SCREENING

Finding cancer's Achilles' heel

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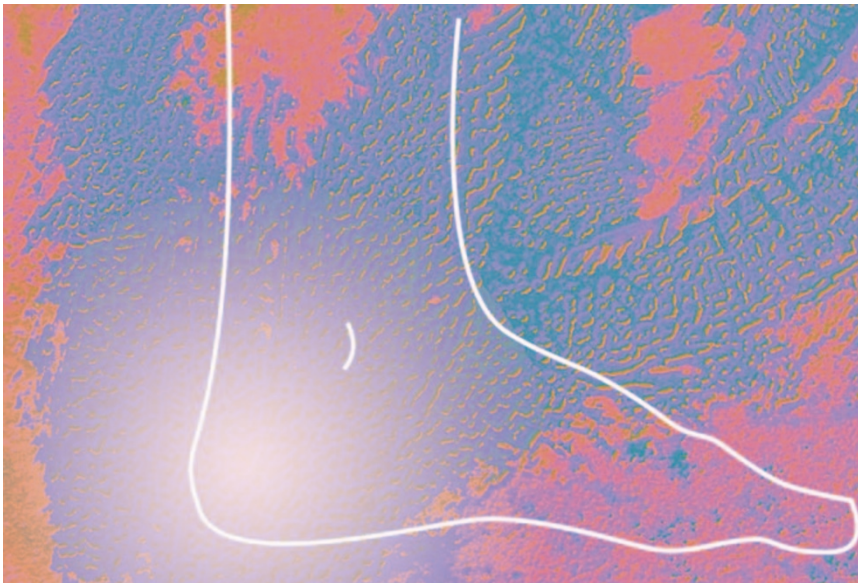

An attractive approach to developing new anticancer drugs is to target genes or proteins that are essential for tumour cell growth and survival. However, this strategy is hindered by the fact that knocking out or inhibiting these genes is usually lethal, which makes it impossible to pinpoint the target responsible for the cytotoxic phenotype.

Louis Staudt and colleagues present a solution to this problem, reporting in *Nature* an inducible loss-of-function RNA interference (RNAi) screen that can identify genes that are crucial for tumour survival. They used this method to classify two closely related tumours on the basis of subtle genetic differences and to identify a new target involved in lymphoma pathogenesis.

The authors generated a library of short hairpin RNA (shRNA) molecules in retroviral vectors and stably transfected these into lymphoma cell lines. The retroviral vector was engineered so that it only expressed the shRNA in the presence of doxycycline, and each construct was also tagged with a unique 60-bp 'bar code' to enable measurement of the abundance of each shRNA construct in different cell populations using a microarray.

Stably transfected cells were then split into two populations: one in which shRNA expression was induced by addition of doxycycline, and an untreated control population. Any shRNA that knocked down the expression of a crucial gene for tumour growth or survival was selectively eliminated from the doxycycline-treated group. A comparison of the relative depletion of cells expressing a particular shRNA therefore provided clues about the necessity of specific genes.

> "An inducible loss-of-function RNA interference (RNAi) screen can identify genes that are crucial for tumour survival."

Two closely related subtypes of diffuse large B-cell lymphoma (DLBCL) were studied: activated B-cell-like DLBCL and germinal centre B-cell-like DLBCL. These subtypes are known to differ in gene expression and clinical outcome; in particular, activated B-cell-like DLBCL is known to require constitutive activation of the nuclear factor-κB (NF-κB) pathway for survival. In the doxycycline-treated population, 17 shRNAs targeting 15 genes were significantly depleted and, of these, shRNAs that were selectively toxic for activated B-cell-like DLBCL were directed towards genes involved in the NF-κB pathway, including *CARD11*, which is required for NF-κB activation.

Having identified *CARD11* as a required gene, the authors studied the toxicity of *CARD11*-directed shRNA in cancer cells over time using a vector expressing *CARD11*-targeted shRNA and green fluorescent protein (GFP). A decrease in the number of GFP-expressing cells was only observed in the activated B-cell-like DLBCL, showing that *CARD11* knockdown is selectively toxic for this lymphoma subtype. *CARD11*-targeted shRNA also upregulated the activity of inhibitor of κB kinase and downregulated the expression of NF-κB target genes.

Although further work is needed to completely understand the role of CARD11 in lymphoma pathogenesis, its confinement to lymphoid tissue makes it an attractive therapeutic target, and its discovery elegantly exemplifies a way to use RNAi to find a cancer cell's Achilles' heel.

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ORIGINAL RESEARCH PAPER Ngo, V. N. *et al.* A loss-of-function screen for molecular targets in cancer. *Nature* 29 March 2006 (doi:10.1038/nature04687)

WEB SITE
Louis Staudt's lab: http://lymphochip.nih.gov/index2.html