

Oncogenic role of Pax5 in the T-lymphoid lineage upon ectopic expression from the immunoglobulin heavy-chain locus

Abdallah Souabni,¹ Wolfram Jochum,² and Meinrad Busslinger¹

¹Research Institute of Molecular Pathology, Vienna Biocenter, Vienna, Austria; and ²Department of Pathology, University Hospital, Zürich, Switzerland

Four of 9 PAX transcription factor genes have been associated with chromosomal translocations in human tumors, although their oncogenic potential has not yet been demonstrated in transgenic mouse models. The B-lymphoid PAX5 gene participates in the generation of the t(9;14)(p13;q32) translocation in germinal center B cells, which leads to deregulated PAX5 expression under the control of the immunoglobulin heavy-chain (*IgH*) locus in a subset of B-cell non-Hodgkin lymphomas. Here we reconstructed a human

t(9;14) translocation in a knock-in mouse by inserting a PAX5 minigene into the *IgH* locus. The *IgH^{P5ki}* allele, which corresponds to a germline rather than somatic mutation, is activated in multipotent hematopoietic progenitors and is subsequently expressed in dendritic cells (DCs) and in natural killer (NK), T, and B cells. Ectopic Pax5 expression interferes with normal T-cell development and causes immature T-lymphoblastic lymphomas in *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice. Aggressive T-cell lymphomas develop even faster in

***Ik^{Pax5/+}* mice expressing Pax5 from the *Ikaros* locus. Pax5 expression in thymocytes activates B-cell-specific genes and represses T-lymphoid genes, suggesting that Pax5 contributes to lymphomagenesis by deregulating the T-cell gene-expression program. These data identify Pax5 as a potent oncogene and demonstrate that the T-lymphoid lineage is particularly sensitive to the oncogenic action of Pax5. (Blood. 2007;109:281-289)**

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Introduction

B-cell commitment critically depends on the transcription factor Pax5 (BSAP), which is required for B-cell development beyond an early pro-B-cell stage in the bone marrow.¹ Pax5^{-/-} pro-B cells are uncommitted lymphoid progenitors with a broad developmental potential and are able to differentiate into mature B cells only once their multilineage potential is suppressed by restoration of Pax5 expression.² Conversely, the loss of Pax5 expression by conditional gene inactivation converts committed pro-B cells with a restricted B-cell potential into lymphoid progenitors with a broad developmental potential.³ Moreover, ectopic Pax5 expression in hematopoietic stem cells (HSCs) and their progeny strongly skews the developmental options of lymphoid progenitors by promoting B-cell development at the expense of T lymphopoiesis.⁴ Consistent with its B-lineage commitment function, Pax5 is exclusively expressed within the hematopoietic system from the pro-B to the mature B-cell stage and is essential for maintaining the identity and function of B lymphocytes.⁵ At the molecular level, Pax5 fulfills a dual role in gene regulation. It activates a multitude of B-cell-specific genes, some of which code for essential components of (pre)BCR signaling.⁶ At the same time, Pax5 represses lineage-inappropriate genes with important functions in receptor signaling, cell adhesion, migration, and transcriptional regulation in other hematopoietic lineages.^{2,7} Hence, Pax5 controls the B-cell phenotype by shutting down inappropriate signaling systems and by simultaneously facilitating B-lymphoid signal transduction from the (pre)BCR.

Tumors of hematopoietic origin are frequently associated with specific chromosomal translocations, which result in the activation of proto-oncogenes controlling differentiation, proliferation, or cell survival.

In B-cell non-Hodgkin lymphomas (B-NHLs), these proto-oncogenes are often deregulated by translocation adjacent to regulatory elements of the immunoglobulin heavy-chain (*IgH*) locus on human chromosome 14q32.⁸ The PAX5 gene on chromosome 9p13 is involved in the recurrent translocation t(9;14)(p13;q32), which juxtaposes the intact coding sequences of PAX5 in opposite orientation next to the constant gene region of the *IgH* locus.⁹⁻¹¹ The molecular analysis of 5 breakpoints revealed that the t(9;14) translocation arises in germinal center B cells as a result of misguided *IgH* class switch recombination or somatic hypermutation. In 3 cases, the breakpoints were found in the switch S μ or S α region of the *IgH* locus and in exon 1B of PAX5, which resulted in replacement of the 2 alternative PAX5 promoters by antisense promoters present in the *IgH* switch region.¹⁰⁻¹² The breakpoints of 2 additional translocations occurred 1.8 kb (KIS-1 lymphoma) or 2 kb upstream of the distal exon 1A of PAX5, which brought the 2 PAX5 promoters under the control of the juxtaposed intronic E μ enhancer or 3' locus control region of the *IgH* locus.^{9,10,13} The regulatory t(9;14)(p13;q32) translocation may participate in lymphomagenesis in 2 ways. Overexpression of the translocated PAX5 gene^{10,11} may deregulate the transcription of Pax5-dependent genes in B cells. The expression of PAX5 is normally down-regulated during terminal B-cell differentiation, whereas the *IgH* gene is transcriptionally most active in immunoglobulin-secreting plasma cells.^{7,14} Hence, the presence of *IgH* control elements forces the translocated PAX5 allele to remain active during terminal differentiation, which may contribute to lymphoma formation by interfering with

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plasma-cell generation or function.⁹⁻¹¹ Consistent with this idea, the *IgH-PAX5* translocation was considered to be a characteristic hallmark of low-grade lymphoplasmacytic lymphomas (LPLs).^{10,15} However, reassessment of the t(9;14) translocation frequency revealed a low incidence in LPL,¹⁶ but a higher prevalence in aggressive B-NHL.^{12,17}

To provide direct evidence that *PAX5* can act as an oncogene in the pathogenesis of NHL, we have reconstructed the KIS-1 t(9;14) translocation in a knock-in mouse by inserting a *PAX5* minigene adjacent to the $E\mu$ enhancer into the *IgH* locus (Figure 1A). We found that the *IgH^{P5ki}* allele is first transcribed in multipotent hematopoietic progenitors and is then expressed in dendritic cells (DCs) and in natural killer (NK), T, and B cells. Forced *Pax5* expression within the T-lymphoid lineage interferes with normal T-cell development and leads to the formation of immature T-lymphoblastic lymphomas in *IgH^{P5ki}* mutant mice. *Pax5* expression under the control of the *Ikaros* locus⁴ generates even more aggressive T-cell lymphomas in *Ik^{Pax5/+}* mice. Ectopic *Pax5* expression in thymocytes activates B-cell-specific genes and represses key T-lymphoid genes, which may contribute to lymphomagenesis. These data identify *Pax5* as a potent oncogene and demonstrate that the T-cell lineage is particularly sensitive to the oncogenic action of *Pax5*.

