TUMOUR CLASSIFICATION

Recognizing differences

Teachers have their job cut out recognizing pupils in their class who are doing better or worse than others and who might need special attention. Separating out subsets of tumours that might benefit from more accurate diagnosis and tailored treatments is also difficult. Using gene-expression profiling, Andreas Rosenwald *et al*. and Kerry Savage *et al*. have now identified a distinct subgroup of the non-Hodgkin's lymphoma diffuse large B-cell lymphoma (DLBCL), called primary mediastinal B-cell lymphoma (PMBL).

Diagnosis of PMBL using clinical and morphological features alone is imprecise, leading to an uncertain prognosis. Both research groups have devised a molecular signature to identify PMBLs and validated their predictor gene sets on samples from patients with DLBCL. Rosenwald *et al*., showed that the patients with molecularly identified PMBL had a better 5-year survival rate (64%) than total DLBCL patients (46%), so confirming previous clinical reports.

Both groups also showed that there are striking similarities between the molecular signature of PMBL and that of Hodgkin's lymphoma (HL) — the similarity in clinical presentation of these B-cell malignancies was already known. Savage *et al*. used a new gene-expression analysis technique, called gene-set enrichment, to confirm the shared molecular features of PMBL and HL. Rosenwald *et al*. found that 34% of the PMBL signature genes were also characteristically expressed in HL. Three genes that have previously been shown to be highly expressed in PBMLs — *MAL*, *FIG1* and *LFA3* — were present in both the PMBL predictor gene sets and in the HL profile.

So, can the individual genes in the molecular signatures tell us anything new about the aberrant signalling pathways in PMBL and HL? Rosenwald *et al*. found that the gene that best discriminated PMBL from other DLBCLs, and is also highly expressed in HL, was a gene close to *JAK2* on chromosome 9, *PDL2*. The *PDL2* locus is amplified in about half of PMBL tumours and it encodes a regulator of T-cell activation. Rosenwald *et al*. suggest that expression of PDL2 might allow a malignant B cell to co-exist with T cells in the thymus, where this lymphoma arises. Both groups noted many other similarities, including low

TUMOUR SUPPRESSORS

Cylindromatosis: cause and treatment

Cylindromatosis is a rare tumour of the skin appendages, and is caused by mutation of the tumour suppressor *CYLD*; however, the function of this gene has only recently been determined. Three papers, published in *Nature*, now report that CYLD is a de-ubiquitylating enzyme that negatively regulates NF-κB activity.

The initial connection between CYLD and the NF-κB pathway was made in different ways by the groups involved. Trompouki *et al*. and Kovalenko *et al*. both performed yeast two-hybrid screens with the IκB kinase-γ (IKKγ) subunit of the IKK complex (see figure), and pulled out CYLD. They then performed co-immunoprecipitation experiments to confirm that the interaction occurred in mammalian cells *in vivo*, and Kovalenko *et al*. showed that CYLD could also interact with another component of the NF-κB pathway — TRAF2.

By contrast, Brummelkamp *et al*. performed an RNA interference (RNAi) screen to knockdown expression of putative de-ubiquitylating enzymes and found that loss of CYLD resulted in increased expression from an NF-κB luciferase reporter construct. The same knockdown in cells resulted in an enhanced response to TNF-induced stimulation of IKKβ activity, and a subsequent decrease in IκBα levels. Both Trompouki *et al*. and Kovalenko *et al*. further analysed the link between CYLD and the NF-κB pathway. Trompouki *et al*. found that wild-type CYLD, but not CYLD mutants, prevented TNF receptors from activating NF-κB, and that RNAi to knockdown CYLD enhanced the activation of NF-κB in response to the CD40 ligand. Kovalenko *et al*. showed that overexpressing CYLD, but not catalytically inactive CYLD, inhibited the induction of NF-κB by the TNF and IL-1 ligands.

The next step was to identify how CYLD might have such an effect on the NF-κB pathway. CYLD has previously been proposed to act as a de-ubiquitylating enzyme, so Trompouki *et al*. expressed wild-type and tumour-associated mutant forms of CYLD and showed that only wild-type CYLD was able to cleave tetraubiquitin. So, what is the target of the de-ubiquitylating activity of CYLD in the NF-κB pathway?

The ability of TRAF2 and TRAF6 to induce NF-κB activity is dependent on their auto-ubiquitylation. Kovalenko *et al*. showed that TRAF2, TRAF6 and IKKγ are all polyubiquitylated, but that their co-expression with CYLD results in the disappearance of these polyubiquitylated forms. Similarly, Trompouki *et al*. and Brummelkamp *et al*. showed that co-expression of TRAF2 and wild-type CYLD caused a decrease in TRAF2 polyubiquitylation. Interestingly, expression of a CYLD mutant increased the ubiquitylation of TRAF2 above background levels.

