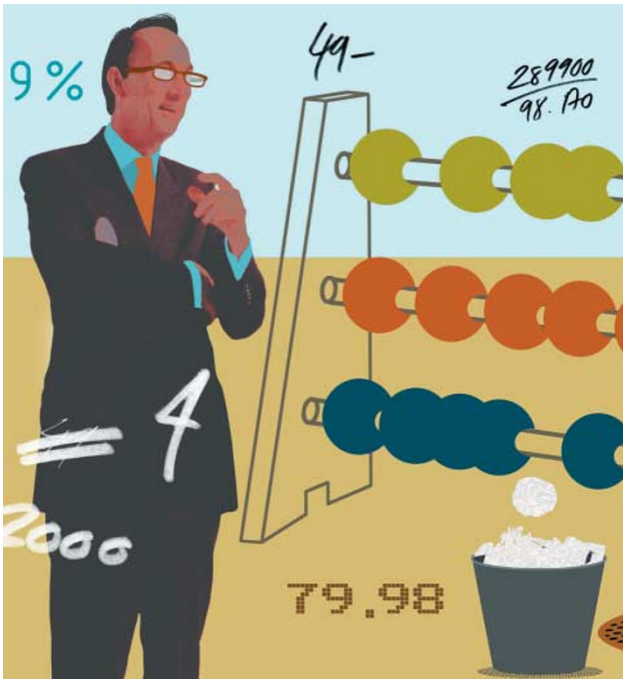
APOPTOSIS

Profit and loss



Suppression of apoptosis is central to the evolution of cancer and the *BCL2* family of pro- and anti-apoptotic genes is a key mediator in this process. BIM is a pro-apoptotic family member and a crucial antagonist of BCL2 survival function. As BCL2 is an oncoprotein, BIM might well be a tumour suppressor — in theory, loss of BIM function is equal to a gain in BCL2 function.

Suzanne Cory and colleagues now show that this is the case in the mouse B-lymphocyte lineage; but, unexpectedly, Bim acts as a tumour suppressor only in mature B cells. The authors investigated how disruption of the *Bim* gene affected tumour development in lymphoma-prone, B-cell-targeted (Eμ)-*Myc* mice. Loss of *Bim* — even one allele — markedly accelerated tumour onset and all the early neoplasms were acute leukaemias of surface IgM-positive B cells. By contrast, earlier results from this lab had demonstrated that the combination of transgenic *Bcl2* and *Myc* in the same system induces lymphomas derived from immature lympho-myeloid progenitor cells.

Pertinently, before tumour onset, Bim loss changed the composition of the B-lymphoid compartment. Eμ-*Myc* transgenic mice have far more B-lymphoid cells than wild-type animals and pre-B cells predominate. By contrast, mature B cells hold sway in Eμ-*Myc Bim*-deficient animals. The authors speculate that the preferential increase in B cells is indicative of a crucial role for Bim in mediating Myc-induced apoptosis in mature B-cells. In the pre-B cells, Bim might be less influential because Myc also activates other pro-apoptotic proteins.

Why do Myc and Bim cooperate in B-cell leukaemia development? The authors found that Bim protein levels were increased in cells expressing the Eμ-*Myc* transgene, especially in mature B cells, and that inactivation of even one *Bim* allele protected the cells against apoptosis. They conclude that Bim mediates Myc-induced apoptosis in B-lymphoid cells, although no evidence was

TARGETED THERAPIES

Layers of necessity

Chronic myeloid leukaemia (CML) and B-cell acute lymphoblastic leukaemia (B-ALL) are both caused by the *BCR–ABL1* oncogene. However, although the ABL tyrosine-kinase inhibitor imatinib (Glivec) is efficacious in chronic-phase CML, it has little effect in BCR–ABL1-positive B-ALL. Yiguo Hu *et al.* now show that this is because B-ALL — unlike CML — also relies on activation of SRC kinases for its development, and that a combination of imatinib and a SRC-kinase inhibitor is effective in this disease.

BCR–ABL1 activates SRC kinases in myeloid cells, from which CML is derived, and Hu *et al.* showed that it also activates SRC kinases in pre-B-lymphoid cells, from which B-ALL is derived. There are eight SRC kinases expressed in haematopoietic cells, but only three — Lyn, Fgr and Hck — were more prominently activated in Bcr–Abl1-induced B-ALL mouse cells, when compared with normal peripheral-blood leukocytes from control mice.

So, are LYN, FGR and HCK involved in leukaemogenesis by BCR–ABL1? The authors had previously developed two mouse models, involving the manipulation of Bcr-Abl1-transduced bone marrow such that mice develop either CML or B-ALL. Triple knockout of Lyn, Fgr and Hck in these two mouse models showed that these Src kinases are required for development of B-ALL, but not CML. Knocking out only one or two of the three Src kinases showed that a combination of two Src kinases, but not all three, was required for induction of B-ALL by Bcr–Abl1. So, Lyn, Fgr and Hck must have overlapping or partially redundant functions in the Bcr–Abl1 signalling pathway in mouse B-lymphoid cells.