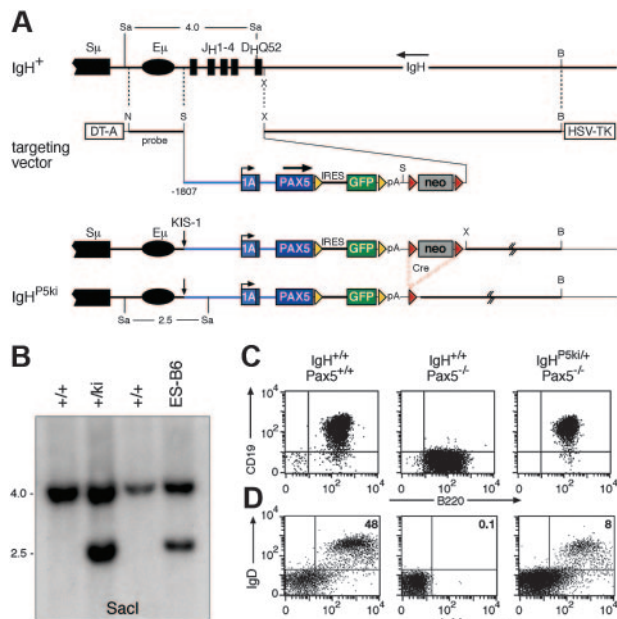


Figure 1. Reconstruction of the KIS-1 translocation by *PAX5* insertion into the *IgH* locus. (A) Structure of the targeted *IgH* locus. The *IgH* sequences from the $J\mu$ 1 to the $D\mu$ Q52 element were replaced by a human *PAX5* minigene linked via an internal ribosomal entry sequence (*IRES*) to a *GFP* gene. The *PAX5* minigene consisted of 1807 bp of 5' flanking sequences, the entire exon 1A, a shortened intron 1, and exon 2 fused in-frame to *PAX5* cDNA containing exons 3 to 10. The *IRES-GFP* gene was flanked by *frt* sites (yellow arrowheads) and the downstream *neomycin* (*neo*) resistance gene by *loxP* sites (red arrowheads). The lengths of *SacI* fragments indicative of correct targeting are shown in kilobases. B indicates *Bam*HI; N, *Not*I; S, *Sal*I; Sa, *Sac*I; X, *Xho*I; DT-A, diphtheria toxin A; HSV-TK, herpes simplex virus thymidine kinase; and pA, polyadenylation site. (B) Southern blot analysis of *SacI*-digested DNA of the indicated mice and targeted ES cell line using a *NotI-SalI* probe shown in panel A. (C) Expression of functional *Pax5* protein from the *IgH^{P5ki/+}* allele. Bone marrow pro-B cells from mice of the indicated genotypes were cultured in vitro prior to fluorescence-activated cell sorting (FACS) analysis of CD19 and B220 expression. (D) Partial rescue of B-cell development in *Pax5^{-/-}* mice by the *IgH^{P5ki}* allele. Splenocytes from 2-month-old mice of the indicated genotypes were analyzed for IgM and IgD expression.

Materials and methods

Detailed methods are provided in Supplemental Document S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article.

IgH^{P5ki} knock-in mouse

The *IgH^{P5ki}* targeting vector was generated based on the *IgH* targeting vector pIVhL2neo,¹⁸ as described in detail in the Supplementary Materials. E14.1 ES cells were electroporated, and correctly targeted ES cells were injected into C57BL/6 blastocysts. The *IgH^{P5ki}* allele was genotyped by amplifying a 780-bp polymerase chain reaction (PCR) fragment from the rabbit β -globin polyadenylation site with the primers 5'-GCGGAGCTCCTCAGGTGCAGGCTGCCTATC-3' and 5'-CGCGTCGACGCTAACATCTGAGGGACTGT-3'.

Mice

The following mice were maintained on the C57BL/6 background: *Pax5^{+/-}*,¹ *Ik^{Pax5/+}*,⁴ *IgH^{uMT/+}*,¹⁹ *lck-cre*,²⁰ and *nestin-cre* mice.²¹

Competitive repopulation assay

C57BL/6 mice (homozygous *Ly5.1/Ly5.1*) were γ -irradiated with 12 Gy 24 hours prior to intravenous injection of 3×10^6 *IgH^{P5ki/+}* bone marrow cells (*Ly5.2/Ly5.2*) and 3×10^6 *IgH^{uMT/+}* bone marrow cells (*Ly5.1/Ly5.2*).

Antibodies and flow cytometry

Antibodies were obtained from PharMingen (San Diego, CA). Single-cell suspensions were stained with the respective antibodies and analyzed on a Calibur or Canto flow cytometer (Becton Dickinson, San Jose, CA). A wide forward/side scatter (FSC/SSC) gate was used for analysis of the different hematopoietic cell types.

In vivo transplantation of T-cell tumors

Single-cell suspensions were prepared from the enlarged thymus and spleen of *IgH^{P5ki/+}* tumor mice, and 10^6 thymocytes or splenocytes were injected into the tail vein of syngeneic wild-type mice.

Tumor cell lines and in vitro culture

Tumor cell lines were derived from *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* lymphomas and cultured like pro-B cells on γ -irradiated ST2 cells in IL-7-containing IMDM medium.¹

Histologic analysis

Dissected tissues were fixed overnight at room temperature in 4% paraformaldehyde/PBS followed by paraffin sectioning and staining with hematoxylin and eosin.

TCR β rearrangement analysis

DNA was prepared from single-cell suspensions of T-cell tumors prior to PCR amplification of the $D\beta$ 2- $J\beta$ 2 rearrangements of the *TCRB* locus.²²

Semiquantitative reverse transcription-PCR analysis

cDNA was prepared from sorted GFP^+ DP thymocytes of *IgH^{P5ki/P5ki}* and *Ik^{Pax5/+}* mice, and semiquantitative PCR was performed using published primers.^{7,22,23}

Results

Reconstruction of the KIS-1 translocation by insertion of *PAX5* into the *IgH* locus

