Molecular pathogenesis of T-cell leukaemia and lymphoma

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Abstract | T-cell acute lymphoblastic leukaemia (T-ALL) is induced by the transformation of T-cell progenitors and mainly occurs in children and adolescents. Although treatment outcome in patients with T-ALL has improved in recent years, patients with relapsed disease continue to have a poor prognosis. It is therefore important to understand the molecular pathways that control both the induction of transformation and the treatment of relapsed disease. In this Review, we focus on the molecular mechanisms responsible for disease induction and maintenance. We also compare the physiological progression of T-cell differentiation with T-cell transformation, highlighting the close relationship between these two processes. Finally, we discuss potential new therapies that target oncogenic pathways in T-ALL.

Karyotype
The complete description of all the chromosomes present in a cell. Most cancers are characterized by numerical and structural abnormalities in karyotype.

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doi:10.1038/nri2304
Published online 18 April 2008

Approximately 15% and 25% of the newly diagnosed cases of acute lymphoblastic leukaemia (ALL) in children and adults, respectively, are T-cell ALL (T-ALL) and are historically linked with a poor prognosis. Compared with the more common B-cell-lineage ALL, T-ALL is defined by distinct clinical and biological characteristics and is generally associated with more unfavourable clinical features, such as a high white-blood-cell count, bulky adenopathy and involvement of the central nervous system[1]. However, despite these features, the outcome for patients with T-ALL has improved markedly in recent years owing to the application of intensive chemotherapy regimens. Although this improvement in cure rates is welcome, such intensive chemotherapy comes at the cost of significant short-term and potentially long-term side effects. So the search for molecular drug targets and the design of tumour-specific therapy is a main goal of current research efforts. To reach this goal, we first need to understand the molecular mechanisms that underlie T-cell leukaemia.

There are significant similarities between T-ALL and T-cell lymphomas. Indeed, most clinical oncologists would agree that these two entities represent a range of the same disease, such that the two conditions are often treated in the same way. In this Review, however, we use the term T-ALL to refer to both T-ALL and T-cell lymphomas.

T-ALL is thought to result from malignant thymocytes that arise at defined stages of intrathymic T-cell differentiation[1]. Transformation events occur in crucial steps in thymocyte development and the expression of certain oncogenes has been closely linked to developmental arrest at particular stages of normal thymocyte development[2]. An abnormal karyotype is found in approximately 50% of T-ALL cases (TABLE 1), however, this is still less than the percentage found in B-cell-lineage ALL cases[3]. The chromosomal translocations that occur in cases of T-ALL frequently involve the juxtaposition of strong promoter and enhancer elements from T-cell receptor (TCR) genes on chromosome 7 (*TCRB* and *TCRG*) and chromosome 14 (*TCRA* and *TCRD*) with transcription factors genes, such as *TAL1* (T-cell acute lymphocytic leukaemia 1; also known as *SCL*), *LYL1* (lymphoblastic leukaemia-derived sequence 1) and *HOX11* (homeobox-11; also known as *TLX1*)[3]. Although some of these transcription factor genes are normally expressed in non-malignant thymocytes and are essential regulators of T-cell ontogeny, others are not expressed in normal thymi, but rather are ectopically expressed by transformed cells in T-ALL. These chromosomal translocations can occur during thymocyte development as a result of illegitimate TCR recombination and lead to aberrant gene expression giving rise to T cells that show abnormal cell-cycle control, proliferation and differentiation.

Additional genetic abnormalities in T cells from individuals with T-ALL include chromosomal translocations that generate fusion genes encoding new chimeric proteins with oncogenic properties, such as the *SIL* (SCL-interrupting locus)–*TAL1* and *MLL*

Table 1 | **Common cytogenetic abnormalities in T-ALL**

Cytogenetic abnormality	Approximate frequency	Involved oncogenes or fusion genes
Translocations involving TCR genes on chromosomes 7q34 (*TCRB* and *TCRG*) and 14q11 (*TCRA* and *TCRD*)	35%	*HOX11*, *HOX11L2*, *TAL1*, *TAL2*, *LYL1*, *BHLHB1*, *LMO1*, *LMO2*, *LCK*, *NOTCH1*, cyclin D2
Abnormal expression	9–30%	*SIL–TAL1* fusion
Fusion gene formation	10%	*CALM–AF10* fusion
	4–8%	*MLL* fusions
	6%	*ABL1* fusions
	Rare	*NUP98* fusions
Chromosomal deletions of 9p21 and 6q	Up to 65% (9p21)	*P15*, *P16*
	20–30% (6q)	Unknown
Activating gene mutations	50–60%	*NOTCH1*, *FLT3*, *NRAS*
Gene duplications	~30% in T-ALL cell lines	*MYB*

ABL1, Abelson 1; BHLHB1, basic helix–loop–helix B1; CALM, clathrin assembly lymphoid-myeloid leukaemia; FLT3, FMS-related tyrosine kinase 3; HOX11, homeobox-11; LMO, LIM-only; LYL1, lymphoblastic leukaemia-derived sequence 1; MLL, mixed-lineage leukaemia; SIL, SCL-interrupting locus; TAL, T-cell acute lymphocytic leukaemia; T-ALL, T-cell acute lymphoblastic leukaemia; TCR, T-cell receptor.

(mixed-lineage leukaemia)–partner-gene fusions. Cryptic deletions leading to the loss of tumour suppressor genes also occur in T-ALL, the most common of which are deletions at chromosome 6q and the deletion of the *INK4* (also known as *ARF* and *CDKN2A*) locus at chromosome 9p21, which contains genes encoding the cyclin-dependent kinase inhibitor p16 and other proteins important in regulation of the cell cycle. In addition, recent reports suggest that gene duplication appears to be another mechanism that could be involved in the transformation of T cells[4–6].

Recently, it has also been found that more than 50% of T-ALL cases involve activating mutations in the key regulator of T-cell fate *NOTCH1* (REF. 7). The Notch proteins — Notch1, Notch2, Notch3 and Notch4 — are essential regulators of the commitment of haematopoietic progenitors to the T-cell lineage, as discussed later. The frequency of activating mutations of *NOTCH1* in T-ALL cells therefore establishes an important relationship between T-cell development and the induction of T-ALL. The activation of many other oncogenes and oncogenic fusions also occurs at defined stages in T-cell development, again providing a close link between T-cell ontogeny and leukaemogenesis, which is highlighted in this Review.

Overview of T-cell development

The molecular mechanisms of regulation of the self-renewal and differentiation of long-term repopulating haematopoietic stem cells (LT-HSCs) are poorly understood but it is widely accepted that stem cells progressively differentiate to generate short-term repopulating HSCs (ST-HSCs) and multipotential progenitors (MPPs)[8]. The initiation of lymphoid commitment is also not well understood with several haematopoietic-cell fractions being proposed to represent bona fide lymphocyte progenitors. Nevertheless, within this population, the cells that are destined to become T cells will exit the bone marrow, migrate through the blood to the thymus in a chemotactic process that is controlled by adhesion molecules[9,10].

