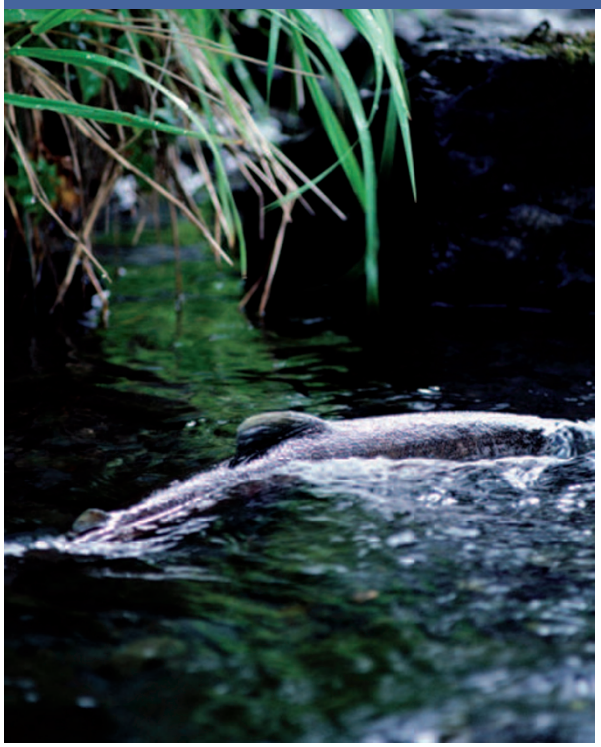
LEUKAEMIA

Wandering downstream



T-cell acute lymphoblastic leukaemia/lymphoma (T-ALL) is associated with an increase in the activity of the receptor Notch 1. Notch 1 is involved in the transcription of a limited set of genes, and Andrew Weng, Jon Aster, Warren Pear and colleagues show that part of the growth-stimulatory effect of Notch 1 in T-ALL cells is based on its upregulation of the proto-oncogene *MYC*.

Using an unbiased expression-profiling screen, the authors identified *MYC* as a gene that is downregulated when Notch 1 function is inhibited in T-ALL cells. Although *MYC* had previously been identified as a gene that is upregulated by the cleaved and active nuclear form of Notch 1, ICN1, evidence that *MYC* was a direct target of Notch 1 was lacking.

Generation of ICN1 involves two successive cleavages in Notch 1, the second of which is mediated by a γ-secretase complex that can be blocked by γ-secretase inhibitors (GSIs). The authors used a T-ALL cell line that accumulates the initial cleaved form of Notch 1 when it is treated with a reversible GSI. Upon washout of GSI, this partially activated form of Notch 1 is rapidly converted to ICN1. The resulting pulse of ICN1 produced an increase in the transcription of *MYC* even when protein synthesis was inhibited, which indicates that the transcription of *MYC* is directly regulated by ICN1.

ICN1 induces gene transcription by forming a complex with the DNA-binding protein CSL. Two possible CSL-binding sites were identified in *MYC*, one of which corresponded to a region that was previously identified as a putative Notch-responsive element. Electrophoretic mobility shift assays showed that CSL and CSL–ICN1 complexes bind to this site, which was also occupied by CSL–ICN1 complexes in T-ALL cells when Notch 1 was active, indicating that this site is important for Notch-1-dependent MYC expression. The authors went on to show that the expression of MYC can reverse the growth arrest caused by Notch 1

IMMUNOTHERAPY

A steady target

As tumour cells are genetically unstable, tumour antigens are not ideal targets for anticancer therapy. By contrast, tumour-associated fibroblasts are genetically more stable, which makes them attractive targets. Ralph Reisfeld and colleagues have used a DNA vaccine against fibroblast activation protein (FAP), which is overexpressed in most stromal fibroblasts associated with colon, breast and lung cancer. They have shown that this vaccine can suppress tumour cell growth both on its own and when given with chemotherapy.

When mice were immunized orally with the pFAP DNA vaccine and then challenged with multidrug-resistant colon or breast carcinoma cells, tumour growth was suppressed. To simulate a therapeutic setting, mice were first challenged with colon carcinoma cells and then vaccinated, and the authors observed a decrease in the growth of established lung metastases.

So, what are the effectors of the anti-tumour immune response? After immunization and subsequent tumour challenge, the authors depleted the $CD4^+$ and $CD8^+$ T cells and natural killer cells in the mice using antibodies. Only the depletion of $CD8^+$ T cells decreased the anti-tumour immune response. Furthermore, $CD8^+$ T cells purified from mice that had been vaccinated with pFAP induced the apoptosis of tumour cells transfected with fluorescently tagged pFAP, but not tumour cells that were transfected with fluorescent label alone. This shows that the pFAP vaccine breaks peripheral T-cell tolerance against the self-antigen FAP. In addition, the tumours that did grow in pFAP-vaccinated mice showed a marked infiltration of $CD8^+$ T cells.

Fibroblasts are the primary source of collagen type 1, and the expression of collagen type 1 has recently been shown to correlate inversely with the intra-tumoral uptake of various chemotherapeutic drugs. The authors therefore investigated whether their pFAP vaccine would decrease the amount of intra-tumoral collagen type 1 and increase the uptake of anticancer drugs. Tumour tissue from vaccinated mice did indeed show decreased expression of both FAP and collagen type 1. Vaccinated mice were first challenged with breast carcinoma cells, which are partially sensitive

inhibitors in 3 out of 5 Notch-1-dependent human T-ALL cell lines and, conversely, that expression of ICN1 can rescue the growth of transgenic *MYC*-driven murine T-ALL cells when expression of the human *MYC* transgene is suppressed. Interestingly, the expression of ICN1 in these cells induces the expression of endogenous *Myc* in the absence of transgenic *MYC* expression, which confirms the functional importance of the Notch-1–MYC pathway.