To study the role of Pax5 in lymphomagenesis, we inserted a human *PAX5* gene into the mouse *IgH* locus in the same configuration as described for the t(9;14)(p13;q32) translocation of the KIS-1 lymphoma.^{9,10,24} To this end, we generated a human *PAX5* minigene containing 1807 bp of its 5' flanking sequences and linked this gene via an internal ribosome entry sequence (*IRES*) to the green fluorescent protein (*GFP*) gene (Figure 1A). The *PAX5-IRES-GFP* expression cassette was inserted into the mouse *IgH* locus adjacent to the intronic $\epsilon\mu$ enhancer in such a way that the *D_HQ52* element and all 4 *J_H* segments were deleted, thus resulting in a nonfunctional *IgH* allele (Figure 1A). Targeted ES cells were subsequently used to generate *IgH^{P5ki/+}* mice (Figure 1B). The *IgH^{P5ki}* allele was crossed into *Pax5* mutant mice to investigate whether Pax5 expression from the *IgH* locus can rescue transcription of the Pax5 target gene *CD19*.¹ Flow cytometric analysis revealed similar CD19 expression on cultured wild-type and *IgH^{P5ki/+} Pax5^{-/-}* pro-B cells in contrast to *Pax5^{-/-}* pro-B cells, indicating that the *IgH^{P5ki}* allele expresses a functional Pax5 protein (Figure 1C). The *IgH^{P5ki}* allele was also able to rescue the early B-cell developmental block in *Pax5^{-/-}* mice because mature IgM⁺IgD⁺ B cells could be detected in the spleens of *IgH^{P5ki/+} Pax5^{-/-}* mice (Figure 1D). However, these mice contained 6-fold fewer splenic B cells than wild-type mice (Figure 1D) due to a partial block of B-cell development at the pro-B- to pre-B-cell transition in the bone marrow (Figure S1). In summary, *Pax5* expression from the *IgH^{P5ki}* allele could restore B lymphopoiesis in *Pax5^{-/-}* mice, although with low efficiency.

Expression of the *IgH^{P5ki}* allele in lymphoid and dendritic cells

The *IgH* locus is transcriptionally active in T cells in addition to B cells, as thymocytes express germline *C μ* transcripts.²⁵ Moreover, the immunoglobulin $\epsilon\mu$ enhancer can direct transgene expression in both B and T cells.²⁶ To investigate the activity of the genetically modified *IgH* locus within the hematopoietic system, we analyzed GFP expression of the *IgH^{P5ki}* allele in bone marrow and spleen cells of heterozygous and homozygous *IgH^{P5ki}* mutant mice (Figure 2A). No GFP expression was detected in granulocytes (Gr1⁺Mac1⁺), monocyte/macrophages (M-CSFR⁺Mac1⁺), and erythroblasts (Ter119⁺). In contrast, the *IgH^{P5ki}* allele was expressed in all lymphoid cell types, because GFP expression was observed in B lymphocytes (B220⁺CD19⁺), peripheral CD4⁺ and CD8⁺ T cells, NK cells (NK1.1⁺TCR β ⁻) as well as in CD11c⁺CD8 α ⁺ and CD11c⁺Mac1⁺ DCs (Figure 2A). As expected, GFP expression was higher in B cells compared to all other lymphoid cell types (Figure 2A). Surprisingly, GFP expression was strongly variegated in peripheral T lymphocytes because a large fraction of these cells failed to express GFP in heterozygous *IgH^{P5ki/+}* mice. Less variegation of GFP expression was, however, observed in T cells of homozygous *IgH^{P5ki/PSki}* mice (Figure 2A). We next studied the initiation of *IgH^{P5ki}* expression in uncommitted bone marrow progenitors by analyzing GFP expression in multipotent progenitors (MPPs or LSKs; IL7R α ⁻Lin⁻Sca1^{hi}c-Kit^{hi}) and common lymphoid progenitors (CLPs; Lin⁻IL7R α ⁺Sca1^{lo}c-Kit^{lo}). GFP expression was absent in 23% of the *IgH^{P5ki/+}* MPPs (Figure 2B), which included the LT-HSCs (Flt3⁻Lin⁻Sca1^{hi}c-Kit^{hi}; data not shown). In contrast, all CLPs in the *IgH^{P5ki/+}* bone

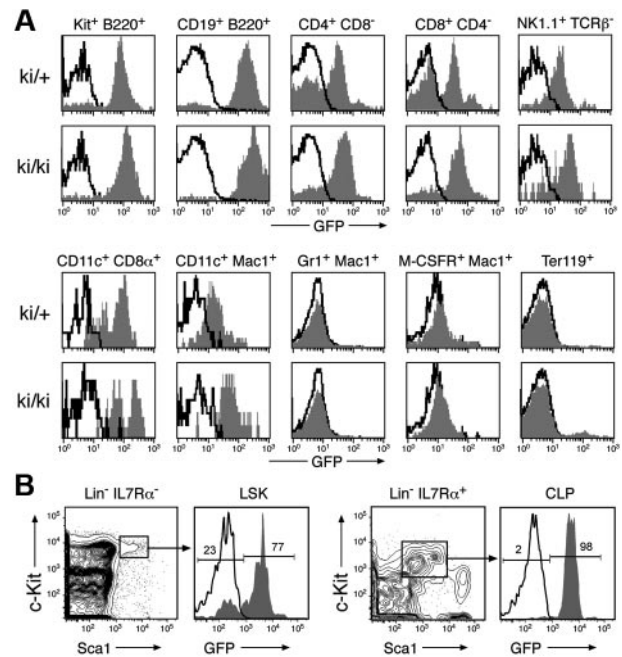


Figure 2. Expression of the *IgH^{P5ki}* allele in lymphoid and dendritic cells. (A) GFP expression in different hematopoietic cell types. Bone marrow (BM) cells and splenocytes (SPL) of 6-week-old *IgH^{P5ki/+}* (*ki/+*) and *IgH^{P5ki/PSki}* (*ki/ki*) mice were stained with the indicated antibodies, and GFP expression of the *IgH^{P5ki}* knock-in alleles (gray area) was displayed for c-Kit⁺B220⁺ pro-B cells (BM), B220⁺CD19⁺ B lymphocytes (BM), CD4⁺ and CD8⁺ T cells (SPL), NK1.1⁺TCR β ⁻ NK cells (SPL), CD11c⁺CD8 α ⁺ and CD11c⁺Mac1⁺ DCs (SPL), Gr1⁺Mac1⁺ granulocytes (BM), M-CSFR⁺Mac1⁺ monocyte/macrophages (BM), and Ter119⁺ erythroblasts (BM). Black lines indicate the absence of GFP expression in the corresponding wild-type cells. (B) Expression of the *IgH^{P5ki}* allele in uncommitted progenitors. GFP expression is displayed for the Lin⁻IL7R α ⁻Sca1^{hi}c-Kit^{hi} MPPs and Lin⁻IL7R α ⁺Sca1^{lo}c-Kit^{lo} CLPs in *IgH^{P5ki/+}* (gray area) and wild-type (black line) BM. The percentage of GFP⁺ and GFP⁻ cells is shown for MPPs and CLPs of the *IgH^{P5ki/+}* mouse.

marrow expressed GFP at a high level (Figure 2B). Together these results indicate that the *IgH^{P5ki}* allele is activated in a subset of MPPs and is subsequently expressed in DCs and all cell types of the lymphoid system.