The thymus requires input from progenitor cells, and this can be either continuous or periodic input to maintain T-cell development and the production of mature, 'educated' T cells[11]. The first thymic immigrants are termed the early T-cell-lineage progenitors (ETPs) or double negative 1 (DN1) cells and phenotypically belong to a $CD3^-CD4^{-/low}CD8^-CD25^-CD44^{hi}KIT^+$ fraction. As the cells commit to the T-cell lineage, they first downregulate their expression of KIT (to become DN2 cells: $CD4^-CD8^-CD25^+CD44^+$), then CD44 (to become DN3 cells: $CD4^-CD8^-CD25^+CD44^-$) and finally CD25 (to become DN4 cells: $CD4^-CD8^-CD25^-CD44^-$)[11]. During this developmental progression, the T-cell progenitors remain in intimate contact with thymic epithelial stromal cells. This contact is of unique importance, as stromal cells express Notch ligands and also produce essential growth factors (such as interleukin-7) and morphogens (such as the sonic hedgehog (SHH) proteins)[12,13]. Upon pre-T-cell receptor (pre-TCR) expression, thymocytes lose their dependence on SHH, Notch and cytokine signalling and differentiate into double positive (DP; $CD4^+CD8^+$) cells. DP cells enter the processes of positive and negative selection, and selected $\alpha\beta TCR^+$ T cells exit the thymus as mature single positive (SP) $CD4^+$ or $CD8^+$ T cells (FIG. 1).

The role of Notch signalling in T-cell commitment. Notch is a master regulator of diverse cellular processes, such as differentiation, proliferation, apoptosis, adhesion and spatial development[14]. The cell-membrane-bound Notch proteins are composed of two polypeptide chains derived from a single protein that is proteolytically processed during its maturation[15]. The resulting extracellular subunit and intracellular subunit are non-covalently linked through the heterodimerization (HD) domains. Extracellular Notch contains multiple epidermal growth factor (EGF)-like repeats through which it binds its ligands Delta-like ligand 1 (DLL1) DLL2, DLL4, Jagged1 and Jagged2. Ligand binding initiates a series of proteolytic cleavage events. The first cleavage of extracellular Notch is catalysed by

Cryptic deletions
Deletions that cannot be detected with standard cytogenetic analysis.

Sonic hedgehog proteins
(SHH proteins). Members of the SHH signalling cascade, an evolutionarily conserved pathway that controls the proliferation and differentiation of multiple cell types.

Pre-T-cell receptor
(Pre-TCR). A receptor that is expressed on pre-T cells. It is formed by a TCR β-chain paired with a surrogate TCR α-chain (known as the invariant pre-Tα protein). The receptor complex includes CD3 proteins and transduces signals that allow further T-cell development.

the ADAM family (a disintegrin and metalloproteinase family) of metalloproteinases, such as ADAM10 and ADAM17. The second cleavage event targets intracellular Notch and is orchestrated by the γ-secretase complex. These two proteolytic events release intracellular Notch from the membrane and, as Notch contains two nuclear localization sequences, this leads to the translocation of Notch to the nucleus (FIG. 2a). The C-terminal region of intracellular Notch contains a PEST domain (proline-, glutamic-acid-, serine- and threonine-rich domain), which is important for the ubiquitylation and stability of the protein (see later). In the nucleus, intracellular Notch associates with the transcriptional repressor CSL (CBF1/suppressor of hairless/Lag1). This interaction promotes the recruitment of co-activator proteins, such as mastermind-like 1 (MAML1) and the histone acetylase p300, resulting in the transcription of Notch-target genes[16] (FIG. 2).

The first suggestion that Notch signalling could be an important element in T-cell differentiation came in the early 1990s when Sklar and colleagues identified a chromosomal translocation t(7;9)(q34;q34.3) that involved the human *NOTCH1* gene in patients with T-ALL[17]. These findings suggested that mutated, 'active' Notch1 could transform T-cell progenitors. Several years later the Robey laboratory suggested that Notch1 signalling is an essential regulator of both αβTCR$^+$ versus γδTCR$^+$ and CD4$^+$ versus CD8$^+$ T-cell-lineage commitment[18]. Although these data were disputed and the experiments revisited by multiple investigators, they represent the first effort of probing for Notch function in T-cell development.

The most conclusive evidence of a crucial role for Notch signalling in T-cell development came from Notch gain-of-function and loss-of-function genetic models[19,20]. Overexpression of the active form of Notch1 (intracellular Notch1) resulted in ectopic development of pre-T cells in the bone marrow, and the deletion of *Notch1* in HSCs led to a total inhibition of T-cell differentiation and to thymic atrophy. Several genetic experiments involving the targeting of multiple regulators of Notch1-mediated transcription (including CSL, Deltex1, MAML1 and Pokemon) have confirmed that Notch1 is an important regulator of haematopoietic progenitor commitment to the T-cell lineage[21].

