ONCOGENES

Dancing the two-step

Regulation by protein degradation has become a recurring theme in cancer biology: several oncoproteins, including β-catenin, are controlled by being targeted for degradation, and proteasome inhibitors have even made it into clinical trials as anticancer drugs. But biological control is seldom as simple as first appears. In the January issue of *EMBO Reports*, Frank Staal and colleagues reveal that phosphorylation, not degradation, might be the key to regulating β-catenin's activity.

β-catenin leads a double life: it's a component of epithelial junctions, but also a co-activator of the TCF family of transcription factors. The β-catenin-mediated induction of TCF's targets stimulates cell proliferation, and the deregulation of this process is an important early step in colorectal tumorigenesis. β-catenin levels are normally kept in check by a 'destruction complex' containing the APC tumour-suppressor protein and glycogen synthase kinase 3β (GSK3β). The accepted view is that phosphorylation of β-catenin by GSK3β allows it to be recognized by a second complex that conjugates β-catenin with ubiquitin — the molecular address tag that sends proteins to their destruction in the proteasome. The physiological switch that blocks β-catenin's destabilization is the extracellar signalling molecule Wnt, which indirectly prevents β-catenin from being phosphorylated by GSK3β.

But is simply increasing the level of β-catenin sufficient to drive transcription of TCF target genes? To find out, the authors blocked β-catenin degradation using a proteasome inhibitor and measured the transcription of TCF-target genes using a reporter construct. To their surprise, stabilizing β-catenin didn't increase transcription of the reporter gene. Experiments in a temperature-sensitive cell line that cannot add ubiquitin to proteins confirmed these results.

All the known oncogenic mutants of β-catenin have mutations in sites that are thought to be phosphorylated by GSK3β, so the authors reasoned that dephosphorylation of β-catenin might be needed not just to protect it from destruction, but also to activate its function as a transcriptional co-activator. To investigate this, the authors measured levels of dephosphorylated β-catenin — using an antibody that recognizes only dephospho-β-catenin — under four different conditions. Wnt addition, transfection with a dominant-positive β-catenin mutant, and the GSK3β inhibitor LiCl increased both the levels of dephospho-β-catenin and TCF-reporter transcription, whereas treatment with a proteasome inhibitor had no effect

THERAPEUTICS

Two for the price of one

AIDS patients who receive protease inhibitors as part of their antiviral therapy not only experience a drop in viral load and an increase in T-cell number, but also a reduced incidence of Kaposi's sarcoma — a cancer that is associated with herpesvirus-8 infection. Although these anticancer effects were originally attributed to the drugs' antiviral activities, researchers have now shown that the protease inhibitors indinavir and saquinavir also have potent anti-angiogenic effects. The discovery that these drugs disrupt proteolytic activity that is not only required for viral replication but also required for tumour growth, reveals a new approach to cancer therapy.

Kaposi's sarcoma is an angio-proliferative cancer that frequently occurs in HIV-infected individuals. The reduced incidence, or regression, of Kaposi's sarcoma in patients treated with indinavir or saquinavir was first reported several years ago. Epidemiologists were unable, however, to show an association between increased T-cell counts, HIV suppression and Kaposi's sarcoma regression. Furthermore, the rate of Kaposi's sarcoma regression was observed to be significantly lower in patients treated with non-protease-inhibitor-containing combination therapies, despite the ability of these drugs to effectively block HIV replication. So scientists have been searching for secondary effects of these drugs, beyond their antiviral activities.

Because Kaposi's sarcoma is characterized by unregulated angiogenesis and upregulation of the angiogenic factors basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), Cecilla Sgadari *et al.* investigated whether indinavir and saquinavir had direct effects on blood-vessel formation. In the March issue of *Nature Medicine*, they report that these drugs block bFGF- and VEGF-induced angiogenesis in a chorioallantoic membrane assay. The drugs also inhibit development of angiogenic lesions that are induced by injection of bFGF or VEGF into nude mice, indicating a direct anti-angiogenic effect.