Abnormal T-cell development in *IgH^{P5ki}* mutant mice

We next investigated whether the early expression of the *IgH^{P5ki}* allele in lymphoid progenitors affects the B- versus T-lineage choice similar to the pan-hematopoietic *Pax5* expression of the *Ik^{Pax5}* allele.⁴ For this purpose, we reconstituted lethally irradiated C57BL/6 mice with a 1:1 mixture of syngeneic *IgH^{P5ki/+}* and control *IgH^{μMT/+}* bone marrow, which could be distinguished by expression of the allogenic markers Ly5.1 and Ly5.2 (Figure S2). Nine months after transplantation, B cells were preferentially derived from the *IgH^{P5ki/+}* HSCs (Figure S2A), whereas thymocytes exclusively developed from the *IgH^{μMT/+}* HSCs (Figure S2B). Hence, precocious *Pax5* expression from the *IgH^{P5ki}* allele prevents T lymphopoiesis at the expense of B-cell development as described for the *Ik^{Pax5}* allele.⁴

Under noncompetitive conditions, the pro-B cell compartment (c-Kit⁺B220⁺) was increased 2-fold in *IgH^{P5ki/+}* and *IgH^{P5ki/PSki}* mice relative to wild-type mice, whereas B-cell numbers were normalized at later developmental stages in *IgH^{P5ki/+}* mice (Figure S3A-C). The thymic cellularity of *IgH^{P5ki/+}* and *IgH^{P5ki/PSki}* mice was, however, reduced on average to 60% and 33% of the wild-type level, respectively (Figure 3A). Flow cytometric analysis revealed an abnormal distribution of the different thymocyte

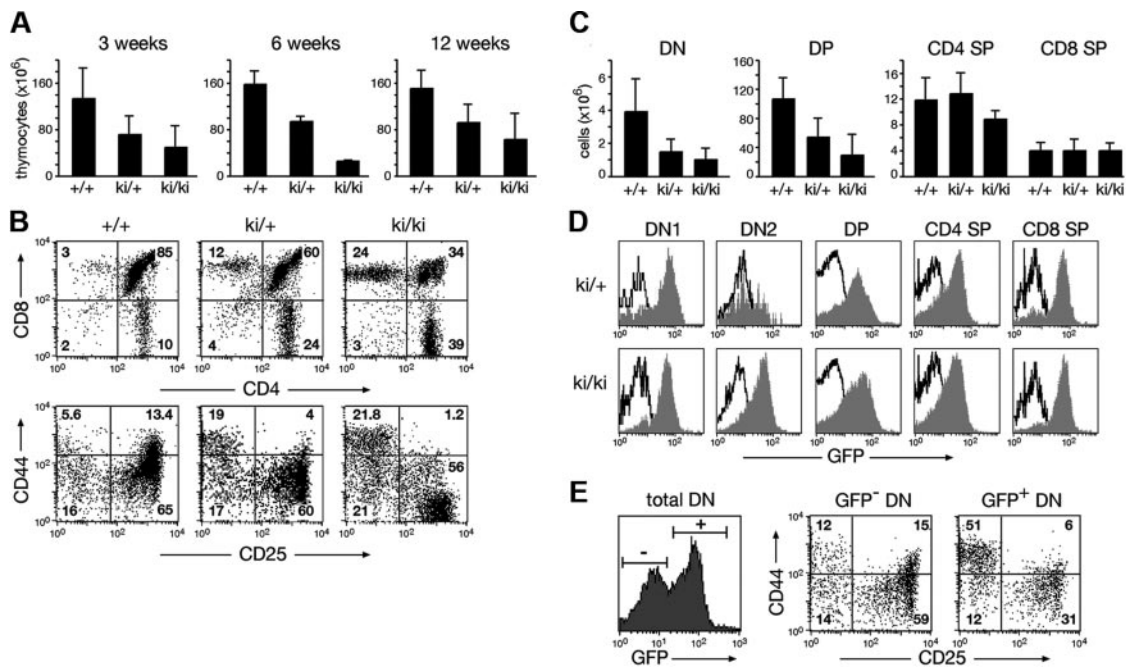


Figure 3. Abnormal T-cell development in *IgH^{P5ki}* mutant mice. (A) Decreased cellularity of the thymus in *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice. The average number of thymocytes ($\times 10^6$) was determined at 3, 6, and 12 weeks with at least 3 mice analyzed per genotype and time point. Error bars represent standard deviations of the mean. (B) Aberrant T lymphopoiesis in *IgH^{P5ki}* mutant mice. Total Thy1.2⁺ thymocytes of 3-week-old mice were examined for CD4 and CD8 expression and the Lin⁻Thy1.2⁺ DN thymocytes for CD44 and CD25 expression. (C) Reduction of DN and DP thymocytes in *IgH^{P5ki}* mutant mice at the age of 3 weeks. Absolute cell numbers ($\times 10^6$) are shown for the indicated mice (at least 4/genotype). (D) GFP expression of the *IgH^{P5ki}* allele (gray area) in the different thymocyte subsets. Black lines indicate the absence of GFP expression in wild-type thymocytes. (E) Variegated phenotype in a 6-week-old *IgH^{P5ki/+}* mouse. The CD44/CD25 expression profile is shown for gated GFP⁻ and GFP⁺ cells of the Thy1.2⁺ DN thymocyte population.

subsets in *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice (Figure 3B). The absolute number of the most immature CD4⁻CD8⁻ double-negative (DN) and CD4⁺CD8⁺ double-positive (DP) thymocytes progressively decreased from heterozygous to homozygous mutant mice, whereas the total number of CD4⁺ and CD8⁺ single-positive (SP) thymocytes remained constant regardless of the genotype (Figure 3B-C). The early phase of T-cell development can be divided into 4 stages, progressing from CD44⁺CD25⁻ (DN1) progenitors through the CD44⁺CD25⁺ (DN2) and CD44⁻CD25⁺ (DN3) stages to CD44⁻CD25⁻ (DN4) cells.²⁷ Analysis of the CD44/CD25 expression profile of Lin⁻ DN thymocytes revealed a partial block at the earliest stage of pro-T cell development, as DN1 cells were 4- to 10-fold increased within the DN cell compartment of *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice compared to wild-type mice (Figure 3B; data not shown). Consistent with an early block in pro-T cell development, most DN1 cells of *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice expressed GFP at a high level (Figure 3D). The GFP expression profile of DN2 cells and thymocytes at later developmental stages was broader and thus more variegated, as a sizeable fraction of the cells failed to express GFP (Figure 3D). Analysis of the GFP⁻ DN thymocytes revealed normal progression through the different stages of early T-cell development in *IgH^{P5ki/+}* mice (Figure 3E). In contrast, the GFP⁺ DN cells were preferentially blocked at the DN1 stage (Figure 3E). We conclude therefore that T-cell development is partially blocked at the earliest pro-T cell stage in *IgH^{P5ki}* mutant mice and that expression of the *IgH^{P5ki}* allele is variegated at later developmental stages.