Although the inhibition of Notch 1 decreased the expression of MYC in all cell lines tested, not all human T-ALL cell lines were rescued from Notch 1 inhibition by MYC alone, which indicates that other targets of ICN1 also contribute to T-ALL cell growth. Nevertheless, these findings are important initial steps towards understanding the oncogenic pathways downstream of Notch 1.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Weng, A. P. *et al.* c-Myc is an important target of Notch1 in T cell acute lymphoblastic leukemia/lymphoma. *Genes Dev.* 17 July 2006 (doi: 10.1101/gad.1450406)
FURTHER READING Grabher, C., von Boehmer, H. & Look, A. T. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. *Nature Rev. Cancer* **6**, 347–359 (2006)

to doxorubicin, and then treated with doxorubicin. Vaccination with pFAP or treatment with doxorubicin suppressed but did not eradicate tumour growth, but vaccination and drug therapy in combination led to complete tumour rejection in 50% of the mice. This experiment was repeated in mice with established metastases, and treatment with pFAP and doxorubicin extended the lifespan of these mice threefold.

As FAP is stably overexpressed in most colon, breast and lung carcinomas, and vaccination increases the intra-tumoral uptake of chemotherapeutic agents, this is an attractive approach to study further.

Ezzie Hutchinson

ORIGINAL RESEARCH PAPER Loeffler, M. *et al.* Targeting tumour-associated fibroblasts improves cancer chemotherapy by increasing intratumoural drug uptake. *J. Clin. Invest.* **116**, 1955–1962 (2006)
FURTHER READING Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. *Nature Rev. Cancer* **6**, 392–401 (2006)

ONCOGENES

Keeping it in the family

The expression of MITF, a member of the MiT family of transcription factors and a master regulator of melanocytic differentiation, is often seen in clear-cell sarcoma (CCS). However, neither the mechanism of MITF regulation in CCS nor its function were known. Now, David Fisher and colleagues show that the CCS fusion protein Ewing sarcoma breakpoint region 1 (EWS)–activating transcription factor 1 (ATF1) is oncogenic because it regulates MITF expression.

CCS is a soft-tissue malignancy that is molecularly defined by the *EWS–ATF1* translocation. Despite being a sarcoma, CCS contains premelanosomes, expresses markers of melanocytic differentiation and, in some cases, produces melanin.

Fisher and colleagues investigated three human CCS lines that express the melanocyte-specific isoform of MITF (M-MITF). Immunoprecipitation using an EWS-selective antibody revealed that EWS–ATF1 occupied the *M-MITF* promoter in all three cell lines. So, does EWS–ATF1 regulate transcription of *M-MITF*? A reporter gene that directs luciferase expression from the *M-MITF* promoter was constitutively active in all three CCS cell lines. Furthermore, expression of dominant-negative EWS–ATF1 in CCS cells resulted in the selective inhibition of endogenous *M-MITF* expression. So, EWS–ATF1 is a necessary transactivator of the *M-MITF* promoter in CCS.

But what is the role of EWS–ATF1-mediated M-MITF expression in CCS? Dominant-negative inhibition of EWS–ATF1 significantly reduced the expression of the M-MITF-target genes *PMEL17* (which encodes the HMB45 antigen used for the pathological identification of CCS) and *MLANA* (a melanocyte marker). Pigmentation was also diminished in EWS–ATF1-inhibited CCS cells, and this could be rescued by M-MITF expression. Together, these findings indicate that EWS–ATF1 functions through M-MITF to mediate the melanocytic differentiation that is characteristic of CCS. Moreover, M-MITF activity was demonstrated to be necessary for CCS cell survival and proliferation: the inhibition of either EWS–ATF1 or M-MITF abrogated CCS colony formation. Interestingly, the expression of MiT-family members TFEB or TFE3 — oncoproteins that are translocated in alveolar soft-part sarcomas and some papillary renal-cell carcinomas — rescued cell viability in M-MITF-inhibited CCS cells in a dose-dependent manner. This indicates that MiT family members can functionally replace each other.

A xenograft model was used to study CCS tumour growth *in vivo*. RNA inhibition of *EWS–ATF1* potently inhibited *in vivo* tumour growth, which could be rescued by co-expression of M-MITF. The authors conclude that in CCS, the EWS–ATF1 fusion protein functions by targeting *M-MITF* to promote tumour cell survival and proliferation.

They propose that CCS could be grouped with melanoma, paediatric renal-cell carcinoma and alveolar soft-part sarcoma to form a family of MiT-associated human cancers, each showing distinct oncogenic deregulation of MiT-family genes. These 'MiT tumours' are clinically and morphologically distinct malignancies that would not otherwise be co-classified, but share characteristics of particular resistance to traditional chemotherapies and radiation therapy. The recognition of a common oncogene family in these seemingly diverse malignancies might aid the discovery of improved therapeutic approaches.

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ORIGINAL RESEARCH PAPER Davis, I. J. *et al.* Oncogenic MITF dysregulation in clear cell sarcoma: defining the MiT family of human cancers. *Cancer Cell* **9**, 473–484 (2006)