The authors then tested whether an inhibitor of Src kinases would be effective alone or in combination with imatinib to treat B-ALL. The SRC kinase inhibitor CGP76030 selectively inhibited Lyn, Fgr and Hck in Bcr–Abl1-positive B-cell or B-ALL mouse cell lines and also inhibited growth and survival of these cells. Mice treated for Bcr–Abl1-induced B-ALL with imatinib or CGP76030 alone showed increased survival, and treatment with both drugs in combination was even more effective. Inhibition of Bcr–Abl1 by imatinib and inhibition of SRC kinases by CGP76030 in leukaemia cells isolated from these mice was confirmed. By contrast, CGP76030 did not improve survival of mice with Bcr–Abl1-induced CML compared with treatment with imatinib alone.

Further understanding of the signalling pathways involved in the transition between chronic and acute phases of leukaemia will help the development of effective combination therapies.

Ezzie Hutchinson

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WEB SITE

Shaoguang Li's lab:
http://www.jax.org/staff/shaoguang_li.html

found that *Bim* is a direct transcriptional target of Myc.

Previously, this group demonstrated that the loss of one allele of *Bim* could correct many of the tissue defects within *Bcl2*-null animals, establishing Bim as a key physiological antagonist of Bcl2. Their most recent data, however, clearly show that the oncogenic impact of loss of Bim in the B-lymphoid compartment is not equivalent to gain of Bcl2. Unraveling this conundrum in the future will undoubtedly tell us more about the sophisticated circuitry regulating cell life and death during B-lymphoid differentiation.

Nicola McCarthy

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Suzanne Cory's lab: http://www.wehi.edu.au/research/divisions/mgc/index.html

TUMORIGENESIS

Right place, wrong time

When members of the FOXO family of transcription factors are located in the nucleus, they activate expression of genes that prevent proliferation and promote apoptosis. Nuclear exclusion of FOXO can therefore contribute to cancer pathogenesis. Hu *et al.* have discovered a new mechanism that regulates this localization, reporting that the IκB kinase-β (IKKβ) regulates FOXO3A access to the nucleus and, therefore, tumorigenesis.

When cells are stimulated with growth factors, signalling pathways become activated that lead to phosphorylation of the kinase AKT (AKT-p), which in turn phosphorylates the transcription factors FOXO1, FOXO3A and FOXO4. This causes their localization to the cytoplasm and subsequent cell proliferation. In the absence of growth or survival signalling, however, AKT remains unphosphorylated and inactivated, resulting in the nuclear retention of FOXO factors and the inhibition of cell division — as well as tumour suppression.

Hu *et al.* investigated the relationships between AKT-p and FOXO3A localization in 131 primary breast tumour specimens. As expected, they observed that FOXO3A was mainly localized to the cytoplasm of tumour cells with a high level of AKT-p and in the nucleus of cells that were AKT-p negative. Surprisingly, they also found a significant number of tumour samples that lacked AKT-p, yet FOXO3A was still confined to the cytoplasm.

So is there an alternative mechanism by which cancer cells exclude FOXO3a from the nucleus? Another cancer-associated kinase that regulates nuclear–cytoplasmic localization of transcription factors is IKKβ, which controls NF-κB activity. When Hu *et al.* examined levels of IKKβ in the tumour samples, they found that the level of nuclear FOXO3A was inversely correlated with the level of this protein. A lack of IKKβ was also correlated with the survival rate of patients with breast cancer. So could IKKβ also contribute to tumorigenesis by keeping FOXO factors out of the nucleus?

Through immunoprecipitation studies, the authors showed that IKKβ physically interacts with and phosphorylates FOXO3A, independently of AKT. Furthermore, IKKβ phosphorylation of FOXO3A leads to its proteolysis through the ubiquitin-dependent proteasome pathway. Hu *et al.* engineered cells to constitutively express IKKβ and showed that this resulted in loss of FOXO3A activity. In these cells, FOXO3A was no longer present in the nucleus, and therefore did not activate transcription of its target genes. This led to cell-cycle progression and proliferation.

Is constitutive IKKβ activity sufficient to cause tumour formation *in vivo*? Injection of IKKβ-stably-transfected cells into the mammary fat pad of nude mice caused tumour formation at that location, whereas control cells did not. Re-expression of FOXO3A in these cells, however, suppressed *in vivo* tumour formation. Therefore, the mechanism underlying IKKβ-mediated tumorigenesis is likely to be through inhibition of FOXO3A.

Hu *et al.* conclude that as there is an inverse correlation between cytoplasmic FOXO3A in tumour cells and survival in patients with breast cancer, this transcription factor might be a useful prognostic factor, as well as a new tool for therapeutic intervention.

Kristine Novak

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Mien-Chie Hung's lab: http://www.bcrfcure.org/rese_meet_hung.html