IN THE NEWS

Double trouble

Scientists are constantly striving to understand the molecular events that predate the onset of cancer.

Now, a study in *Science* has shown that an increase in the number of immature cells in the lining of the bowel predisposes to the development of colon cancer. This novel phenotype was seen in mice that had been genetically manipulated to express twice the normal amount of the imprinted gene insulin-like growth factor *(Igf)2*.

In humans, loss of imprinting of *IGF2* is associated with several tumour types, including colorectal cancer. Surprisingly, in the mutant mice, doubling the amount of this growth factor did not affect the turnover of intestinal cells but instead seemed to block their maturation, which increased the numbers of immature cells in the gut lining. Importantly, mice that had both defective *Igf2* imprinting and an additional tumour-promoting mutation developed twice as many bowel tumours as mice with the tumour-promoting mutation alone. This indicates that the immature bowel cells are a 'hot-spot' for neoplastic transformation.

This study shows how a combination of epigenetic and genetic misfortune can have catastrophic results. As co-author Christine Iacobuzio-Donahue explains: "In the mice with a double dose of IGF2, everything is pretty normal except for the extra precursor cells … But when the genetic mutation is present, too, we found a clear cost for what otherwise appears to be a benign effect of extra IGF2." (*myDNA.com*, 25 February 2005).

Andrew Feinberg, main author, is excited by the prospect that "…this discovery should expand attention in colon cancer to earlier events, situations present well before tumors appear." (*bio.com*, 24 February 2005). The hope is that this will lead to effective cancer prevention strategies.

Shannon Amoils

EPIGENETICS

RB1 goes global

The retinoblastoma 1 (RB1) protein is a well-studied tumour suppressor protein. It regulates the transcription of cell cycle genes through physical interaction with the E2F family of transcription factors and by recruiting chromatin-modifying enzymes to specific promoters. A new study by Blasco and colleagues in the April issue of *Nature Cell Biology* now shows that RB1 also maintains constitutive pericentric and telomeric heterochromatin — the inactive form of chromatin — with the potential of non-selectively repressing gene expression. This adds a new and more global tool to the repertoire of tumour-suppressor functions of RB1.

To address the role of the RB1 family — which comprises RB1, retinoblastoma-like 1 (RBL1) and RBL2 — in genome stability and chromosome structure, the authors used mouse embryonic fibroblasts (MEFs) from single-, double- (DKO) and triple-knockout (TKO) animals. After several passages, aneuploid cells were already evident in DKO and TKO MEFs, and after 20 passages, TKO cells established tetraploidy. These chromosome segregation defects were confirmed by fluorescence *in situ* hybridization (FISH) analysis, which showed chromosomes with four sister chromatids ('butterfly chromosomes').

Investigating changes in the chromatin structure of TKO cells in more detail, Blasco and colleagues found reduced DNA methylation, increased acetylation of histone 3 (H3), and decreased tri-methylation of histone 4 at lysine 20 (H4K20). As hypermethylation of DNA and histones, as well as the hypoacetylation of histones, are hallmarks of constitutive heterochromatin, the authors investigated the effect of RB1 proteins on pericentric and telomeric heterochromatin. Indeed, they found decreased DNA methylation and tri-methylation of H4K20 at pericentric heterochromatin in TKO cells. Using a dominant-negative mutant of E2F, the authors excluded a possible involvement of transcriptional changes caused by the absence of an RB1–E2F interaction.

As tri-methylation of H4K20 at pericentric chromatin is known to be mediated by the histone methyltransferases (HMTases) Suv4-20h1 and Suv4-20h2, the authors focused on potential interactions of these enzymes with RB1. First, they showed

SIGNALLING

Reassessing Ras routes

It's easy to group all isoforms of a certain protein under the same umbrella. But a closer look at the Ras GTPases H-ras, N-ras and K-ras reminds us that they generate isoform-specific biological outputs, and that this is influenced by their differential localization at the plasma membrane (PM) and subcellular membranes. This, in turn, reflects their C-terminal lipid modifications: after farnesylation, AAX proteolysis and methylation of the farnesylated cysteine, H-ras and N-ras are palmitoylated, which enables them to localize to the PM and Golgi membranes; by contrast, K-ras bypasses the secretory pathway and localizes to the PM.

Both the PM and the Golgi are sites of active Ras signalling, but how this compartmentalized localization and activity of palmitoylated Ras isoforms is accomplished has remained unclear. Work by Rocks *et al.*, reported in *Science*, now shows that a constitutive de- and re-palmitoylation cycle maintains the specific compartmentalization.

Golgi-localized Ras was thought to comprise nascent proteins trafficking to the PM. But by inhibiting protein synthesis, and thereby removing nascent proteins, the authors showed that H-ras and N-ras still localized at the Golgi. Moreover, photobleaching and photo-activation studies showed that palmitoylated Ras cycled between the PM and the Golgi, and that Golgi Ras was replenished by retrograde transport of PM-localized Ras. Using a hexadecylated version of N-ras — HDFar — that could not undergo de- and re-palmitoylation, the authors showed that this localization wasn't mediated by clathrin, caveolae or cholesterol, but that de- and re-palmitoylation events were required. Micro-injected HDFar localized nonspecifically throughout the membrane system.

The authors also noticed that the kinetics of H-ras and N-ras trafficking were different. H-ras is palmitoylated on two cysteines, whereas palmitate moieties are added to only one cysteine in N-ras. N-ras trafficked faster than H-ras, so Rock *et al.* studied monopalmitoylated H-ras mutants. C181S and C184S H-ras had an increased preference for Golgi localization relative to wild-type H-ras, and showed faster PM–Golgi exchange. By developing a new assay to compare the dynamics of Ras activation at the PM and Golgi, Rock *et al.* showed H-ras to be rapidly and transiently activated at the PM in response to growth factor stimulation, and to have a delayed onset but to be sustained at the Golgi. By contrast, active H-ras C184S and N-ras were detectable

© 2005 Nature Publishing Group