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A new tumour suppressor enters the network of intestinal progenitor cell homeostasis

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In this issue of *The EMBO Journal*, Wilson *et al* (2012) elegantly discovered an important new axis for intestinal homeostasis and cancer, using an RNAi screen to enhance the RAS-induced multivulva (MUV) phenotype in *Caenorhabditis elegans*.

Originally identified in their *C. elegans* screen, the authors subsequently show that conditional deletion of Nrbp1 throughout the adult mouse results in an intestinal progenitor cell phenotype that is associated with increased proliferation and perturbed differentiation (Batlle *et al*, 2002; Sansom *et al*, 2004; Finch *et al*, 2009). These data recapitulate the key features associated with deletion of the adenomatous polyposis coli (*Apc*) tumour suppressor gene in the murine intestine. *APC* is the most frequently mutated gene in colorectal cancer in humans, suggesting a common mechanism. The major tumour-suppressive function of *Apc* is to negatively regulate Wnt signalling and, consistent with this, NRBP1-deficient intestines showed a marked increase in Wnt target genes, including *Ccnd1*, *CD44*, *Mmp7*, *Sox9* and *Tnfrsf12a*. Mechanistically it appears that Nrbp1 negatively regulates Sall4, possibly through an Elongin B–C ubiquitin complex; hence, loss of Nrbp1 allows the upregulation of Sall4, which can then activate Wnt signalling (Figure 1).

Over the past 5 years, there has been a remarkable progress regarding the identification of stem cell markers in the intestine (Barker *et al*, 2007). Therefore, one question is whether loss of Nrbp1and downstream Sall4 activation drive a stem-cell-like phenotype in the intestine. In support of this is the marked increase in Lgr5 (the canonical stem cell marker) levels following Nrbp1 loss. Moreover, a recent work by Hobbs *et al* (2012) showed that Sall4 has essential roles in the maintenance of embryonic germ cells and the differentiation of spermatogonial progenitor cells, and a previous work has shown its requirement for early embryonic development (Warren *et al*, 2007). However, the finding that long-term deletion of Nrbp1 in the intestine is not tolerated strongly argues against a stem-cell-like phenotype in Nrbp1 cells. Moreover, the main cell type that is produced in the crypts of Nrbp1 are highly proliferative cells that fail to differentiate and therefore are presumably not *bona fide* stem cells. It is interesting to note that OCT4 overexpression in the murine intestine also drives a progenitor rather than a stem cell phenotype (Hochedlinger *et al*, 2005) and it is only in the *Drosophila* intestine when Notch is overexpressed that tumours composed of purely stem cells arise (Hayward *et al*, 2005). It should be noted that human colorectal cancers retain many features of the intestinal crypt and predominantly consist of progenitor cells, while cancer cells expressing the intestinal stem cell (ISC) signature represent only a fraction of the tumour (Batlle *et al*, 2002). Given the phenotypic and transcriptome similarities with *Apc* gene deletion, it is rather surprising that Nrbp1-deficient cells are outcompeted by wild-type cells and crypts cannot grow

