IMAGING

Glowing success

Although many targeted anticancer drugs prove effective when tested in biochemical assays, it is another matter to determine how they affect their molecular targets *in vivo*. In the June issue of *Nature Medicine*, William Kaelin's lab reports a bioluminescent method of monitoring the *in vivo* efficacy of drugs designed to inhibit the cyclin-dependent kinase CDK2.

CDK2 regulates cell-cycle progression and is therefore a potential anticancer-drug target. Among its many substrates, it phosphorylates the cyclin-dependent kinase inhibitor p27 (also known as KIP1). This phosphorylation leads to ubiquitylation and eventual proteolytic degradation of p27. Kaelin's group created a plasmid vector that expressed a fusion of p27 and the enzyme luciferase, which can be tracked *in vivo* with bioluminescent imaging. This protein, termed p27Luc, behaves in a manner similar to p27 and can therefore be used as a marker of CDK2 activity.

The authors transfected several tumour cell lines with the p27Luc-expressing vector. They observed that the luciferase activity in these cells increased when cells were treated with CDK2-inhibitory proteins, peptides or small inhibitory RNA, meaning that p27Luc was no longer degraded. Treatment of p27Luc-expressing cells with CDK2-inhibitory drugs such as flavopiridol and *R*-roscovitine caused a dose-dependent increase in luciferase activity in these cell lines, whereas they had no effect in cells that simply expressed the luciferase gene.

But can this system be used to monitor CDK2 activity *in vivo*? Kaelin's group injected p27Luc-expressing lung carcinoma cells subcutaneously into nude mice, and imaged the resulting tumours 6 weeks later (see figure). They observed that flavopiridol induced p27Luc-mediated luminescence at the xenograft site. A particularly useful feature of this approach is the ability to take repeated measurements over extended time periods, allowing analysis of tumour growth and spread without sacrificing animals. So,

luciferase reporters will be useful in monitoring the pharmacokinetics of CDK2 inhibitors, as well as other targeted therapeutics.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Zhang, G.-J. *et al.* Bioluminescent imaging of Cdk2 inhibition *in vivo*. *Nature Med.* 2 May 2004 (doi:10.1038/nm1047)

WEB SITE

William Kaelin's lab: http://www.dana-farber.org/abo/danafarber/detail.asp?PersonID=111&RD=True

BREAST CANCER

Establishing normality

Microarray studies have been used to classify breast tumours according to their expression of markers characteristic of different normal mammary epithelial cells. However, such classifications are limited by the fact that normal breast tissue has rarely been used for comparison with tumour samples. Researchers from the United Kingdom and Italy have now established a 'baseline' for gene expression in normal mammary epithelial cells, paving the way for advances in understanding and treating breast cancer.

Jones *et al.* used tissue left over from breast-reduction surgery to isolate the two main cell types that give rise to breast carcinomas — the luminal and myoepithelial cells of the ductal-lobular system — and carried out microarray analysis of gene expression in these cells. Initial analysis using an unsupervised hierarchical-clustering method revealed sets of genes that are expressed differentially between the two cell types. Supervised analysis using a trained algorithm then identified the genes with the greatest predictive value, establishing a set of 33 genes that can be used as markers to distinguish between myoepithelial and luminal cells. These differential expression patterns were confirmed by PCR after reverse transcription of RNA and antibody staining of tissue sections.

To evaluate the prognostic usefulness of some of these markers, the authors used microarrays of breast tumour samples for which the clinical outcome was already known. Several showed potential for future use in predicting disease outcome. For example, nuclear expression of galectin 3, a protein with several proposed roles in cancer, and increased expression of SPARC, an extracellular-matrix protein associated with tumour invasiveness, both correlated with decreased survival rates.

This study also raises concerns about the use of cultured cell lines to represent normal breast tissue in microarray experiments. Commercially available human mammary epithelial cells (HMECs) — derived from unsorted breast epithelium — have been used for comparisons with tumour cells in breast cancer microarray studies. However, it has been suggested that HMECs are derived mainly from myoepithelial rather than luminal cells, which could bias the interpretation of results. This was confirmed by comparing gene expression in HMECs from previous studies with the data obtained for normal luminal and myoepithelial cells. HMECs were found to express mainly myoepithelial markers, indicating that results from previous gene-expression studies using these cells should be re-interpreted.

The gene-expression profiles established in this study now provide an accurate baseline for future microarray experiments. This should speed up progress in determining important changes during breast cancer development and identifying new targets for anticancer drugs.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPER Jones, C. *et al.* Expression profiling of purified normal human luminal and myoepithelial breast cells: identification of novel prognostic markers for breast cancer. *Cancer Res.* **64**, 3037–3045 (2004)