Brummelkamp *et al*. propose that the tumour-suppressive activity of CYLD is related to its ability to inhibit the anti-apoptotic activity of NF-κB. When CYLD is depleted by RNAi, the ability of cells to survive apoptotic stimulants increases. These results indicate an interesting possibility for treating cylindromatosis. If loss of CYLD increases the activity of NF-κB, drugs such as aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit IKKβ — might counteract this effect. A clinical trial of the topical application of aspirin derivatives on cylindromas has recently been initiated to test this.

Emma Greenwood

expression of B-cell receptor signalling molecules, and high expression of interleukin-13 signalling molecules (including JAK2 and STAT1), tumour necrosis factor family members and several targets of the NF-κB transcription factor (including TRAF1). Savage *et al.* went on to show that the NF-κB pathway was activated in almost all cases of PMBL, and suggest that this might be the mechanism by which PMBL and HL cells resist apoptosis. Despite these similarities, the two lymphoma types were clearly distinguishable by the expression of other genes — such as several mature B-cell genes that were only detectable in the PMBL cells.

Molecular diagnosis of PMBL could help guide management of patients with DLBCL and lead to development of targeted treatments.

Ezzie Hutchinson

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WEB SITE

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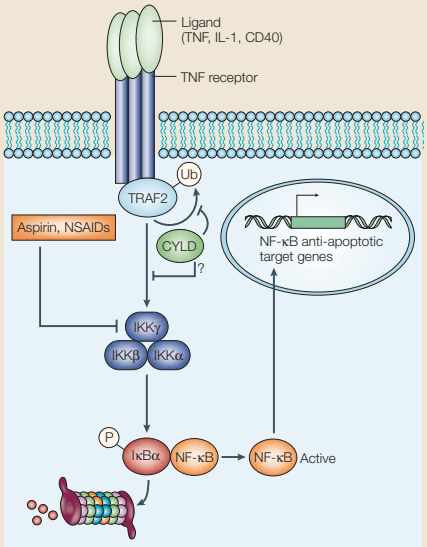

DRUG RESISTANCE

Clinging on

If you manage to cling on for dear life, as the saying goes, you survive, but in the case of acute myelogenous leukaemia (AML) cells, clinging on to the bone-marrow stromal cells seems to allow AML cells to resist cell death caused by chemotherapy, so decreasing the survival chances of the patient. Matsunaga *et al.* report in *Nature Medicine* that interaction between a β1 integrin, VLA4, on AML cells and fibronectin on stromal cells is crucial in the development of minimal residual disease (MRD) and prognosis in patients with AML.

Studies of two AML cell lines, U937 and HL60, which express the β1 integrins VLA4 and VLA5, showed that they were more resistant to loss-of-anchorage induced apoptosis (anoikis) after treatment with the anticancer drugs daunorubicin and AraC if attached to fibronectin-coated plates than those incubated on plates with no fibronectin coating. Antibodies to VLA4, but not to VLA5, reversed this resistance — as measured by the effect on cell viability and the increase of expression of the apoptosis pathway proteins annexin V, caspase 3, BCL2 and phosphatidylinositol 3-kinase.

Anti-VLA4 antibodies also abrogated MRD in severe combined immunodeficient (SCID) mice innoculated with the AML cell lines. If VLA4 antibodies were given with AraC to the mouse model of MRD, the mean survival of the mice was significantly higher than those receiving AraC alone.

So, what is the relevance of VLA4 expression to patients with MRD of AML? The authors showed that VLA4-positive leukaemic cells taken from 20 patients were more viable when cultured with fibronectin in the presence of daunorubicin or AraC than the cells taken from 10 patients whose leukaemic cells were VLA4 negative. When the VLA4-positive cells were incubated with VLA4 antibodies, they were more susceptible to drugs than those cultured without antibodies. When SCID mice transplanted with VLA4-positive leukaemic cells taken from patients were treated with VLA4 antibodies and AraC, no MRD was detected, unlike when VLA4-positive cells without antibody were used.

Furthermore, analysis of 25 AML patients showed that the complete response rate for the 15 VLA4-positive patients was significantly lower (60%) than that of the 10 VLA4-negative patients (100%) and the relapse rate was higher in the VLA4-positive patients (55.6%) than in the VLA4-negative patients (0%). Of all the possible risk factors tested — including age, gender, performance status, white blood cell count and blast count — only VLA4 positivity was a significant unfavourable variable for response, relapse and survival.

VLA4 might, therefore, be a useful clinical marker and antibodies to VLA4 might be of value for treating MRD. Further work needs to be done with larger patient numbers to confirm these results.

Ezzie Hutchinson

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