THERAPEUTICS

Breaking down resistance

The kinase inhibitor imatinib (Glivec) can induce complete remission in patients with chronic-phase chronic myelogenous leukaemia (CML). Patients whose disease has advanced to blast crisis, however, frequently become resistant to the drug, due to mutations in the *BCR–ABL* kinase domain. Azam *et al.* have developed an *in vitro* screen to survey mutagenized forms of BCR–ABL, and obtained a more comprehensive picture of mutations that confer drug resistance.

Kinases typically exist in equilibrium between 'open' (active) and 'closed' (autoinhibited) states. Co-crystallization studies of imatinib and the ABL kinase domain have shown that the drug achieves its specificity by trapping the kinase in the closed conformation. Most patients who become resistant to imatinib therefore harbour mutations within the *BCR–ABL* kinase domain. For example, mutations have been discovered that sterically hinder drug occupancy of the kinase active site, alter the phosphate-binding P loop, or influence the conformation of the loop that surrounds the active site. Different mutations confer different levels of resistance to the drug.

Azam *et al.* reasoned that mutations in other domains of the protein, in addition to the kinase domain, might also mediate resistance. To look for these, they randomly mutagenized the *BCR–ABL* gene by propagating it in a bacterial strain that is deficient in DNA repair. The screen led to the isolation of 112 protein variants with distinct amino-acid substitutions in 90 residues. The kinase activity of 65 variants was confirmed, and 59 of these were found to be resistant to imatinib treatment. Only 13 of these mutations had been previously identified in patients with drug-resistant CML.

So, how do these mutations confer drug resistance? A total of 26 resistance-associated mutations were found to lie outside the kinase domain. These were instead located in the cap region (which is implicated in ABL autoinhibition), as well as in the Src-homology domains 2 (SH2) and 3 (SH3), and the linker region between the SH2 and the catalytic domains. Structural modelling studies indicated that many of these mutations destabilize the closed conformation of the ABL kinase, shifting the protein equilibrium towards the open, active kinase state. This conformation precludes drug binding.

Analysis of these drug-resistant forms of BCR–ABL will improve the ability to predict patient drug responsiveness. For example, mutations in the SH3 domain, and some regions of the SH2 domain, generated only low levels of drug resistance. It should therefore be possible to treat patients who are found to have mutations in these regions with escalating doses of imatinib. The authors also discovered some P-loop mutations that confer very high levels of drug resistance. These have not yet been reported in patients, but any individuals who are found to carry these variants are unlikely to respond to imatinib. Some of the mutations were also associated with increased kinase activity and accelerated disease progression. These will be useful in determining patient prognosis.

Accompanying papers by Nagar *et al.* and Hantschel *et al.* provide a structure–function analysis of c-ABL, and a rationale for the mechanisms of many of the drug-resistant variants recovered from this screen.

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References and links

ORIGINAL RESEARCH PAPER Azam, M., Latek, R. R. & Daley, G. R. Mechanisms of autoinhibition and STI-571/Imatinib resistance revealed by mutagenesis of *BCR–ABL*. *Cell* **112**, 831–843 (2003)

FURTHER READING Hantschel, O. *et al.* A myristoyl/phosphotyrosin switch regulates c-Abl. *Cell* **112**, 845–857 (2003) | Nagar, B. *et al.* Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* **112**, 859–871 (2003)

WEB SITE

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IN BRIEF

IMMUNOTHERAPY

Nonredundant roles of antibody, cytokines, and perforin in the eradication of established Her-2/neu carcinomas.

Curcio, C. *et al. J. Clin. Invest.* **111**, 1161–1170 (2003)

A DNA vaccine that encodes ERBB2 (also known as HER2/neu) has been shown to stimulate immune rejection of established breast tumours in mice. Curcio *et al.* analysed this mechanism and showed that successful immunotherapy requires the actions of $CD4^+$ and $CD8^+$ T cells, CD1d-restricted natural-killer T cells, neutrophils, macrophages, antibodies, Fc receptors, IFN-γ and perforin.

CARCINOGENESIS

Immune enhancement of skin carcinogenesis by $CD4^+$ T cells.

Daniel, D. *et al. J. Exp. Med.* **197**, 1017–1028 (2003)

Daniel *et al.* investigated the immune response during epithelial carcinogenesis in K14-HPV16 mice. Although pro-inflammatory $CD4^+$ T cells were found to infiltrate tumours, they targeted the bacterial infections associated with dysplastic skin, rather than the cancer cells. Surprisingly, the ensuing inflammatory response promoted epithelial carcinogensis. Mice that lacked $CD4^+$ T cells were found to have delayed neoplastic progression and a lower incidence of tumours, indicating that the inflammatory response can, in some instances, enhance neoplastic progression.

TARGET DISCOVERY

Identification of Down's syndrome critical locus gene *SIM2-s* as a drug therapy target for solid tumours.

DeYoung, M. P. *et al. Proc. Natl Acad. Sci. USA* **100**, 4760–4765 (2003)

By combining data-mining and antisense technologies, DeYoung *et al.* have shown that the short *Single Minded 2* (*SIM2-s*) gene — found in the Down's syndrome critical region of chromosome 21 — is expressed in certain solid tumours. Antisense *SIM2-s* caused growth inhibition and apoptosis *in vitro*, and inhibition of tumour growth *in vivo*. These findings have important implications for diagnosis and treatment of solid tumours, and could aid understanding of the cancer risk in Down's syndrome patients.

PROSTATE CANCER

Cancer-related changes in prostate DNA as men age and early identification of metastasis in primary prostate tumours.

Malins, D. C. *et al. Proc. Natl Acad. Sci. USA* 15 Apr 2003 (epub ahead of print)

Malins *et al.* have shown that the DNA of histologically normal prostate tissue changes with age. 42% of older men have structural changes that resemble those from primary prostate tumours; these are caused by age-related DNA damage. Importantly, this analysis could distinguish primary prostate tumours from which metastases have developed, with 90% sensitivity and specificity. This could help identify men at risk of developing prostate cancer, and the chances of metastasis of those who already have it.