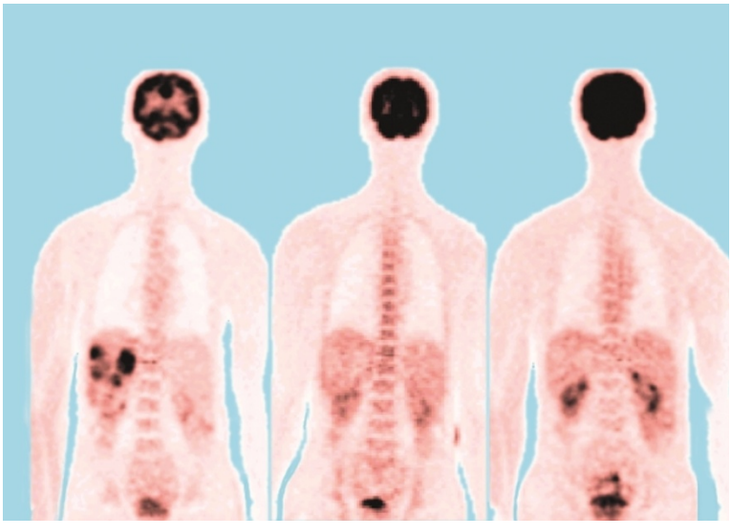
ONCOGENES

Gut reaction



False-coloured ^{18}fluorodeoxyglucose positron emission tomography scans of a patient who was enrolled in the study, showing gastrointestinal stromal tumour status at the start of the study (left), after 8 days (middle) and after 4 weeks (right) of treatment with imatinib. Courtesy of Allan van Oosterom, Catholic University, Leuven, Belgium.

Soft-tissue carcinomas (tumours of mesodermal tissue) are — thankfully — rare. But when they do occur, the only option is surgery. Survival of individuals with soft-tissue sarcomas is usually about 53 weeks, and treatment with conventional chemotherapy has so far been unsuccessful in promoting survival. But now, encouraging news from van Oosterom and colleagues, reporting in the 27 October issue of *The Lancet*, shows that imatinib (STI-571 or Glivec) is both safe and effective for the treatment of gastrointestinal stromal tumours (GISTs).

The United States Food and Drug Administration approval of imatinib for the treatment of chronic myelogenous leukaemia (CML) has created much excitement. As well as inhibiting BCR–ABL, it also blocks other oncogenic tyrosine kinases, including KIT, the receptor for stem-cell factor. KIT is overexpressed on many — but not all — GISTs, so would Glivec's success in CML be repeated for this tumour? This Phase I study aimed to establish a safe dose level for treating GISTs.

van Oosterom and colleagues enrolled 40 patients with sarcomas, 36 of which were GISTs. The GIST patients were all KIT positive. Patients were given one of four dose levels: either 400 mg daily, 300 mg twice daily, 400 mg twice daily or 500 mg twice daily. Treatment was given until the tumours progressed, side effects became too severe or the patients refused treatment. Otherwise, treatment continued for at least 1 year.

The main side effects during the first 8 weeks of treatment were skin rash, oedema, diarrhoea, nausea and vomiting. More serious side effects, such as intratumoral bleeding, myelosuppression and neutropaenia occurred in a small minority of patients, but most importantly, side effects were dose limiting at 500 mg imatinib twice daily; at 400 mg imatinib twice daily or less, side effects were manageable and diminished as treatment continued. So, 400 mg twice

TUMOUR SUPPRESSORS

One-hit wonders?

Dogma would have it that both copies of a tumour-suppressor gene must be inactivated to promote tumour formation. But a new breed of tumour suppressor is now emerging — for which inactivation of one allele is enough. In 1998, James Roberts, Christopher Kemp and their collaborators established that *CDKN1B*, which encodes the cyclin-dependent kinase inhibitor Kip1, is haplo-insufficient for tumour suppression. Loss of just one copy of the Pten tumour suppressor is also sufficient to promote tumour progression in a transgenic model of prostate cancer. Now, Kazushi Inoue and colleagues define a new one-hit tumour suppressor in the 15 November issue of *Genes & Development*. Most tumours downregulate the p53 pathway by mutating or deleting both *TP53* alleles, expediting the disposal of p53 by upregulating MDM2, or inactivating ARF, which inhibits MDM2. These proteins are kept in check by a complex network of controls: ARF, for example, is regulated by several transcriptional activators and repressors, including the transcriptional activator DMP1.

