Cell cycle independent activity of cyclin D1

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The deconvolution of gene expression patterns in human tumours has identified additional mechanisms of action thought to be responsible for the role of cyclin D1 in cancer development [1]. Gene expression profiling is well established as a tool to classify human cancers enabling a prediction of prognosis and it now shows promise in assigning function to genes.

Why cyclin D1?

Justin Lamb and colleagues at the Dana-Farber Cancer Institute (http://www.dfci.harvard.edu) were interested in the *cyclin D1* gene because it is overexpressed in a range of human tumour types and is proposed to contribute to cancer development. Early studies demonstrated that D-type cyclins positively regulated progression through the G1 phase of the cell cycle; this is achieved via the activation of the cyclin dependent kinases (cdk) 4 and 6, phosphorylation of the tumour suppressor protein pRb and derepression of the E2F transcription factors. Subsequently, the cdks became subject to intense drug development programs, with drugs such as flavopiridol and CYC202 undergoing clinical trials [2; http://www.aventis-oncology.net/cell-cycle-inhibition.htm; http://www.cyclacel.com/home.htm]. However, more recent research has implicated Cyclin D1 in cdk-independent modulation of other cellular transcription factors, including the oestrogen and androgen receptors, STAT3 and BETA/NeuroD [3].

Gene expression profiling

To investigate cyclin D, Lamb *et al.* infected MCF-7 and MDA-MB468 human breast cancer cells with either recombinant adenoviruses expressing cyclin D1 or a cyclin D1 mutant unable to activate cdk4: green fluorescent protein was used as a control. Ectopic protein expression was detectable 6 hours after infection, and RNA was isolated at intervals up to 24 hours post infection. Hybridization to microarrays containing ~7000 unique oligonucleotide probe-sets enabled the team to identify genes that consistently had a greater than threefold change in expression following cyclin D1 overexpression (Figure 1). Reassuringly, the HSP70-2 gene, known to be activated by cyclin D1, was amongst the 21 target genes identified. These target genes – identified as induced by both wild type and mutant cyclin D1 – were used as the definitive cyclin D1 expression signature for the rest of the study.

But, as second author of the study, Sridhar Ramaswamy, points out, 'the open question has been whether what is found in the test tube is what really