But how do viral protease inhibitors block angiogenesis? Angiogenesis requires invasion of the blood-vessel basement membrane by vascular endothelial cells, and this process is mediated by the matrix metalloproteinase MMP-2. MMP-2 is highly expressed by angiogenic endothelial cells and by Kaposi's sarcoma cells. It is released as a proenzyme that is proteolytically activated by a complex mechanism involving several other proteases. Sgadari *et al.* showed that although indinavir and saquinavir did not affect MMP-2 expression, they did block conversion of MMP-2 to its active form. These drugs therefore prevent activation of proteases that are involved in cell invasion and tumour angiogenesis.

So do indinavir and saquinavir have effects on cancers that are not associated with viral infection? Sgadari *et al.* found that the drugs inhibited growth of angiogenic lung adenocarcinoma cells implanted in nude mice. Because these drugs have already been shown to be safe and effective for human use, they might be quickly developed for treatment of Kaposi's sarcoma and other types of cancer in HIV-seronegative individuals.

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WEB SITE

Istituto Superiore di Sanità web site: http://www.iss.it/english/laboratori/index.htm

on either, indicating that dephosphorylation of β-catenin is required for activation of β-catenin as a transcription factor. Immunofluorescence revealed that most of the dephospho-β-catenin was nuclear.

These findings are also supported by previously published clinical data: patients whose tumours have high levels of phosphorylated β-catenin have a better prognosis than those with low levels. This extra level of β-catenin regulation might also turn out to be therapeutically useful because it offers hope of blocking β-catenin's transcriptional function even in the absence of active APC.

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PROGNOSTICS

Tailor-made therapy

A diagnosis of lymph-node-negative breast cancer can result in very different long-term prognoses. The ability to correctly predict the outcome would influence treatment decisions, but how can this be achieved? Laura van't Veer *et al.* report in the 31 January issue of *Nature* that a 'prognosis classifier', identified using microarray analysis, can outperform other clinical methods that are currently used to forecast disease outcome, and could be used to determine whether patients would benefit from adjuvant therapy.

In order to identify a gene-expression signature that could be used to predict disease outcome, the authors isolated RNA from 98 tumours of patients with lymph-node-negative, primary breast cancer. Microarray analysis revealed that 5,000 genes had significant alterations in expression level. An unsupervised cluster analysis — which grouped tumours according to their similarities over these 5,000 genes — then showed that the tumours fell into one of two groups, and that these could, to some extent, distinguish between tumours with a good prognosis and a bad prognosis.

In order to establish a more effective prognostic signature, tumours taken from 78 patients diagnosed with sporadic cancer — 44 of whom had remained disease free for at least 5 years and 34 of whom had developed distant metastases within 5 years — were subjected to a three-step supervised classification method. This allowed identification of 70 genes — the 'prognosis classifier' — that could correctly predict disease outcome in 83% of cases. However, even if this sacrifices overall accuracy, because failure to treat a patient with a poor prognosis is more dangerous than over-treating a patient with a good prognosis, it is more important that the poor-prognosis patients are diagnosed correctly. Altering the threshold to achieve this aim allowed more than 90% of the tumours with a poor prognosis to be assigned correctly.

So which genes predict tumour prognosis? Unsurprisingly, those involved in cell-cycle progression, invasion and metastasis, angiogenesis and signal transduction are upregulated in tumours with a poor prognosis. But interestingly, genes that have previously been suggested to be predictive of breast cancer outcome did not appear in the prognosis classifier, perhaps indicating that single genes are lacking in predictive power and validating the 'multigene' approach.

So how effective is the prognosis classifier compared with more conventional methods of classifying tumours? When tested on an independent set of primary tumours, the disease outcome was correctly predicted for 17/19 patients. Moreover, the poor prognosis signature was shown to result in an odds ratio of 15 for a short time to metastasis (as compared with the good signature tumours). This is a significant improvement on prognostic factors that are currently used, such as tumour size, grade and angioinvasion.

Such prognosis classifiers could be used to aid in treatment decisions — at present, 70–80% of patients that receive adjuvant therapy would have survived without it, and chemotherapy has significant side effects and long-term consequences. This classification method can predict those that should receive treatment as effectively as other methods, while reducing the number who receive treatment unnecessarily. Gene signatures therefore seem to be the way forward in predicting outcome, and should pave the way for new therapies that are tailored to the patient.

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