Might DMP1 be a tumour suppressor in its own right? Inoue and colleagues previously reported that Dmp1$^{-/-}$ mice didn't spontaneously develop tumours in their first year of life, but now the mice are older and we're all a little wiser. In their second year, *Dmp1*$^{-/-}$ mice spontaneously developed a variety of tumour types but, intriguingly, so did *Dmp1*$^{+/-}$ mice. This was not due to loss of heterozygosity or epigenetic silencing of the wild-type *Dmp1* allele because, in all tumours tested, the second allele was retained and mRNA and protein were produced.

To explore whether *Dmp1* mutant tumours select for loss of other genes in the p53 pathway, the authors crossed *Dmp1* mutant mice with Eμ-Myc mice, which spontaneously develop Burkitt's lymphomas. Around half of these tumours usually contain either p53 mutations or Arf mutations. Both Eμ-Myc/*Dmp1*$^{+/-}$ mice and Eμ-Myc/*Dmp*$^{-/-}$ mice developed tumours with a much shorter latency than Eμ-Myc/*Dmp*$^{+/+}$ mice — 12 weeks rather than 6 months. Most of the tumours in the Eμ-Myc/*Dmp*$^{+/-}$ mice produced detectable wild-type Dmp1 protein, supporting the notion that losing just one *Dmp1* allele promotes tumour formation. Furthermore, only a small percentage of these mice (9% for *Dmp1*$^{-/-}$ mice; 14% for *Dmp1*$^{+/-}$ mice) sustained mutations in p53 or Arf.

Dmp1, then, is a *bona fide* tumour suppressor and its downregulation, even by 50%, reduces the selection pressure for loss of p53 or Arf function. One of the central tenets of cancer biology — Knudson's two-hit hypothesis — might just have sustained another hefty blow.

Cath Brooksbank

References and links

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daily was defined as the recommended dose for further studies.

Most encouragingly, objective tumour responses — assessed using the Response Evaluation Criteria in Solid Tumours (RECIST) and ^{18}fluorodeoxyglucose positron emission tomography (see picture) — showed that tumour regression occurred in 69% of patients and, in most patients, within 8 days of beginning treatment. Imatinib was particularly successful in treating patients with advanced GISTs.

So, although still at an early clinical trial stage, imatinib shows great potential for the treatment of this previously intractable tumour. Future studies will hopefully clarify the optimum recommended dosage and duration of treatment.

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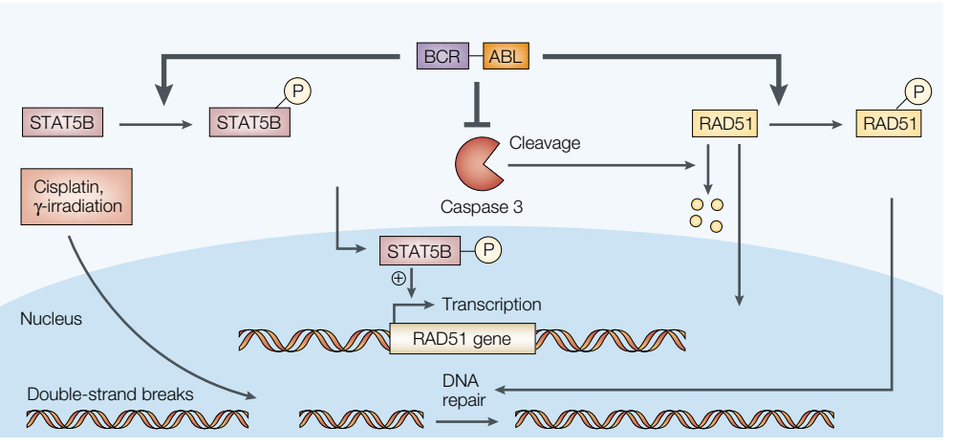

DRUG RESISTANCE

ABL to resist

We think of cancer cells as having unstable genomes, so it seems counterintuitive that they might increase their ability to repair DNA. But Artur Slupianek and co-workers now describe how some leukaemia cells do just that to resist attack by DNA-damaging drugs.