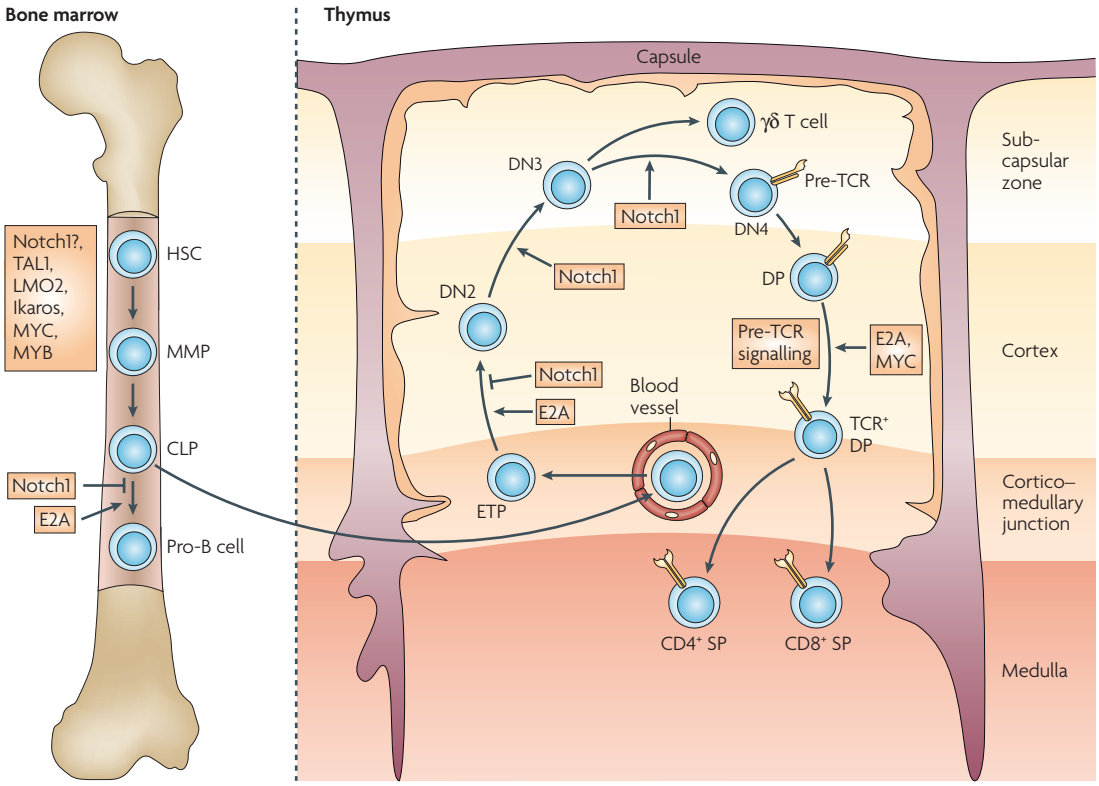

Figure 1 | **Stages of haematopoiesis and T-cell development and T-cell-leukaemia-related oncogenes.** Bone-marrow haematopoietic stem cells (HSCs) exit the quiescent 'niche' and differentiate to become multipotent progenitors (MPPs). MPPs further commit to the lymphoid lineage generating common lymphoid progenitors (CLPs). Several progenitor subsets (including MPPs and CLPs) have been suggested to represent the progenitor of thymic pro-T cells. These subsets migrate to the thymus (as early T-cell-lineage progenitors (ETPs)) and commit to the T-cell lineage, progressing through the double negative (DN; CD4$^-$CD8$^-$) stages, DN2, DN3 and DN4. Upon successful recombination at the T-cell receptor β (*TCRB*) locus, pre-T cells acquire surface expression of the pre-TCR that promotes differentiation to the DN4 stage. Pre-TCR-selected cells reach the double positive (DP; CD4$^+$CD8$^+$) stage, at which point they are subjected to the processes of positive and negative selection. Selected cells then exit the thymus as single positive (SP) CD4$^+$ or CD8$^+$ T cells. The stages of differentiation at which oncogenes that are known to be associated with T-cell acute lymphoblastic leukaemia and required in the bone marrow and thymus are also depicted. LMO2, LIM-only 2; TAL1, T-cell acute lymphocytic leukaemia 1.

ADAM family
(A disintegrin and metalloproteinase family). Members of this family contain disintegrin-like and metalloproteinase-like domains and are involved in the regulation of developmental processes, cell–cell interactions and protein processing, including ectodomain shedding.

γ-secretase complex
The enzyme complex that is responsible for cleavage at the S3 site of Notch proteins, thereby releasing the intracellular domain.

PEST domain
(Proline-, glutamic-acid-, serine- and threonine-rich domain). A protein sequence that is found in unstable cytosolic proteins that contain unusually high frequencies of proline, glutamine, serine and threonine residues. It results in rapid, proteasome-mediated degradation.

However, Notch signalling is not only involved in cell-fate decisions but it also provides an important homeostatic mechanism for differentiating DN thymocytes. Normal homeostasis (survival, proliferation and metabolism) is usually disturbed by the expression of oncogenes. Recent evidence suggests that Notch1 signalling could affect pre-T-cell metabolism through the activation of the PI3K (phosphoinositide 3-kinase)–AKT signalling cascade[22]. The role of Notch signalling in cell metabolism has been further supported by a recent genome-wide study of Notch-regulated genes[23]. Although the molecular mechanisms remain under intense study, it is currently accepted that Notch signalling is essential for the DN to DP transition and for the differentiation of the αβTCR$^+$ T-cell lineage[24].

