PROGNOSIS

Prediction power

Wouldn't we all like the power to predict the future? For breast cancer patients, the accuracy with which the progress of their disease can be mapped can make the difference between whether they are overtreated or undertreated and can also affect predictions of their survival. Current criteria used for prognosis in breast cancer include age, the size of the tumour, axillary-node status, histological type, pathological grade and hormone-receptor status. van de Vijver and colleagues now add a gene-expression profile to the list of useful prognostic indicators, as they report in the 19 December issue of *New England Journal of Medicine*.

The authors used a 70-gene prognosis profile that was first described by the same research team earlier in 2002 to classify 295 patients with primary breast cancer — which included 61 of the 78 patients, all lymph-node negative, from the first study — into poor-prognosis and good-prognosis groups. The prognosis signature assigned 115 tumours to the good-prognosis category and 180 to the poor-prognosis category. There was a strong correlation between the probability of remaining free of metastases and of surviving, and the good-prognosis signature.

Interestingly, the prognostic profile was independent of lymph-node status, but was also highly predictive of the risk of distant metastases in the lymph-node-positive subgroup. This could be useful for identifying patients with lymph-node-positive disease who have an unexpectedly good prognosis and indicates that lymph-node metastases develop independently of distant metastases.

The gene-expression profile was also predictive of overall survival — 94.5% of patients in the good-prognosis group survived for 10 years compared with 54.6% in the poor-prognosis group. The hazard ratio for distant metastases in the poor-

THERAPEUTICS

Mass destruction

The urokinase pathway is involved in physiological and pathological tissue remodelling processes, including cancer invasion and metastasis. Both urokinase plasminogen activator (uPA) and its receptor (uPAR) are overexpressed in nearly all human cancers, but are only expressed at very low levels in normal tissues, except as a rapid response to tissue injury. Bugge, Leppla and colleagues have exploited this tumour-associated uPA system to activate a targeted immunotoxin, based on anthrax, which causes potent destruction of tumours.

Anthrax toxin is secreted by bacteria as three antigens and is activated when the protective antigen (PrAg) binds to a ubiquitously expressed cell-surface receptor (tumour endothelium marker 8), is cleaved by furin proteases and forms a complex with the two other antigens. Binding of the active part of the complex with fusion protein 59 (FP59) kills the cell by inhibiting translation elongation factor 2. The authors modified PrAg so that it is cleaved by uPA and not by furin, so that only cancer cells would be targeted.

So, how toxic are these toxins? When just 2 μg of the native anthrax toxin, PrAg, was given to mice with FB59, extreme toxicity was observed — widespread organ damage and death occurred within a few hours. By contrast, up to 30 μg of the modified toxin, PrAg-U2, resulted in no toxicity. Next, the authors used mice with complete deficiencies in key components of the urokinase pathway — including plasminogen (Plg), which is activated by urokinase — to show that the uPA system is crucial in activation of PrAg-U2. Whereas wild-type mice died when given 40–200 μg PrAg-U2 plus FP59, *uPA*$^{-/-}$, *uPAR*$^{-/-}$ and *Plg*$^{-/-}$ mice remained healthy, and mice null for uPA inhibitor were hypersensitive to the toxin. This shows that the components of the uPA pathway are crucial to the activation of PrAg-U2 *in vivo*.

The authors now knew that PrAg-U2 was specifically activated by the tumour-associated urokinase system, so they next tested the tumoricidal activity of the toxin in mice bearing established fibrosarcomas, melano-mas and lung carcinomas — tumours that have a poor response to conventional treatment. PrAg-U2 reduced the size of the tumours by more than 85%, and completely eradicated all fibrosarcomas in 67% of mice after only one treatment. No increase in apoptosis was seen, indicating that the toxin triggered necrotic cell death.

The engineered toxin suppressed tumour growth and destroyed established tumours with no toxicity to normal tissues. Because uPA is overexpressed on epithelial, mesenchymal and haematopoietic tumours, this strategy has promise as a broad-acting antitumour therapy. The authors suggest that modifications could be made to increase protease specificity or to use other toxin proteins instead of anthrax. The therapeutic index of immunotoxins already in clinical use might also be improved by adapting this strategy.