Development of immature T-lymphoblastic lymphoma in *IgH^{P5ki}* mutant mice

Kaplan-Meier survival analysis demonstrated that half of the *IgH^{P5ki/P5ki}* mice died within 4.4 months (Figure 4A). As shown by

macroscopic or histologic analysis, the homozygous *IgH^{P5ki}* mutant mice developed disseminated lymphoma with complete penetrance by the age of 10 months. Lymphomas also occurred in heterozygous *IgH^{P5ki/+}* mice, although with a longer latency and at a lower incidence, because only 16% of all heterozygous mice died within 1 year (Figure 4A). All diseased mice showed splenomegaly and enlarged lymph nodes, whereas the majority of moribund mice also had a huge thymus (Figure 4B-D). Histologic analysis of the thymus, spleen, and lymph nodes revealed complete disruption of the normal architecture by a monomorphic population of medium-sized lymphoid cells, as shown for the thymus of a diseased mouse (Figure 4F) compared to a wild-type mouse (Figure 4E). Tumor cells were also found outside the capsule of the thymus and lymph nodes, where they infiltrated into adjacent tissues (Figure S4A). Extensive tumor infiltrates were furthermore detected in the lung (Figure 4H), liver (Figure 4I), kidney (Figure 4K), salivary gland, testis, and heart of moribund mice, which demonstrates efficient spreading of the tumor cells into nonlymphoid organs. Cytologically, the tumor cells displayed a high nuclear-to-cytoplasmic ratio; their nuclei were round to ovoid in shape and contained a predominantly fine chromatin (Figure 4G). The tumor cells furthermore showed high mitotic and apoptotic activity (Figure 4G).

Immunophenotyping of the lymphoma cells isolated from the thymus, bone marrow, spleen, and lymph nodes of homozygous and heterozygous *IgH^{P5ki}* mutant mice revealed that most tumor cells were CD4⁺CD8⁺ in all lymphoid organs with a varying contribution of CD8 SP cells (Figure 5A; data not shown). In about 20% of moribund mice, the lymphoma cells in the bone marrow and peripheral lymphoid organs consisted mainly of CD8 SP T cells, although the corresponding lymphoma in the thymus predominantly contained DP T cells. Detailed flow cytometric analysis demonstrated that the DP and CD8 SP lymphoma cells expressed

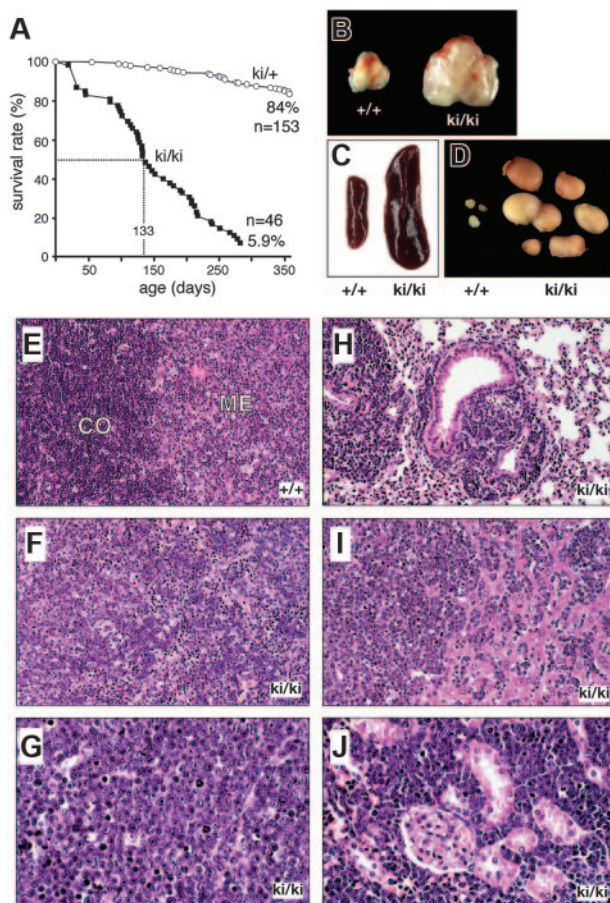


Figure 4. Lymphoma development in *IgH^{P5ki}* mutant mice. (A) Kaplan-Meier survival curves. The survival of *IgH^{P5ki/+}* mice (n = 153) and *IgH^{P5ki/P5ki}* mice (n = 46) was followed over time. Lymphomas were detected in all dead *IgH^{P5ki/P5ki}* mice, which were subjected to macroscopic or histologic analysis. Because we were unable to analyze 36% of the *IgH^{P5ki/P5ki}* mice at death, we conclude that at least 64% of all homozygous mutant mice died due to lymphoma development. (B-D) Gross aspect of the thymus (B), spleen (C), and lymph nodes (D) of the moribund *IgH^{P5ki/P5ki}* (ki/ki) tumor mouse 11 and a wild-type (+/+) littermate. (E-G) Disrupted architecture of the thymus in the *IgH^{P5ki/P5ki}* tumor mouse 11. Histologic sections of the thymus from a wild-type mouse (E) and the *IgH^{P5ki/P5ki}* tumor mouse (F-G) were stained with eosin and hematoxylin. The cytologic details of the tumor cells are shown at higher magnification in panel G. CO indicates cortex; ME, medulla. (H-K) Infiltration of the lung (H), liver (I), and kidney (J) by lymphoma cells in the diseased *IgH^{P5ki/P5ki}* tumor mouse 11.

TCR β at a low (tumor 15) to intermediate (tumor 11) level, were large in size, and had an activated CD25⁺ phenotype (Figure 5A). Importantly, the lymphomas expressed GFP and thus Pax5 (Figure 5A). Moreover, some of the tumors also expressed the Pax5 target gene *CD19* at a low level, as exemplified by tumor 15 (Figure 5A). We conclude, therefore, that forced *Pax5* expression in thymocytes induces the formation of immature T-cell lymphomas.