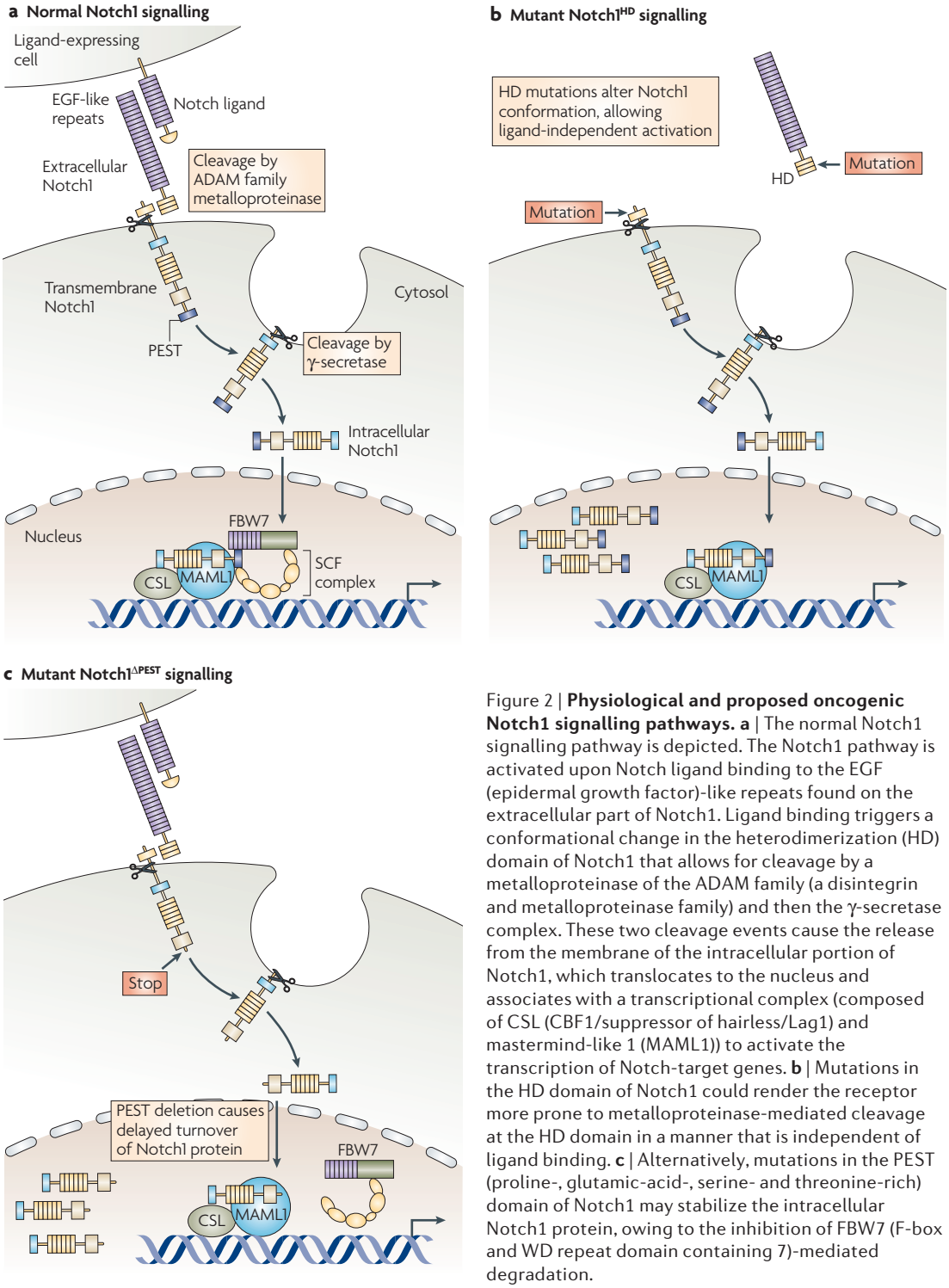

Figure 2 | **Physiological and proposed oncogenic Notch1 signalling pathways. a** | The normal Notch1 signalling pathway is depicted. The Notch1 pathway is activated upon Notch ligand binding to the EGF (epidermal growth factor)-like repeats found on the extracellular part of Notch1. Ligand binding triggers a conformational change in the heterodimerization (HD) domain of Notch1 that allows for cleavage by a metalloproteinase of the ADAM family (a disintegrin and metalloproteinase family) and then the γ-secretase complex. These two cleavage events cause the release from the membrane of the intracellular portion of Notch1, which translocates to the nucleus and associates with a transcriptional complex (composed of CSL (CBF1/suppressor of hairless/Lag1) and mastermind-like 1 (MAML1)) to activate the transcription of Notch-target genes. **b** | Mutations in the HD domain of Notch1 could render the receptor more prone to metalloproteinase-mediated cleavage at the HD domain in a manner that is independent of ligand binding. **c** | Alternatively, mutations in the PEST (proline-, glutamic-acid-, serine- and threonine-rich) domain of Notch1 may stabilize the intracellular Notch1 protein, owing to the inhibition of FBW7 (F-box and WD repeat domain containing 7)-mediated degradation.

The role of the pre-TCR in T-cell commitment. DN3 thymocytes initiate gene rearrangement at the *TCRB* locus, and following productive V(D)J recombination they express a TCR β-chain. The β-chain then pairs with the pre-TCR α-chain (which is encoded by *PTCRA*) and members of the CD3 complex to form the pre-TCR[25]. Recent elegant experiments have demonstrated that the pre-TCR signals in a ligand-independent manner due to its ability to oligomerize and induce the phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) of CD3 (REF. 26). Pre-TCR-derived signals are important for progression of DN3 cells to the DP stage, cell-cycle entry, suppression of cell death, inhibition of further TCRβ rearrangement (known as 'allelic exclusion') and commitment of the developing progenitors to the αβ (instead of the γδ) T-cell lineage[25]. Although multiple signalling intermediates have been implicated in these processes, we are still far from the complete understanding of the molecular mechanisms of pre-TCR-mediated T-cell differentiation.

Interaction of the Notch and pre-TCR signalling pathways. Two studies[24,27] have shown that the Notch and pre-TCR signalling pathways interact during T-cell development and transformation. Initially, the Zúñiga-Pflücker group showed that pre-TCR$^+$ progenitors cannot develop optimally in the absence of Notch signalling[27]. In addition, pre-TCR-deficient thymocytes (owing to the deletion of recombination-activating gene 2 (*Rag2*)) were unable to differentiate in the presence of Notch signalling, even if a rearranged TCR β-chain was introduced into the cells. These results were recently supported by studies of mice in which Notch signalling was suppressed using a dominant-negative form of the transcriptional co-activator MAML1 (REF. 24). Compared with these mice, Notch1-deficient mice showed a milder block in T-cell development, suggesting that other Notch-family members can participate in the DN to DP transition[28], although the deletion of *Notch3* or *Notch2* has not been reported to cause any developmental defect. An involvementof both the Notch and pre-TCR signalling pathways in T-cell transformation was shown by studies of the induction of T-cell leukaemia in mice that lack the pre-TCR (*Rag2*$^{-/-}$ or *Ptcra*$^{-/-}$ mice) or downstream pre-TCR signalling regulators (*Slp76*$^{-/-}$ and *Ccnd3*$^{-/-}$ mice). In all of these mice, the expression of activated forms of human or mouse Notch1 failed to induce (or was less efficient at inducing) the transformation of T-cell progenitors into leukaemic cells[29]. Finally, recent *in vitro* data further support a synergistic role between Notch and pre-TCR signalling in tumour maintenance by showing that silencing of pre-TCR activity in T-ALL cell lines that carry Notch1 mutations significantly suppresses their growth[30].

These observations beg the question: what might be the mechanism of cooperation between the two signalling pathways? There are two non-mutually exclusive scenarios that can be proposed. First, the two pathways could act in a linear manner, with one pathway downstream of the other. This is supported by several lines of evidence. The *Ptcra* gene has been shown both *in vitro* and *in vivo* to be a transcriptional target of Notch activity[31]. Similarly, the *CD3* genes have also been suggested to be regulated by Notch[23]. Also, *TCRB* gene rearrangement and expression of the TCR β-chain is promoted by Notch1 activation[32], further suggesting that the Notch pathway could be upstream of pre-TCR assembly and cell-surface expression. In the second scenario, the two pathways could act in parallel and converge at one or multiple signalling stages. This hypothesis is supported by recent evidence that the two pathways share common signalling intermediates. Indeed, both pathways are able to activate common kinases (LCK, the PI3K–AKT pathway and IKK (IκB kinase)) and transcription factors (MYC, NF-κB (nuclear factor-κB) and NFAT (nuclear factor of activated T cells))[22,33–35]. Also, both pathways appear to share common gene targets (for example, cyclin D3 and B-cell lymphoma 2A1 (BCL-2A1))[36,37]. It is therefore possible that synergistic signalling between the Notch and pre-TCR pathways is essential for the optimal regulation of cell-cycle entry, survival and further differentiation of DN3 cells. Of course, these two proposed models do not exclude the possibility of other alternative scenarios.

Finally, gene-expression profiling experiments suggest a potential negative correlation between the two pathways. Indeed, comparison of gene expression between pre-TCR$^-$ and pre-TCR$^+$ thymocytes shows that several elements of the Notch pathway (Notch1, Notch3, Deltex1, CD3δ and CD25) are downregulated following pre-TCR expression (I.A., unpublished observations). These data would suggest that pre-TCR signalling (or the transition from pre-TCR$^-$ to pre-TCR$^+$ DN3 cells) is able to restrict Notch signalling because aberrant Notch activation could lead to progenitor transformation. Although these studies are in their infancy, they propose an interestingly complicated regulation mechanism between the two pathways.