Tumours expressing oncogenic tyrosine kinases such as BCR–ABL — the fusion protein that is a hallmark of chronic myelogenous leukaemia — are resistant to DNA-damaging drugs. To find out why, Slupianek and colleagues expressed a deletion mutant of BCR–ABL that lacked the SH2 and SH3 domains of ABL (BCR–ABLΔΔ), in a myeloid cell line. Cells expressing this mutant were sensitive to cisplatin and mitomycin C, whereas cells expressing full-length BCR–ABL were resistant to these drugs. The SH2 and SH3 domains of ABL are required to activate the transcriptional activator STAT5B, and drug resistance could be reinstated in BCR–ABLΔΔ cells by expressing a dominant-active mutant of STAT5B (STAT5B-DAM). So which of STAT5B's many target genes is responsible for drug resistance? Expression of the DNA-repair gene RAD51 and some of its paralogues was dysregulated in cells expressing either BCR–ABL or the combination of BCR–ABLΔΔ and STAT5B-DAM, but not in the parental cell line or in cells overexpressing BCR–ABLΔΔ alone.

Is activation of *RAD51* expression controlled directly by STAT5B? STAT5B could drive transcription of a luciferase reporter gene fused to *RAD51*'s promoter in cells expressing BCR–ABL, but not in cells expressing BCR–ABLΔΔ. But increased transcription might not be the whole story: RAD51 is a substrate of the apoptotic protease caspase 3, which is inhibited by BCR–ABL. Western blots to detect activated fragments of caspase 3 and a proteolytic product of RAD51 revealed that caspase 3 was activated by cisplatin in BCR–ABLΔΔ-expressing cells and the parental cell line, but not in cells expressing BCR–ABL. *RAD51* overexpression is sufficient to cause drug resistance because expression of *RAD51* in BCR–ABLΔΔ cells restored most of their ability to resist cisplatin and mitomycin C treatment, whereas expression of a *RAD51* antisense sequence in BCR–ABL-expressing cells sensitized them to the drugs.

Is drug resistance caused by increased ability to repair a lethal accumulation of double-strand breaks, or some other property of RAD51? The repair of double-strand breaks can be measured by transfecting cells with two constructs that, when repaired, yield an intact gene for green-fluorescent protein (GFP) and hence a fluorescent signal. In BCR–ABL-expressing cells, introduction of RAD51 increased levels of repair, whereas a RAD51 antisense construct decreased it. This effect was not seen in cells expressing a kinase-dead mutant of BCR–ABL.

But increasing RAD51 levels is not the only way in which BCR–ABL bolsters DNA repair: coimmunoprecipitations revealed that both c-ABL and BCR–ABL interact with RAD51. Phosphorylation of RAD51 was increased by cisplatin or mitomycin C in the parental cell line, which expresses c-ABL. By contrast, RAD51 was constitutively phosphorylated in cells expressing BCR–ABL. RAD51 has previously been reported to be phosphorylated by c-ABL on two tyrosine (Y) residues — Y54 and Y315. Tyrosine-to-phenylalanine (F) mutations at these two residues indicated that Y315 is the main site of phosphorylation by BCR–ABL. Transfection of BCR–ABL-positive cells with the Y315F mutant increased their sensitivity to cisplatin and mitomycin C, indicating that phosphorylation of Y315 by BCR–ABL controls drug resistance.

So, BCR–ABL has three different ways of boosting RAD51's activity (see picture): by increasing its expression, decreasing its degradation and activating it through post-translational modification. It's an intriguing possibility that other oncogenic tyrosine kinases might also be able to activate one or more of these mechanisms. Could we resentitize resistant tumours to DNA-damaging agents by treating them with tyrosine kinase inhibitors? And does aberrant expression of *RAD51* and its paralogues contribute to genomic instability in malignant cells?

Cath Brooksbank

References and links

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WEB SITES

Richard Fishel's lab: http://www.kcc.tju.edu/staff/fishel/

Tomasz Skorski's lab: http://www.temple.edu/biology/faculty/skorski.html