Cell lines of different lymphomas could be readily established in vitro within 2 to 3 weeks in coculture with stromal ST2 cells. These cell lines largely maintained the phenotype of the original tumor (Figure S5). Moreover, the tumor cells of heterozygous and homozygous *IgH^{P5ki}* mutant mice rapidly gave rise to secondary lymphomas in different organs within 7 weeks after intravenous injection into wild-type syngeneic mice (Figures S4D-F and S5). We next studied the clonal origin of the different tumors. PCR analysis of the *TCR β* recombination status of *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* lymphomas revealed that each tumor was clonal because it contained maximally 2 distinct D β 2-J β 2 rearrangements (Figure 5B). Every tumor was therefore derived from a single T cell

that, in addition to ectopic *Pax5* expression, had to acquire further genetic alterations to undergo malignant transformation. We identified the same clonal origin for the lymphomas in the spleen and thymus of tumor mouse 7 (Figure 5B). Surprisingly however, the rearrangement pattern differed for the lymphomas present in the same 2 organs of tumor mouse 19 or 34 (Figure 5B; Figure S6A-B). This difference could result from 2 independent oncogenic events giving rise to distinct monoclonal tumors in different organs of these mice. Alternatively, the different rearrangement patterns may arise from ongoing V(D)J recombination in CD4⁺CD8⁺ lymphoma cells due to continuous RAG protein expression. Together these data demonstrate that ectopic *Pax5* expression in the T-lymphoid lineage leads to the development of aggressive immature T-cell lymphomas.

Ik^{Pax5/+} mutant mice also die of aggressive T-lymphoblastic lymphomas

Previously, we have shown that *Pax5* expression under the control of the *Ikaros* (*Ik*) locus results in grossly abnormal T-cell development in *Ik^{Pax5/+}* mice.⁴ Already at 2 weeks, the thymic cellularity and the number of DP thymocytes were more strongly reduced in these mice⁴ compared to *IgH^{P5ki/P5ki}* mice (Figure 3A). Consistent with more severe developmental abnormalities, all *Ik^{Pax5/+}* mice died between 2 and 4 months (data not shown). Moribund *Ik^{Pax5/+}* mice had enlarged thymus, spleen, and lymph nodes, and histologic analysis of these lymphoid organs revealed diffuse infiltration by lymphoblastic lymphoma (Figure 6A). Extensive tumor infiltrates were found in many nonlymphoid organs such as the heart (Figure 6B), lung (Figure 6C), liver, kidney, intestine, and testis. The tumor cells in the thymus and spleen were monoclonal and mostly CD25⁺CD19⁺TCR β ^{int}CD4⁺CD8⁻ (Figure 6D-E), although some tumors predominantly consisted of either DN or DP T cells (data not shown). Immature T-cell lymphomas even developed in *Ik^{neol/+} lck-cre* mice (Figure 6D-E), where ectopic *Pax5* expression was exclusively activated within the T-lymphoid lineage by Cre-mediated deletion of a floxed neomycin stop cassette.⁴ Together these data indicate that ectopic *Pax5* expression in *Ik^{Pax5/+}* thymocytes also results in the formation of immature T-cell lymphomas, although with shorter latency compared to *IgH^{P5ki}* mutant mice.

Gene expression changes in Pax5-expressing thymocytes

To gain molecular insight into the oncogenic role of Pax5 in T cells, we investigated the transcriptional changes in Pax5-expressing thymocytes. To this end, we compared the expression of candidate genes by semiquantitative reverse transcription-PCR (RT-PCR) in sorted GFP⁺ DP thymocytes from *IgH^{P5ki/P5ki}* and *Ik^{Pax5/+}* mice with DP thymocytes and pro-B cells from wild-type mice. PCR conditions, which detect the human and mouse *Pax5* mRNAs with equal efficiency, revealed that the human *PAX5* minigene of the *IgH^{P5ki}* and *Ik^{Pax5}* alleles was expressed in DP thymocytes at a similar level compared to the endogenous *Pax5* gene in wild-type pro-B cells (Figure 7A). Because we previously measured a 5-fold higher expression level of the *Ik^{Pax5}* alleles in DN thymocytes,²² we confirmed this result by flow cytometric analysis of GFP expression, indicating that the transcription of the *Ik^{Pax5}* allele is down-regulated during the transition from DN to DP thymocytes (Figure S7). Previous expression analyses revealed that ectopic Pax5 expression activates the transcription of several B-cell-specific genes

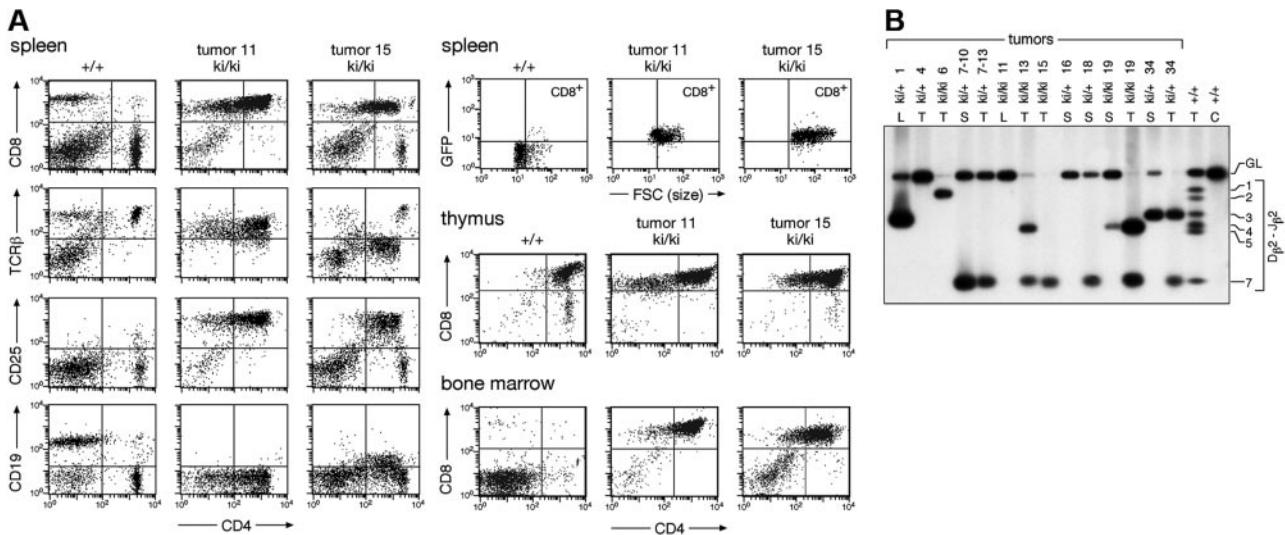


Figure 5. Ectopic Pax5 expression results in immature T-cell lymphomas. (A) Immunophenotype of lymphoma cells. The spleen, thymus, and bone marrow of a wild-type (+/+) and 2 *IgH^{P5ki/P5ki}* (ki/ki) tumor mice were analyzed for CD4 and CD8 expression; TCRβ, CD19, CD25, and GFP expression is also shown for the splenocytes. (B) Clonal *TCRβ* rearrangements in Pax5-induced T-cell lymphomas. *D_{β2}-J_{β2}* rearrangements were analyzed by PCR amplification of tumor DNA isolated from the thymus (T), lymph node (L), or spleen (S) of *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice. The different tumor mice are indicated by their numbers. The designations 7-10 and 7-13 refer to secondary lymphomas in the spleen of the recipient mice 10 and 13, which were intravenously injected with lymphoma cells from the spleen or thymus of tumor mouse 7, respectively. C indicates control tail DNA. Numbers to the right refer to the different *J_{β2}* segments and GL to the germline PCR product.