Animal models of T-cell leukaemia

Specific interchromosomal translocations are frequently associated with particular subtypes of leukaemia and lymphoma. However, although cytogenetic analysis of T-ALL lymphoblasts reveals recurrent translocations that activate several oncogenes in approximately 50% of cases, there is a large portion of individuals with T-ALL that have a normal karyotype. Furthermore, fluorescence *in situ* hybridization (FISH) analysis frequently indicates cryptic abnormalities, such as microdeletions that induce the loss of tumour suppressor genes. In 35% of T-ALL cases, the TCR loci are involved in translocations, probably occurring at the stage of T-cell differentiation when V(D)J recombination takes place. During TCR rearrangement, other genetic loci may be present in an 'open' chromatin configuration and therefore may be susceptible to the activity of the recombinase enzymes RAG1 and RAG2. Among these are oncogenes including: the basic helix–loop–helix (bHLH) family members *TAL1*, *TAL2*, *LYL1* and *BHLHB1*; the LIM-only (LMO) domain genes *LMO1* and *LMO2*; and the homeobox genes *HOXA–HOXD*, *HOX11* and *HOX11L2* (also known as *TLX3*). However, as discussed later, these translocations are normally associated with other chromosomal abnormalities or oncogene overexpression, suggesting that multiple pathways have to be disrupted for the induction or progression of leukaemia.

V(D)J recombination
Somatic rearrangement of variable (V), diversity (D) and joining (J) regions of the genes that encode antigen receptors, leading to repertoire diversity of both T-cell and B-cell receptors.

Allelic exclusion
A mechanism by which antigen receptors of a single specificity are expressed at the cell surface of a lymphocyte. This is an integral step in the clonal commitment of a cell lineage.

Fluorescence *in situ* hybridization
(FISH). The use of fluorescent probes to label specific DNA sequences in the nuclei of cells that are in the interphase or metaphase stages of mitosis.

Basic helix–loop–helix proteins
(bHLH proteins). A family of transcription factors with a basic domain that binds to a hexanucleotide sequence called the E-box, and a hydrophobic domain (the helix–loop–helix) that allows the formation of homodimers and heterodimers. They can also have leucine repeats called a leucine zipper.

LIM-only (LMO) domain genes
A family of genes encoding DNA-binding factors that include several blood oncogenes (LMO1, LMO2, LMO3 and LMO4). The LMO domain is a unique double-zinc finger motif that is found in a variety of proteins such as homeodomain-containing transcription factors, kinases and adaptors.

CD2 enhancer
A lymphocyte-specific promoter that is usually active at the common lymphoid progenitor stage.

Much of our current understanding of the molecular basis of T-cell malignancies has come from a detailed analysis of mouse models carrying the same translocations observed in the human disease[38] (TABLE 2). Here, we summarize the existing animal models of T-cell leukaemia and the information gained on the importance of each oncogenic lesion.

Basic helix–loop–helix proteins. Alterations in several bHLH proteins have been found in cases of T-ALL, the most common of these being the transcriptional regulator that is known to be essential for haematopoiesis, TAL1. The *TAL1* gene is normally expressed in a subset of haematopoietic cells (haematopoietic progenitors, erythroid lineage cells, mast-cell lineage cells and megakaryocytic lineage cells), endothelial cells and cells of the central nervous system. Mice lacking a functional TAL1 protein die of anaemia between embryonic day 8.5 and 10.5; these embryos contain no red blood cells. The *TAL1* gene is constitutively activated in up to 25% of T-ALL cases, but only in 3% of these does the activation result from the translocation of *TAL1* to the TCR loci[39]. The remaining cases have a deletion that replaces the 5′ regulatory sequence of *TAL1* with that of the upstream gene that is known as *SIL*, and this leads to dysregulation of *TAL1* expression[40,41]. Although there is convincing evidence for a role for TAL1 in human T-ALL, efforts to demonstrate a similar role for TAL1 in mouse models have been relatively unsuccessful. For example, transgenic mice that express TAL1 under the control of the CD2 enhancer do not develop leukaemia despite having high levels of TAL1 protein in their thymocytes[42]. Similarly, using a retrovirus encoding *TAL1* and a bone-marrow reconstitution model, there was no evidence of leukaemia despite TAL1 expression in the transferred bone-marrow cells[43]. These results suggest that additional genetic abnormalities are required to stimulate leukaemogenesis. Indeed, several reports have shown that abnormal expression of TAL1, in collaboration with casein kinase IIα (CKIIα; a serine/threonine protein kinase that is known to modulate the activity of bHLH transcription factors) and the transcriptional regulators LMO1 or LMO2, leads to the development of aggressive T-ALL in transgenic mice at a young age[44–49]. Importantly, these genes encoding CKIIα, LMO1 and LMO2 are found to be activated in individuals with T-ALL because of their translocation to the TCR loci[50,51] (TABLE 1). In addition, an independent study has shown that expression of *TAL1* under the control of the promoter for the T-cell-specific protein kinase LCK is able to induce T-ALL with long latency in only 30% of mice[51], whereas co-expression with LMO1 or loss of the *Ink4* locus results in the rapid development of leukaemia in all *TAL1*-transgenic mice[49,52–54].

Also, as observed for the TAL1 overexpression mouse models, transgenic mice expressing full-length or an amino-terminal truncated version of TAL1 fused to a carboxy-terminal truncated SIL, which is therefore driven by the *SIL* promoter (mimicking the SIL–TAL1 oncogenic fusion protein found in human T-ALL) showed only a block at the DN1 stage of thymocyte development or bone abnormalities and growth retardation, but no leukaemia development[55]. However, when crossed with transgenic mice that overexpress LMO1[49], all of these mice developed leukaemia, again suggesting that TAL1 expression under the control of *SIL* regulatory elements can provide an abnormal environment in which additional genetic events occur, resulting in complete malignant transformation. Despite these observations, the Croce group reported that about 80% of mice expressing transgenic *TAL1* under the control of the *LCK* promoter developed lymphoblastic leukaemia (of a T-cell-type, often with a minor B-cell component), suggesting a strong correlation between the abnormal TAL1 expression and T-ALL induction[51].

Two models have been proposed for the mechanism by which TAL1 alterations lead to leukaemogenesis. First, it is thought that TAL1 acts as a transcriptional repressor by forming heterodimers with the class I bHLH factors E2A and HEB (HeLa E-box-binding protein), thereby preventing the formation of E2A homodimers, as well as by actively recruiting transcriptional repressors, which leads to a block in transcriptional activity[56–59]. Support

Table 2 | **Mouse models of T-cell leukaemia**

Gene expressed	Mouse model	Phenotype	References
TAL1 (driven by the *CD2* promoter)	Transgenic	No leukaemia	42
TAL1	BMT	No leukaemia	43
TAL1 (driven by the *LCK* promoter)	Transgenic	Leukaemia in 30% of mice with long latency (9–10 months)	51
SIL–TAL1	Transgenic	No leukaemia	55
SIL–TAL1	Knock in	No leukaemia	46
LYL1 (driven by the *EF1A* promoter)	Transgenic	T-cell- and B-cell-type leukaemia in 30% of mice at 12 months	64
LMO1	Transgenic	Leukaemia with long latency (10 months)	49
LMO2	Transgenic	Leukaemia with long latency (10 months)	45
HOX11	BMT	Leukaemia with long latency (10 months)	81–83
Intracellular *Notch1*	BMT	Aggressive leukaemia	33
Intracellular *Notch3*	BMT	Aggressive leukaemia	85–86

BMT, bone-marrow transplant; *EF1A*, elongation factor 1α; HOX11, homeobox-11; LMO, LIM-only; LYL1, lymphoblastic leukaemia-derived sequence 1; SIL, SCL-interrupting locus; TAL1, T-cell acute lymphocytic leukaemia 1.

of this regulatory activity of TAL1 comes from a study in which the development of disease in *TAL1*-transgenic mice was accelerated when E2A or HEB were lacking[60]. Moreover, Palomero *et al.* recently proposed that TAL1 acts not only as a repressor but also as an activator of transcription, suggesting that TAL1 induces a complex transcriptional network that results in the disruption of crucial mechanisms that control cell homeostasis during thymocyte development[61].

