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.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Sgadari, C. *et al.* HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi's sarcoma. *Nature Med.* **7**, 225–232 (2002)

FURTHER READING Sturzl, M., Zeitz, C., Monini, P. & Ensoli, B. Human herpesvirus-8 and Kaposi's sarcoma: relationship with the multistep concept of tumorigenesis. *Adv. Cancer Res.* **81**, 125–159 (2001)

WEB SITE

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