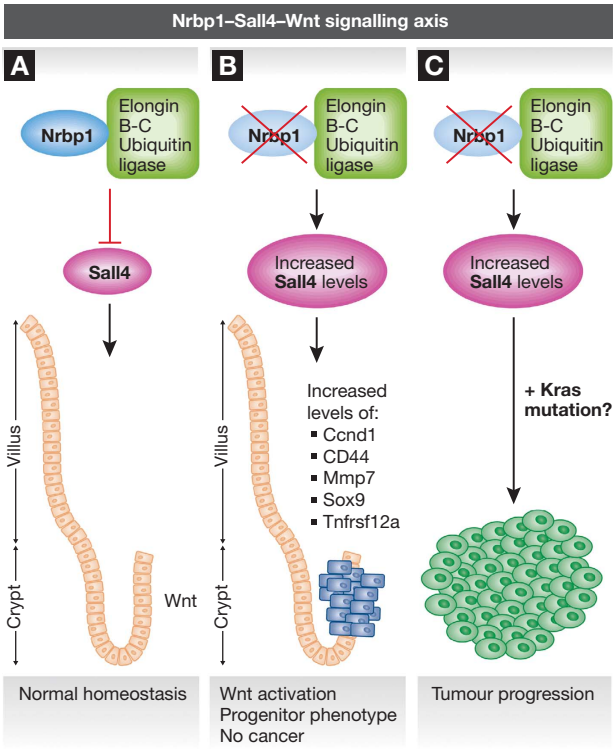

Figure 1 A novel Nrbp1–Sall4–Wnt signalling axis. (**A**) Nrbp1 regulates Sall4 levels through its interaction with the Elongin B–C E3 ubiquitin ligase complex, allowing the maintenance of intestinal progenitor cell homeostasis. (**B**) Loss of Nrbp1 increases Sall4 levels, leading to Wnt activation and an expansion of the crypt progenitor-like cells with aberrant proliferation, differentiation and localisation of cells along the crypt villus axis. (**C**) Nrbp1 loss following other mutational events such as KRAS drives tumour progression and confers a poor prognosis.

over longer periods in culture. This highlights that Nrbp1 is unlikely to be an initiating tumour suppressor mutation and would not be able to substitute for *Apc* gene mutation. One clue to why cells may be lost is the finding that low-level deletion of Nrbp1 can initiate tumourigenesis, suggesting that the presence of surrounding wild-type cells may provide survival advantages to cells lacking Nrbp1.

In contrast to the initiating properties of NRBP1 loss, the data suggesting a cooperative role for Nrbp1 downregulation in cancer appear compelling. Wilson *et al* (2012) show that Nrbp1 is downregulated in a number of diverse cancer types, including colorectal tissue and lung. It is interesting to note that many cancers show a deregulation of Wnt signalling without loss of the *Apc* gene or activating mutations in β-catenin. It is possible that Nrbp1 downregulation may contribute to this; thus, future studies correlating Nrbp1 downregulation to Wnt signalling activation in a number of human cancers can provide information on how widespread a function this could be. This may have therapeutic implications, as a number of Wnt signalling inhibitors exist, such as cell surface-blocking antibodies, that are unlikely to work well if *Apc* is mutated or β-catenin is activated, as there will then be ligand-independent signalling (Ettenberg *et al*, 2010). In contrast, Wilson *et al* (2012) show that Nrbp1-deficient cells are still dependent on ligand signalling as removal of R-Spondin, a Wnt-signalling agonist in intestinal cultures, leads rapidly to crypt death.

One prediction from the promotion of the MUV phenotype in *C. elegans* would be that Nrbp1 downregulation might be associated with tumours that harbour KRAS mutation and loss might cooperate in mouse models carrying KRAS mutation. It is possible that KRAS mutation may overcome this long-term selection against Nrbp1-deficient cells, and it will be of interest to see what extra properties Nrbp1 downregulation confers on KRAS mutant cells. To this end, one could predict that increased levels of Sall4 and Wnt signalling might increase the numbers of cells expressing the stem cell/progenitor cell signature within tumours, a feature conferred by Wnt signalling in a number of tumours (Zheng *et al*, 2010).

In summary, this study represents the discovery of an exciting novel tumour suppressor axis that plays a key role in intestinal homeostasis and that may be of therapeutic relevance to cancer. As the polyomic and sequencing data continue to proliferate, the validation of these targets remains key. None would have predicted that a screen from promotion of a KRAS phenotype in *C. elegans* would have implications on intestinal homeostasis. This emphasises the requirement for functional screens and, if strong cooperation exists, physiologically relevant findings for the mammalian/human situation might rapidly emerge.

Conflict of interest

The authors declare that they have no conflict of interest.

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