Another bHLH protein that is expressed specifically by adult haematopoietic cells and is overexpressed in T-ALL is LYL1 (lymphoblastic leukaemia-derived sequence 1)[62]. The *TAL1* and *LYL1* genes share 90% sequence identity in their bHLH motif. However, Giroux *et al.* recently demonstrated, by comparing the expression patterns of *LYL1* and *TAL1* using *in situ* hybridization in mouse embryos from 7 to 14 days post coitus, that these genes have a largely overlapping pattern of expression. In particular, they are expressed by the developing vasculature, the endocardium, the developing haematopoietic system, the fetal liver and spleen, but not in the thymus[63].

Only recently has the oncogenicity of LYL1 been proved using a transgenic mouse strain that overexpresses full-length LYL1 driven by the elongation factor 1α (*EF1A*) promoter, which results in the ubiquitous, high expression of LYL1. Of these transgenic mice, 30% developed T-cell and B-cell leukaemia in 1 year that was associated with infiltration in multiple organs. However, because of the ubiquitous and strong overexpression of LYL1 in these mice, one must be cautious of drawing conclusions regarding its direct role in human cancer[64]. Immunohistochemical analysis showed that the infiltrated tumour cells were mainly DP T cells and mature B cells. These studies suggested that LYL1 is not only involved in the induction of T-cell-type leukaemia but also in B-cell-type leukaemia. The observation that LYL1 is highly expressed in the majority of cases of acute myeloblastic leukaemia (AML) and myelodysplastic syndrome (MDS) indicates that overexpression of LYL1 might also be involved in the development of a wide range of blood tumours[65].

LIM domain proteins. As mentioned above, co-expression of TAL1 and the LMO domain proteins LMO1 and LMO2 leads to the development of aggressive leukaemia in mice. Indeed, *LMO1* was discovered because it was shown to be associated with the chromosomal translocation t(11;14)(p15;q11) found in cases of T-ALL[66]. Similarly, *LMO2* was also found to be involved in the chromosomal translocation t(11;14)(p13;q11) observed in T-ALL[67,68]. Moreover, aberrant T-cell-specific expression of LMO1 and LMO2 has been found in 45% of T-ALL cases, with or without chromosomal translocations. It has also been reported that 4 of 11 patients with X-linked severe combined immunodeficiency treated with retrovirus-based gene therapy developed a T-ALL-like disease owing to retroviral integration into the *LMO2* locus[69].

To evaluate the role of abnormal expression of the LMO protein family, several mouse models have been generated. Both *LMO1*-transgenic mice and mice expressing a T-cell-specific *LMO2* transgene develop leukaemia with an accumulation of immature DN T cells, although the cancer had a long latency. These results indicate that the transgenes are necessary but not sufficient to cause tumours in these models, which is consistent with the observation that the combination of LMO1 or LMO2 and TAL1 accelerates the onset of leukaemia[45,49,62].

There are two hypotheses for the mechanism of leukaemia induction by abnormal overexpression of LMO1 and LMO2. First, several groups suggest that the LMO proteins form an aberrant DNA-binding multiprotein complex that may control the expression of target genes by direct binding to DNA[70–73]. Second, Grutz *et al.* have suggested that this abnormal complex would displace LMO4 from its normal association with LIM- domain binding 1 (LDB1; also known as CLIM2 or NLI) that occurs in DP T cells, thereby interfering with T-cell differentiation before the occurrence of T-cell transformation[74]. These findings indicate that one function of LMO–LBD1 complexes is to maintain the proliferative rather than the differentiation state of T cells. Changes in the stoichiometry of these complexes may perturb the normal pathways occurring in these cells and may promote the immature phenotype that is characteristic of transformed cells in T-ALL.

Homeobox genes. The homeobox genes encode a family of highly conserved master regulators of transcription that were first identified for their function in early development and are strongly implicated in the regulation of haematopoiesis[75,76]. So far, only the HOXA genes (especially *HOXA10* and *HOXA11*) have been shown to be involved in cryptic inversions or translocations associated with T-ALL[77,78]. The upregulation of expression of these genes is probably due to the juxtaposition of *TCRB* regulatory elements in the vicinity of the *HOXA* genes or it may be due to the separation of the *HOXA* locus into two parts, which may distance the *HOXA* genes from the normal regulatory elements of the cluster[79].

Several orphan HOX proteins have been shown to function as cofactors for HOX proteins, and they are implicated in organogenesis and in the differentiation of certain cell types[75]. For example, mice deficient in *HOX11* fail to develop a spleen, suggesting that this gene is important for the survival of splenic precursors and is required for spleen organogenesis[80]. Only two of the orphan HOX genes, *HOX11* and *HOX11L2*, have been associated with T-ALL[81]. The *HOX11* gene was originally identified because it was found to be involved in the translocation t(10;14) found in 7–10% of paediatric patients with T-ALL, and subsequently, was also found to be involved in the t(7;10) translocation[92,93]. In addition, the expression of this gene is frequently upregulated in T-ALL cells in the absence of a genetic rearrangement[93,94]; in these cases demethylation of its promoter might induce *HOX11* gene activation[95]. Importantly, the *HOX11* gene is not normally expressed in thymocytes, but during leukaemogenesis its expression can become controlled by the regulatory regions of the TCR loci, thereby inducing aberrant expression in T cells and causing a block at the DP stage. The oncogenic potential of HOX11 has also been shown by the induction of

Latency
The time that separates the carcinogenic insult from the clinical detection of the tumour. A portion of the latency period can be attributed to the slow accumulation of genetic alterations that lead to immortalization and transformation.

LCK promoter
A T-cell-specific promoter that drives the expression of transgenes early in double-negative thymocytes.

leukaemia in mice in which HOX11 was overexpressed by bone-marrow progenitors. Nevertheless, as is the case for several other oncogenic mutations, the leukaemia in these mice had a long latency (7–12 months), indicating that progression to a fully malignant state requires additional mutations[82–84].