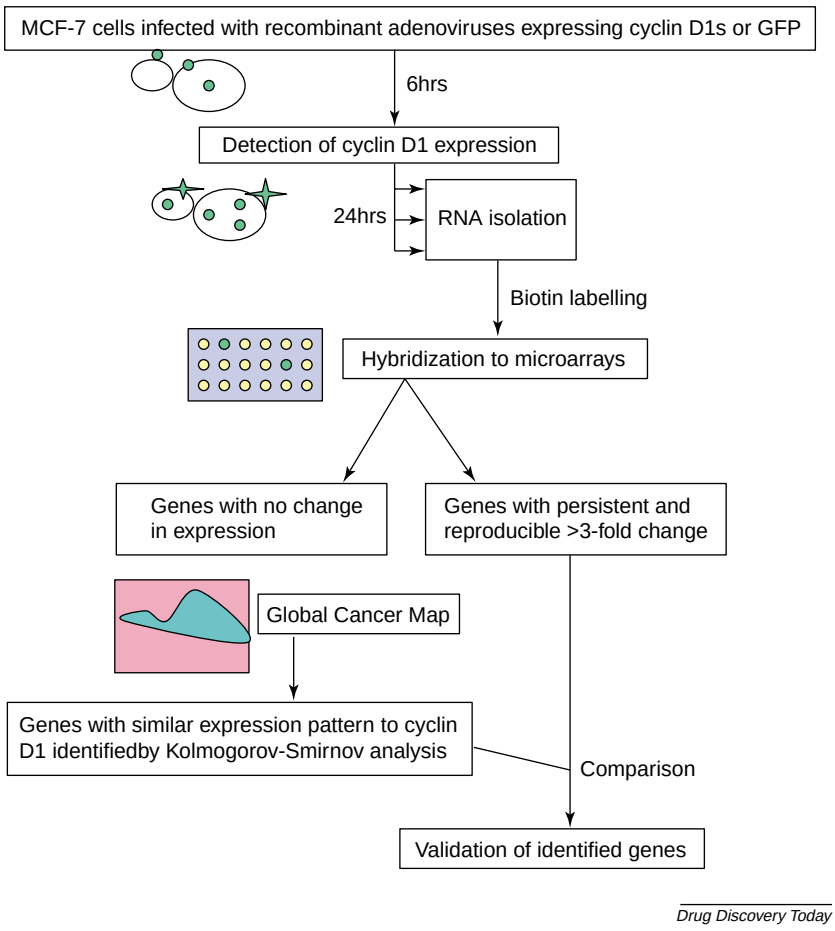

Figure 1. Schematic showing methodology used by Lamb and colleagues at the Dana-Farber Cancer Institute to identify cyclin D1-dependent gene expression ([1]; http://www.dfci.harvard.edu).

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happens in human tumours'. Concerned that their expression signature might be an artefact of their experimental cell culture system, the researchers analyzed the Global Cancer Map (GCM) database, which contains gene expression profiles from 190 primary human tumours of 14 different histological types [4]. They ordered the genes according to how closely their expression pattern approximated that of cyclin D1 and used the Kolmogorov-Smirnov (KS) nonparametric rank statistic [5] to capture the position of the set of 21 genes identified *in vitro* within this ordered list. This ranking is a similar process to that employed by Internet search engines. They found a significant correlation between the expression patterns of their set of target genes and the expression of cyclin D1 in human tumours. Further comparisons showed that cyclin D1 expression did not correlate with E2F target genes, but cyclin D3 expression did, highlighting differences between the cyclin D subtypes.

The rapid changes in gene expression indicated direct involvement between cyclin D1 and a transcription factor. C/EBPβ (CCAAT/enhancer-binding protein) alternatively named Nf-116 or LAP, was identified as a candidate. Further results obtained strongly supported the hypothesis that C/EBPβ is involved in regulating genes affected by cyclin D1 overexpression and an important player in previously unappreciated mechanisms of cyclin D1 action. Functional studies confirmed endogenous C/EBPβ as a crucial constitutive repressor of cyclin D1 target promoters and the existence of a functional interdependency between cyclin D1 and C/EBPβ for gene transcription, mediated by physical contact.

Controversially a study published simultaneously by Wang *et al.* identified peroxisome proliferator-activated receptor-γ (PPAR γ) as a target of cyclin D1 repression [6]. Using different methodology, their study compared wild type and cyclin D1 knockout mice.

Importance of independent activity

Earlier failures to correlate cdk-dependent cyclin D1 activity with tumour development has led to suggestions that cyclin D1 oncogenesis is cdk-independent [7]. Disruption of signalling through C/EBPβ is already known to contribute to malignant transformation in the murine mammary gland [8], thus this new work suggests cyclin D1 mediates its oncogenic effects via C/EBPβ. Nevertheless an involvement of PPAR γ in cancer is not unprecedented, as PPAR γ agonists have been shown to inhibit the growth of human colorectal cancer cells [9]. Thus, as stated by cell cycle researcher Richard Pestell, senior author of the Wang *et al.* study, 'the central issue is whether cyclin D1 inhibition of C/EBPβ or PPAR γ is the functionally relevant target'.

The research of Lamb *et al.* has wider implications because it highlights the role tumour gene expression databases could have in uncovering the functions of many other human oncogenes. Their methodology could become a prototype for future studies, as stated by Sridhar Ramaswamy, 'the paper represents a proof-of-concept of the approach'.

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Minding the Ps and Qs of genomewide analysis

Henry Nicholls, BMN News

The much-loved p-value is inadequate for testing hypotheses on a genomewide scale, say statisticians, who argue that a new measure of statistical significance – the q-value – could help in these wide-ranging analyses.

Patterns for drug discovery

Mining genomes for significant patterns is one of the first steps in drug

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