in *Ik^{Pax5/+}* DN thymocytes.²² Consistent with this finding, the Pax5 target gene *CD19* was also expressed in DP thymocytes of *Ik^{Pax5/+}* and *IgH^{P5ki/P5ki}* mice (Figure 7A). We next focused our attention on T-cell-specific genes that are repressed by Pax5 in lymphoid progenitors during B-cell commitment.⁷ The Pax5-repressed genes *Cd28*, *Grap2* (*Gads*), *Cd47*, *Itgal* (*Cd11a*), *Ramp1*, and *Satb1* were similarly expressed or only minimally affected in DP thymocytes of *IgH^{P5ki/P5ki}* mice compared to wild-type mice (Figure 7A). However, the same genes were efficiently down-regulated in DP thymocytes of *Ik^{Pax5/+}* mice (Figure 7A), suggesting that the 5-fold higher Pax5 expression in *Ik^{Pax5/+}* DN thymocytes may be essential for initiating gene repression in early T-cell development. Flow cytometric analysis furthermore confirmed that the expression of the cell surface proteins CD28 and CD11a (*Itgal*) was down-regulated on DP thymocytes of *Ik^{Pax5/+}* mice relative to control *Ik^{neo/+}* mice

(Figure 7B). This experiment also demonstrated that ectopic Pax5 expression rather than *Ikaros* heterozygosity is responsible for the down-regulation of CD28 and CD11a (Figure 7B). Surprisingly, *Notch1* transcription, which is normally repressed by Pax5 in the B-cell lineage,⁴ was even increased by ectopic Pax5 expression in DP thymocytes of *IgH^{P5ki/P5ki}* and *Ik^{Pax5/+}* mice (Figure 7A). The *Mint* gene coding for a repressor of Notch signaling was, however, similarly expressed in DP cells irrespective of the genotype (Figure 7A). In contrast, *Deltex1*, a direct target of Notch signaling,²³ was expressed in DP thymocytes of all mutant mice compared to wild-type controls, suggesting that ectopic Pax5 expression promotes Notch signaling within the T-cell lineage. Hence, Pax5 appears to contribute to the rapid development of T-cell lymphomas in *Ik^{Pax5/+}* mice by activating B-cell-specific genes, repressing T-lymphoid genes and possibly enhancing Notch signaling.

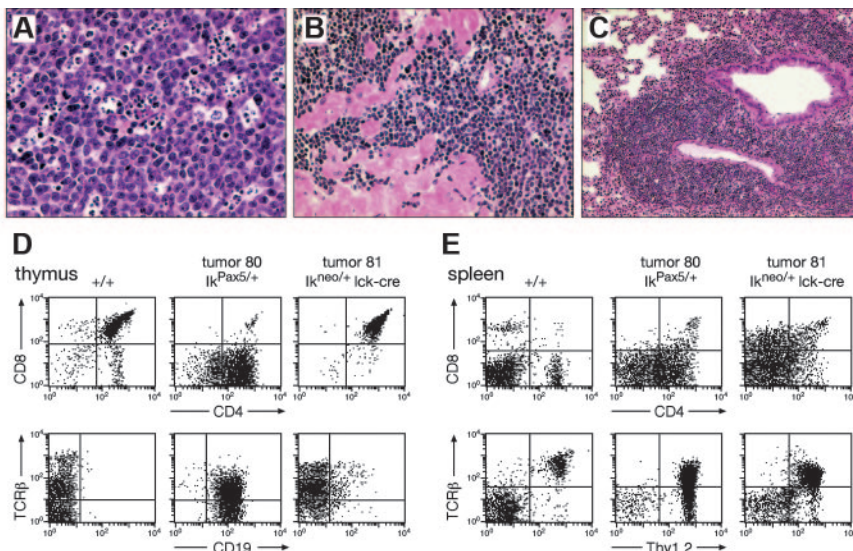
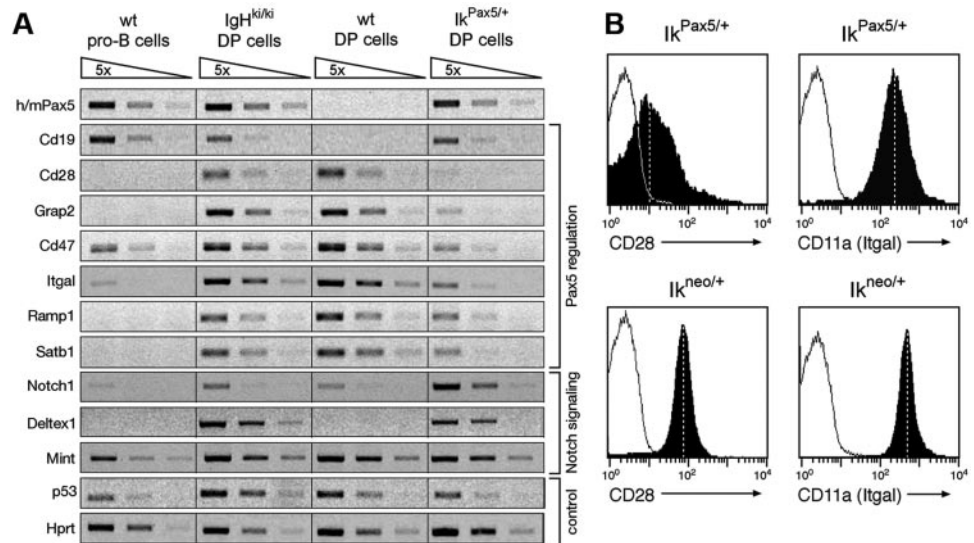


Figure 6. Development of immature T-cell lymphomas in *Ik^{Pax5}* mutant mice. (A-C) Histologic analysis of the lymphomas in the *Ik^{Pax5/+}* tumor mouse 80. A hematoxylin and eosin-stained section of the thymus (A) reveals the characteristic appearance of lymphoblastic lymphoma. The heart (B) and lung (C) are massively infiltrated by lymphoma cells. (D-E) Immunophenotype of immature T-cell lymphomas in the thymus (D) and spleen (E) of *Ik^{Pax5/+}* and *Ik^{neo/+}* *lck-cre* mice.

