TRIAL WATCH

Anti-angiogenics

At the American Society for Clinical Oncology meeting in Orlando, Florida, 18–21 May, National Cancer Institute researchers reported that the angiogenesis inhibitor bevacizumab (Avastatin) — a recombinant humanized monoclonal antibody that binds vascular endothelial growth factor — slows tumour growth in patients with metastatic renal-cell carcinoma. James C. Yang and colleagues showed that in patients who received the highest doses of bevacizumab (10 mg/kg), tumours took 2.5 times longer to grow than in patients who did not receive the drug (five versus two months) — a statistically significant difference. There was also a smaller, but still significant, effect from a lower dose of bevacizumab (3 mg/kg). The Phase II trial involved 116 patients with advanced cancer who had failed other treatment approaches, and only minimal side effects were associated with the drug. These findings provide important support for the efficacy of anti-angiogenic agents — the clinical potential of which has been questioned. The drug is currently being tested in Phase III trials for breast and colorectal cancer, as well as Phase II trials for prostate, breast, colorectal, cervical, ovarian, pancreatic and lung cancers. It is also being tested for its effects on mesothelioma and several types of leukaemia.

WEB SITE

<http://www.gene.com/gene/pipeline/status/oncology/avastin/index.jsp>

DRUG RESISTANCE

Resisting arrest

Like criminals, cancer cells can resist arrest. Clemens Schmitt *et al.*, reporting in the 3 May issue of *Cell*, show that cancer cells can respond to chemotherapy by arresting, as well as dying, and drug resistance requires the abolition of both pathways. Different proteins are involved in these pathways, so their mutation during tumorigenesis can determine the response to therapy.

The authors examined the response to chemotherapy in *Eμ-myc* transgenic mice, which overexpress the *c-Myc* oncogene and are prone to B-cell lymphoma; defined genetic lesions can also be introduced into these mice to allow further dissection of the pathways involved. Lymphomas that arose in *Eμ-myc* mice regressed rapidly following treatment with the chemotherapeutic agent cyclophosphamide (CTX), indicating that CTX induced apoptosis. However, in *Eμ-myc* mice that were heterozygous for *Trp53* (these frequently lose the wild-type allele to become *Trp53* null) despite an initial, albeit delayed, p53-independent apoptotic response, the tumours soon progressed and the mice died.

But p53 can also induce cell-cycle arrest, so could cells arrest following CTX treatment if the apoptotic response is inhibited? Haematopoietic cells derived from *Eμ-myc* and *Eμ-myc Trp53^{+/-}* mice were transduced with the apoptotic inhibitor Bcl2 to test this hypothesis. Expression of Bcl2 in *Eμ-myc* mice prevented tumour regression in response to CTX, but the tumours did not rapidly progress, as occurred in p53-null mice; instead, they entered a condition of stasis. The induction of cell-cycle arrest was confirmed by showing that untreated Bcl2-expressing cells continued to proliferate, but cells treated with CTX did not. Expression of Bcl2 also suppressed loss of the wild-type allele in *Trp53^{+/-}* tumours, but not following treatment with CTX, indicating that p53 confers a selective advantage for resistance to CTX, presumably by inducing cell-cycle arrest.

So could proteins other than p53 determine the response to CTX? The authors investigated the effect of the tumour suppressors Ink4a and Arf — which are encoded by the *Cdkn2a* locus — by crossing *Eμ-myc* mice with *Arf^{+/-}* mice (deleted for exon 1β) and *Ink4a/Arf^{+/-}* mice (deleted for exon 2). All mice developed tumours more rapidly than *Eμ-myc* mice, with a consequent reduction in apoptosis, and the wild-type *Cdkn2a* allele was lost in all cases for *Ink4a/Arf^{+/-}* mice. In *Arf^{+/-}* mice, ~65% had lost exon 1β and the rest displayed susceptibility to a heterozygous deletion in exon 2. As deletion of Ink4a alone does not increase tumorigenesis in *Eμ-myc* mice, Arf, not Ink4a, seems to be important for suppressing formation of Myc-induced lymphomas. However, the response to CTX treatment was surprisingly different according to whether Ink4a was present: *Ink4a/Arf*-null mice have a

worse prognosis than *Arf*-null mice. Survival was further reduced when Bcl2 was expressed, indicating that Ink4a and Bcl2 act in different pathways to induce resistance to CTX.

Spontaneous mutations in the *Cdkn2a* locus also occur frequently in cancer, and by correlating the response to therapy with polymerase chain reaction analysis of these mutations, the authors confirmed that the loss of Ink4a provides a growth advantage to cancer cells. Interestingly, expression of Bcl2 prevents loss of the Ink4a allele until the cells are treated with CTX, which indicates that Ink4a suppresses growth following CTX treatment via a non-apoptotic pathway.

Ink4a can accumulate following treatment with CTX in p53-null cells that continue to proliferate. This indicates that p53 is required for Ink4a's cytostatic function. In fact, the two proteins seem to cooperate: in either p53-null *Ink4a/Arf* heterozygotes, or *vice versa*, the wild-type allele was never lost, indicating that the selection pressure to mutate the other gene is alleviated and they act together to induce cell-cycle arrest. The arrest was shown to be premature senescence because Bcl2-expressing *Eμ-myc* cells also expressed a senescence-associated marker — acidic β-galactosidase. *Arf*-null cells, but not p53-null or *Ink4a/Arf*-null cells, also expressed SA-β-gal following CTX treatment.

A few key genes can therefore drastically alter the response to the same drug, which can only strengthen the argument for a patient-tailored approach to cancer treatment.

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References and links

ORIGINAL RESEARCH PAPER Schmitt, C. A. *et al.* A senescence program controlled by p53 and p16^{Ink4a} contributes to the outcome of cancer therapy. *Cell* **109**, 335–346 (2002)

WEB SITE

Scott Lowe's lab: <http://www.cshl.org/public/SCIENCE/lowe.html>