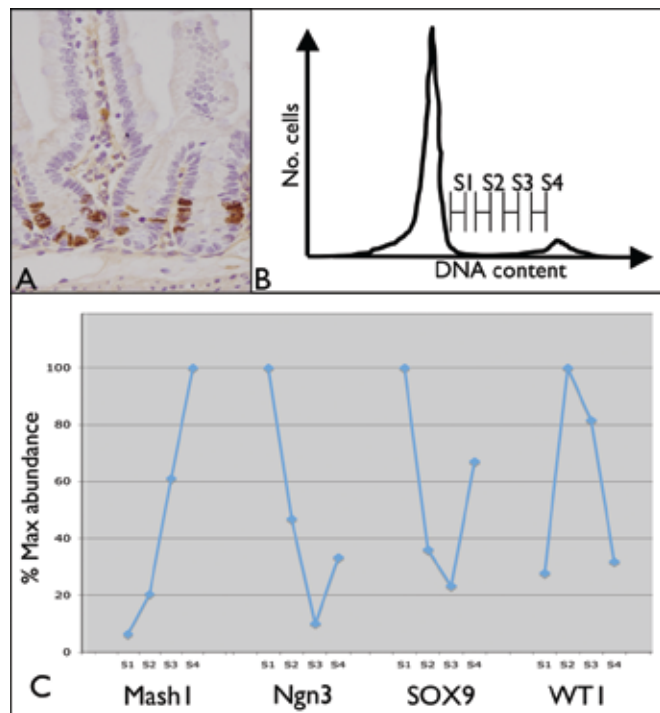


Figure 1. (a) BrdU incorporation into proliferating cells of the intestinal epithelium. (b) FACS analysis by DNA content of disaggregated epithelial cells showing four S-phase fractions. (c) Graphic representation of qPCR analysis of the four genes shown in immunoprecipitated, bromodeoxyuridine incorporated DNA in the four S-phase fractions.

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