Ezzie Hutchinson

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WEB SITE

Thomas Bugge's lab:
http://wwwdir.nidcr.nih.gov/dirweb/opcb/ptru.asp

prognosis signature group compared with the good-prognosis signature group was 5.1, and the hazard ratio for overall survival was 8.6. van de Vijver *et al.* compare their classification with that made by standard consensus criteria, and suggest that the current criteria misclassify many patients, which results in some patients getting unnecessary treatment and others missing out on adjuvant treatment that would be beneficial. This has important implications for the future of breast cancer management, especially if the prediction technique is validated in older patients who have more advanced disease.

Ezzie Hutchinson

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WEB SITE

Marc van de Vijver's lab: http://www.nki.nl/nkidep/vdvijver/index.html

CARCINOGENESIS

Double whammy

Tobacco smoke contains more than 40 carcinogenic chemicals, most of which are believed to cause lung cancer through the induction of DNA damage. In the January issue of *The Journal of Clinical Investigation*, Kip West *et al.* show that tobacco also has a direct biochemical effect on cells through activation of the anti-apoptotic signalling protein AKT.

West *et al.* began investigating how lung epithelial cells that have undergone tobacco-induced DNA damage evade apoptosis and eventually become resistant to chemotherapeutic drugs. The anti-apoptotic protein AKT was previously observed to be constitutively activated in non-small-cell lung cancer cell lines. This serine/threonine kinase controls several cellular processes, including glucose metabolism, cell-cycle progression and apoptosis.

To test whether tobacco-derived carcinogens could activate this pathway in human lung epithelial cells, West *et al.* examined normal human bronchial epithelial cells derived from large airways, which can become squamous-cell carcinomas, and small airway epithelial cells, which can become adenocarcinomas. These cells were treated with two components of tobacco — nicotine, which is the addictive compound of tobacco and a precursor to many carcinogens, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is a potent tobacco-specific carcinogen.

Nicotine and NNK both induced AKT phosphorylation, which is required for activation, in these cell lines. This led to phosphorylation of downstream targets that control the cell cycle and protein translation, such as GSK3, forkhead transcription factor family (FKHR), eukaryotic translation initiation factor 4E (EIF4B) and the ribosomal kinase protein S6. After treatment, cell adhesion and dependence on exogenous growth factors — two indicators of transformation — GSK3 were reduced . Most importantly, nicotine/NNK activation of this pathway increased cell survival after treatment with etoposide, ultraviolet irradiation or H_2O_2. So, this might be a mechanism by which lung cancer cells become resistant to chemotherapy.

Nicotine and NNK had similar effects *in vivo*, as treatment of mice with these agents led to AKT phosphorylation in airway epithelial cells and the development of aggressive lung tumours. AKT phosphorylation was also detected in ten lung cancer specimens derived from smokers.

But how do nicotine and NNK activate AKT signalling? They both bind to specific subgroups of nicotinic acetylcholine receptors (AchRs) that are expressed by bronchial epithelial and endothelial cells. West *et al.* showed that treatment of bronchial epithelial cells with inhibitors of these receptors blocked nicotine- and NNK-induced AKT phosphorylation, whereas an AchR agonist increased AKT phosphorylation. So, nicotine and NNK seem to activate AKT by signalling through an AchR, although other undiscovered signalling pathways might exist.

Kinetic studies in the cells showed that AKT activation preceded DNA-damage induction, so nicotine and NNK might deliver a 1–2 punch, exerting not only genotoxic effects but also protecting cells from subsequent induction of apoptosis. Nicotine has also been shown to stimulate endothelial-cell proliferation and angiogenesis, so the authors suggest that nicotine replacement therapy for smoking cessation could have long-term carcinogenic effects. GlaxoSmithKline and Pharmacia, which make smoking cessation products, have both issued press releases stating that there is no clinical evidence that nicotine replacement therapy causes cancer.

Kristine Novak

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