Notch proteins. In rare cases of T-ALL, Notch1 activation is caused by a t(7;9) translocation of the *NOTCH1* gene with the TCR locus. This translocation juxtaposes the 3′ end of *NOTCH1*, starting from the coding sequences of EGF-like repeats, with the *TCRB* promoter and enhancer sequences, which drive the expression of a series of abnormally truncated mRNAs that encode Notch1 polypeptides with an amino-terminal deletion[17]. Direct proof that aberrant Notch signalling caused leukaemia was provided by studies of a bone-marrow transplant reconstitution model, in which LIN^- bone-marrow cells expressing the active form of Notch1 (intracellular Notch1) were transferred into irradiated recipient mice[32,85]. The overexpression of Notch1 in these mice led to an accumulation of immature DP T cells in the bone marrow, blood and secondary lymphoid tissues as early as two weeks post-transplantation[33].

Other Notch isoforms can also be oncogenic. Rohn *et al.*[86] isolated recombinant feline leukaemia virus (FeLV) proviruses from FeLV-induced lymphomas in cats and showed that they contained active Notch2 sequences, which implies a correlation between Notch2 and leukaemogenesis. The Screpanti group generated transgenic mice, in which intracellular Notch3 was overexpressed specifically in T cells, and found that these mice developed multi-organ infiltration of T lymphoblasts of variable phenotypes and died at 10–14 weeks of age, suggesting that Notch3 is able to induce T-cell malignancy similar to Notch1[87,88]. An intriguing question is whether there is a role for Notch3 in human Notch1-induced T-ALL. However, mutations of Notch3 in T-ALL have not yet been described.

In the remaining cases of human T-ALL, however, activated Notch1 is caused by mutations in the HD domain and/or the PEST domain of Notch1. As we discuss later, mutations in the PEST domain stabilize the intracellular Notch1 protein as they affect its recognition by a ubiquitin ligase. The specific effect of mutations in the HD domains is unknown; it appears that such mutations alter the HD domain conformation and make the receptor susceptible to ligand-independent proteolysis and activation. Whether this conformational change causes the receptor subunits to fall apart or merely alters the protein sufficiently to allow ADAM to access its cleavage site has not yet been determined[89] (FIG. 2b).

Together, these findings indicate that it is possible to model T-cell leukaemia in mice using gain-of-function mutants of several oncogenes. Each of these genes can contribute to the transformation of developing T cells. Although the use of animal models has enriched our understanding of the molecular mechanisms of disease induction in humans, a note of caution should be added. Indeed, most of the described experiments involve overexpression transgenes driven by T-cell-specific but heterologous promoters and this could affect both the mechanistic interpretations and treatment options. The use of gene-targeting techniques and the design of 'knock-in' and cell-type specific approaches are therefore recommended.

Phenotypic analysis of leukaemia and lymphomas in multiple animal models has revealed that oncogenes can arrest lymphoid cells at specific stages of differentiation. This suggests an interesting interplay between normal differentiation and leukaemic transformation driven by oncogenes that function at specific stages of T-cell development (FIG. 1). Look and colleagues were the first to study this notion by carrying out molecular profiling studies on a large number of T-ALL samples[90]. Their analysis showed that there is indeed a correlation between oncogenic signatures and specific stages of T-cell development. Subsequent data mining and profiling of physiological T-cell development further validated this correlation and uncovered interesting patterns of gene activation. For example, the anti-apoptotic protein BCL-2 and the cell-cycle regulator cyclin D2 are highly expressed in pro-T cells and pro-T-cell leukaemias (such as those expressing LYL1). By contrast, the expression of both BCL-2 and cyclin D2 is suppressed in later, pre-TCR-selected stages of development (as in T-ALL caused by dysregulated TAL1 expression). Interestingly, at these developmental stages two other members of the same protein family (BCL-2A1 and cyclin D3) are expressed[29].

Targets of Notch1 signalling in T-cell leukaemia

The identification of Notch1 as the key T-ALL oncogene revolutionized the field and has led several laboratories to address important questions of Notch1 regulation. One of the first issues was the identification of signalling pathways and target genes regulated by oncogenic Notch1 in T-ALL cases. Initially, several groups identified the oncogenic transcription factor MYC as a direct transcriptional target of Notch1 in both T-ALL and breast cancer[23,91–93]. Indeed, using gene array and chromatin immunoprecipitation (ChIP) approaches, it was shown that the *MYC* promoter contains Notch1-binding sites that are important for the induction of MYC expression in T-ALL cell lines. Moreover, our laboratory, using gene array analysis of haematopoietic progenitors expressing intracellular Notch1, found that its activation leads to that of the NF-κB signalling pathway[33]. Indeed, intracellular Notch1 could induce the nuclear localization of NF-κB and the expression of several NF-κB components (such as RELB and NF-κB2) and NF-κB targets (BCL-2A1, ICAM1 (intercellular adhesion molecule 1) and IκBα (inhibitor of NF-κBα)). This is particularly interesting because the NF-κB pathway has been implicated in cell transformation, potentially owing to its ability to counter cell death and promote proliferation. Similar NF-κB induction was caused by vectors carrying human T-ALL *NOTCH1* mutations, and attenuation of the NF-κB pathway resulted in the suppression of

T-cell leukaemia both *in vitro* and *in vivo*. As studies have shown that MYC expression is regulated by two NF-κB sites located in the *MYC* promoter, future studies addressing a potential link between Notch1, NF-κB and MYC could provide exciting results.

Nevertheless, NF-κB and MYC are not the only downstream targets of Notch signalling in leukaemia. Ferrando and colleagues have performed a comprehensive analysis of the direct targets of the active Notch1–CSL complex using a genome-wide ChIP-on-chip approach in T-ALL cell lines treated with inhibitors of the γ-secretase complex[23]. Surprisingly, they found that more than 40% of Notch-responsive genes were regulators of cell metabolism and protein biosynthesis. More recent analysis from the same group revealed that an important metabolic pathway, the PI3K–AKT cascade, is controlled by Notch signalling through the function of the phosphatase PTEN (phosphatase and tensin homologue). Indeed, PTEN expression is negatively regulated by Notch1 through the activity of HES1 and MYC[94]. The importance of this interaction for the induction and maintenance of T-ALL remains to be tested *in vivo* using PTEN-deficient mice. Finally, another signalling cascade, the NFAT signalling pathway, was recently identified as a Notch1 target through the activation of calcineurin, a calcium-activated phosphatase that is essential for the processing and activation of NFAT factors. Treatment of leukaemic mice with the calcineurin inhibitors cyclosporine A (CsA) and FK506 induced cell death of the leukaemic cells *in vitro*, and rapid tumour clearance, and substantially prolonged animal survival[35]. It is therefore currently accepted that Notch1 mutations could induce cell transformation through multiple signalling pathways that alter cell survival, proliferation and metabolism.

