which develop endometrial cancers and phaeochromocytomas that frequently show loss of heterozygosity of the second *Pten* allele. Treatment of $Pten^{+/-}$ mice with CCI-779 for 20 weeks significantly reduced tumour volume and, again, this seemed to be due to growth inhibition rather than cell killing.

So, by preventing $PTEN^{-/-}$ cancers — which include prostate and endometrial tumours and glioblastomas — from activating FRAP1 through the PI3K pathway, we might be able to halt their growth. AKT remains an enticing therapeutic target as its inhibition should block PI3K-mediated growth *and* survival signals. But, in the meantime, CCI-779 is worthy of clinical investigation.

Cath Brooksbank

References and links

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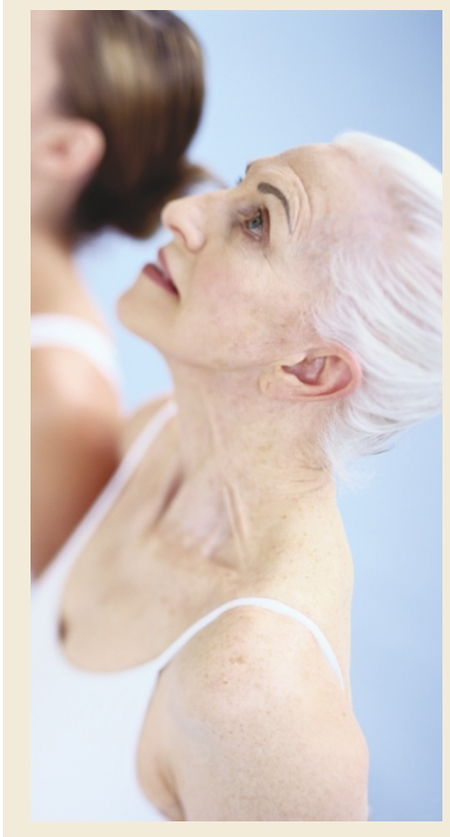

CHEMOTHERAPY

Smoking gun

5-fluorouracil (5-FU) has proved to be one of the most effective chemotherapeutics for colon cancer. It induces apoptosis in rapidly dividing cells, but little is known about the molecular mechanism by which it causes them to self-destruct. In the October issue of *Nature Medicine*, Bert Vogelstein and colleagues report that 5-FU depends on ferredoxin reductase (FR) — an 'electron gun' — to kill cells through oxidative stress.

Previous studies have shown that apoptosis induction by 5-FU involves the transcription factor p53, but what else? Vogelstein's group used serial analysis of gene expression (SAGE) to identify 5-FU-responsive genes. By comparing gene-expression patterns between 5-FU-sensitive $TP53^{+/+}$ human colon cancer cells and 5-FU-resistant $TP53^{-/-}$ cells, they could isolate genes that were only transcribed in association with p53-mediated cell death.

SAGE libraries were constructed from mRNA purified from each cell type after 18 hours of treatment with 5-FU — long enough for transciption to be activated, but well before the cellular apoptotic programme starts. Surprisingly, only a few genes were induced by 5-FU in a p53-dependent manner. One unexpected finding was that the gene that encodes FR, which had not been previously associated with p53 or apoptosis, was upregulated within 3 hours of drug treatment, indicating that it could be a direct transcriptional target of p53. Sure enough, the authors found that the FR gene promoter contained a p53-binding site and that its expression was indeed activated after 5-FU treatment. Furthermore, p53 activates FR expression in three different colon cancer cell lines.

Next, Vogelstein's group investigated the biological functions of FR. The colon cancer cell line studied carried three copies of the gene that encodes FR, but deleting all three copies was lethal, indicating its essential role in cell survival. Disruption of two of the three copies reduced 5-FU-induced apoptosis, so it is likely to be an important mediator of cell death.

But how does FR contribute to the apoptotic pathway? FR sits on the matrix side of the inner mitochondrial membrane, where it is involved in transferring electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to substrates such as cholesterol. Under substrate-limiting conditions, FR releases electrons, which can then generate reactive oxygen species (ROS) such as superoxide. So, too much FR and not enough substrate can generate oxidative stress. Several previous studies had already implicated mitochondrial-derived ROS in p53-mediated cell death, so FR might be one of the missing links in this process.

To prove that FR's role in p53-mediated cell death involves the generation of oxidative stress, the authors used a chemical indicator to measure ROS production after 5-FU treatment. Colon cancer cells with intact *TP53* and *FR* genes produced high levels of ROS after exposure to 5-FU, whereas colon cancer cells with disruptions in *TP53* or *FR* did not. Furthermore, a pharmacological antioxidant prevented 5-FU-induced apoptosis, showing that killing cells by 5-FU depends on ROS generation.

So, is this definitive proof that 5-FU induces toxicity strictly through p53 and generation of oxidative stress? Apparently not, as the authors showed that neither disruption of *TP53* nor *FR* allowed colon cancer cells to survive 5-FU treatment when measured by clonogenic assays, a more stringent test of chemosensitivity than the apoptosis assays used in this study. Therefore, 5-FU seems to have other toxic effects in cells, not involving p53, that await discovery.

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WEB SITES

Bert Vogelstein's lab: http://www.med.jhu.edu/pharmacology/pages/faculty/vogelstein.html

The SAGE home page: www.sagenet.org/