Notch1 regulation by ubiquitylation

Another issue with important therapeutic implications is the identification of genes and/or proteins that can suppress Notch1 activation. The recently identified PEST domain mutations of Notch1 in T-ALL were shown to generate mutant Notch1 proteins that had extended half-lives, suggesting that ubiquitylation and proteasome-mediated degradation are processes that might regulate Notch1 signalling. We identified an ubiquitin ligase, FBW7 (F-box and WD repeat domain containing 7; also known as AGO and SEL10), that interacts with Notch1 in the nucleus[95,96]. FBW7 is an E3 ubiquitin ligase and forms part of the SCF complex (SKP1–Cullin-1–F-box protein complex) that can target several important cell-cycle regulators, including MYC, JUN and cyclin E[97] (FIG. 3). FBW7 binds a phosphothreonine centred degron (Thr[2512]) in the most distal part of the Notch1 PEST domain. This FBW7-binding degron is conserved in all four members of the mammalian Notch family (I.A., unpublished observations). The importance of this degron is illustrated by the finding that it is deleted in all Notch1 PEST mutants found in T-ALL. As FBW7 could potentially function as a tumour suppressor by regulating the expression of Notch proteins, we sequenced FBW7 transcripts from a large number of T-ALL patients. This analysis showed that a significant portion (~20%) of T-ALL patients tested had inactivating mutations that destroyed the degron-binding pocket in FBW7 and generated a dominant-negative form of the molecule. Interestingly, the majority of T-ALL patients with mutations in the *FBW7* locus also had mutations in the PEST or HD domains of Notch1, suggesting that the mutations have a synergistic effect in the induction of disease[98–100]. Also, most FBW7 mutations could be found in samples from patients undergoing disease relapse but not in samples used for initial diagnosis, suggesting that *FBW7* mutations could be selected for resistance to treatment. In agreement with this hypothesis, the majority of *FBW7*-mutated human T-ALL cell lines were resistant to treatment with γ-secretase inhibitors, which are drugs that are currently in clinical trials for the treatment of T-ALL.

So can FBW7 be considered a bona fide tumour suppressor in T-ALL? To address this issue, we have generated mice, in which *Fbw7* could be inducibly deleted in T cells. Preliminary studies of these mice reveal that deletion of *Fbw7* in T cells stabilizes the Notch1 protein in thymocytes. Moreover, these mice

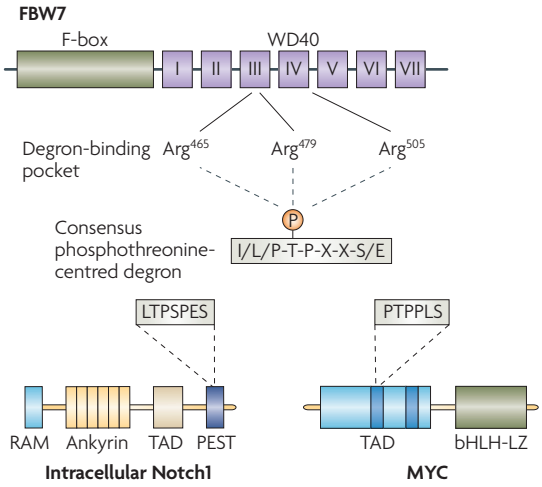

Figure 3 | **Mechanism of T-ALL oncogene recognition and degradation by FBW7.** The E3 ubiquitin ligase FBW7 (F-box and WD repeat domain containing 7) is able to bind, ubiquitylate and induce the proteasome-mediated degradation of intracellular Notch1 and MYC. FBW7 recognizes its substrates using a conserved binding pocket in its WD40 domain. The F-box of FBW7 is essential for interactions with the SCF (SKP1–Cullin-1–F-box protein) ubiquitin ligase complex. Three conserved arginine residues within the WD40 domain of FBW7 are essential for binding its target proteins. Significantly, these three residues have been shown to be mutated in patients with T-cell acute lymphoblastic leukaemia (T-ALL). Each arginine mutation is sufficient to abolish FBW7 binding to Notch1, MYC and cyclin E, which contain a consensus FBW7-binding degron (I/L/P-T-P-X-X-S/E; where X denotes any amino acid). The two FBW7 targets that have been implicated in T-ALL are shown here. The exact FBW7-binding degrons in Notch1 and MYC are also shown. bHLH, basic helix–loop–helix; LZ, leucine zipper; RAM, RBP-J-associated molecule; TAD, transactivation domain; PEST, proline-, glutamic-acid-, serine- and threonine-rich domain.

Chromatin immunoprecipitation
(ChIP). The use of antibodies specific for transcription factors to precipitate nucleic-acid sequences from chromatin for amplification.

E3 ubiquitin ligase
The enzyme that is required to attach the molecular tag ubiquitin to proteins that are destined for degradation by the proteasomal complex.

SCF complex
(SKP1–Cullin-1–F-box protein complex). A multisubunit ubiquitin ligase that contains SKP1, a member of the Cullin family (CUL1), and an F-box protein, as well as a RING-finger-containing protein (ROC1 or RBX1).

Degron
A signal within a protein that targets it for rapid degradation.

Bortezomib
(Also known as Velcade). A proteasome inhibitor approved by the Food and Drug Administration for the treatment of multiple myeloma.

develop T-cell leukaemia and lymphomas, although with a longer latency compared with mice overexpressing active Notch1 (REF. 101) (I.A., unpublished observations). These data support the hypothesis that FBW7 acts as a tumour suppressor in T-ALL.

Conclusions

The study of the molecular mechanisms that control T-cell development and T-cell leukaemia has uncovered a close connection between these two processes. Indeed, oncogenes and oncogenic pathways are essential regulators of physiological T-cell development, suggesting that there is a crucial balance between normal differentiation and malignant transformation. However, this close mechanistic connection could prove to be a weapon against leukaemia, as the accumulated knowledge on the function of genes and pathways during T-cell development could offer valuable therapeutic solutions.

Although current treatment protocols have improved the overall outcome for patients with T-ALL, a significant number of patients remain at a high risk of relapse, and few individuals survive when the disease recurs. The newly acquired knowledge of the molecular pathology of the disease will facilitate the design of novel targeted therapies. The frequency of *NOTCH1* activating mutations in T-cell leukaemia provides a compelling rationale for the use of either inhibitors of the Notch pathway, such as γ-secretase antagonists, or inhibitors of the NF-κB pathway, such as bortezomib[33]. Preclinical studies and early phase clinical trials exploring these agents and approaches are presently underway and offer promise for targeted and more effective therapy in the future.

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Acknowledgements
We thank W. Carroll, B. Mar and the members of the Aifantis laboratory for stimulating discussions and help with the preparation of the manuscript. Our work has been supported by the US National Institutes of Health (RO1CA105129), the American Cancer Society (RSG0806801), the Edward Mallinckrodt, Jr Foundation, the Sidney Kimmel Foundation for Cancer Research, The Cancer Research Institute, The Penelope London Fund, The Friedman Fund for Childhood Leukemia, and the G&P Foundation for Cancer Research. S.B. is supported by the New York University Molecular Oncology and Immunology training grant (T32 CA-09161) and by an American Society of Haematology scholar award. We apologize for any omissions of key citations due to space constraints.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BHLHB1 | E2A | FBW7 | HEB | HOX11 | LMO1 | LMO2 | LYL1 | MAML1 | NOTCH1 | TAL1

FURTHER INFORMATION

Iannis Aifantis' homepage: http://www.aifantislab.com

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