Figure 7. Repression of T-lymphoid genes in *Pax5*-expressing thymocytes. (A) RT-PCR analysis of the indicated genes in sorted pro-B cells from wild-type (wt) bone marrow and in sorted DP thymocytes from wild-type, *IgH^{P5ki/P5ki}*, and *Ik^{Pax5/+}* mice. The cDNA was normalized for equal expression of the hypoxanthine phosphoribosyltransferase (*Hprt*) gene prior to semiquantitative PCR of 5-fold serial cDNA dilutions. (B) CD28 and CD11a (Itgal) expression on gated DP thymocytes of *Ik^{Pax5/+}* and control *Ik^{neo/+}* mice (filled areas) as compared with wild-type DP thymocytes (black lines). The mean fluorescence is indicated by vertical dashed lines.



Discussion

Four of the 9 developmental control genes of the human *PAX* family have been associated with specific chromosomal translocations in human tumors. These translocations generate novel transcription factors by fusing the N-terminal DNA-binding domain of the Pax protein with the C-terminal regulatory sequences of a second transcription factor.^{28,29} *PAX3-FKHR* and *PAX7-FKHR* translocations are found in alveolar rhabdomyosarcoma, a pediatric muscle tumor,²⁸ the *PAX8-PPARG1* translocation in thyroid follicular carcinoma,³⁰ and the *PAX5-ETV6* translocation in B-progenitor acute lymphoblastic leukemia.³¹ Where analyzed, the fusion protein proved to be a more potent transcriptional activator than the corresponding wild-type Pax protein, suggesting that the fusion proteins contribute to oncogenesis by deregulating the expression of Pax target genes.²⁸ *PAX5* also participates in a second, regulatory translocation, t(9;14)(p13;q32), which leads to deregulated expression of a structurally intact *PAX5* gene under the control of the *IgH* locus in a subset of B-NHLs. Ectopic expression of *PAX5* was also observed in medulloblastoma,³² glioblastoma,³³ and neuroblastoma,³⁴ although the molecular mechanisms causing *PAX5* deregulation in these common tumors of the nervous system are still unknown. Despite the compelling association of *PAX* genes with human tumors, the oncogenic potential of these genes has not yet been demonstrated in transgenic mouse models in vivo.²⁹

Here we have described the characterization of an *IgH^{P5ki}* knock-in mouse that reproduces the specific structural and regulatory features of the t(9;14)(p13;q32) translocation identified in the human KIS-1 lymphoma.^{9,10,24} One important difference between this knock-in mouse and the corresponding human lymphoma is the fact that the *PAX5* insertion in the *IgH^{P5ki}* allele corresponds to a germline mutation, whereas the *IgH-PAX5* translocation in human lymphomas arises in germinal center B cells during late B-cell development.⁹⁻¹¹ Consequently, the inserted *PAX5* gene is transcribed in all *IgH*-expressing cells of the knock-in mouse, which contrasts with the exclusive expression of the endogenous *Pax5* gene in the B-lymphoid lineage of wild-type mice.¹⁴ The expression of the *IgH^{P5ki}* allele is initiated in multipotent hematopoietic progenitors and is subsequently expressed in DCs and in NK, T, and B cells. Interestingly, the T-lymphoid lineage proved to be particularly sensitive to the oncogenic action of Pax5 because the

IgH^{P5ki/+} and *IgH^{P5ki/P5ki}* mice die of immature T-lymphoblastic lymphoma. Although the *IgH^{P5ki}* knock-in mouse unequivocally identifies *Pax5* as an oncogene, the development of T-lymphoblastic lymphomas was surprising because the *PAX5* gene has not been implicated in the genesis of human T-cell tumors so far.³⁵ One possible reason for the absence of *PAX5* in chromosomal translocations involving the *TCRB* or *TCRA* loci is provided by the observation that all 3 genes are present in the same centromere-to-telomere orientation in the human genome. Hence, any head-to-head rearrangement between *PAX5* and a *TCR* locus would result in a translocation chromosome lacking any centromere and thus in its rapid loss in contrast to the *IgH-PAX5* translocation. However, the transcription factor genes *TALI(SCL)*, *LYL1*, *LMO1*, *LMO2*, *HOX11*, and *HOX11L2* are activated as T-cell oncogenes by translocations involving the *TCRA* or *TCRB* locus.³⁵ Importantly, the forced T-cell-specific expression of these transcriptional regulators, which like Pax5 are normally not expressed in the T-lymphoid lineage, is the most prevalent cause for human T-cell leukemia.³⁵

The substitution of the *J_H* gene segments by the *PAX5* minigene interferes with V(D)J recombination of the *IgH* gene and thus arrests B-cell development at the pro-B cell stage in homozygous *IgH^{P5ki/P5ki}* mice similar to previously published mice lacking the *J_H* domain.³⁶ The absence of B cells can lead to opportunistic infections, which may explain the significantly higher mortality of *IgH^{P5ki/P5ki}* mice compared to heterozygous *IgH^{P5ki/+}* mice. However, we could detect lymphomas in all dead *IgH^{P5ki/P5ki}* mice analyzed, indicating that death by opportunistic infections is an unlikely reason for the lower survival of these mice. An alternative explanation is provided by the observed variegated expression of the *IgH^{P5ki}* allele within the T-lymphoid lineage. T cells, which fail to express the *IgH^{P5ki}* allele and thus evade the oncogenic action of Pax5, are more abundant in heterozygous *IgH^{P5ki/+}* mice compared to homozygous *IgH^{P5ki/P5ki}* mice. The variegated expression of the *IgH^{P5ki}* allele could reflect the preferential location of the *IgH* locus at the nuclear periphery of thymocytes, which constitutes a repressive chromatin compartment.²² In addition, the *IgH* locus may be subject to classic position-effect variegation in T cells in agreement with the finding that the *E_μ* enhancer is able to induce variegated expression of a *CD2* transgene in T lymphocytes.³⁷ The probability that 1 of the 2 *IgH* alleles escapes the negative influence of repressive chromatin is thus increased in homozygous *IgH^{P5ki/P5ki}* mice, which could explain their higher tumor incidence.

Pax5 expression from the *IgH^{P5ki}* allele interferes with T-cell development prior to lymphoma formation, because DN and DP thymocytes are reduced in *IgH^{P5ki/+}* and *IgH^{P5ki/P5ki}* mice. In particular, Pax5 expression leads to an incomplete block at the earliest DN1 progenitor cell stage of T-cell development. This early block is a likely reason that the *IgH^{P5ki/+}* HSCs in competition with wild-type HSCs fail to give rise to T-cell development in bone marrow chimeras. T lymphopoiesis is even more severely affected in *Ik^{Pax5/+}* mice,⁴ which express *Pax5* from the *Ikaros* locus at approximately a 5-fold higher level in early T-cell development compared to the *IgH^{P5ki}* allele²² (Figure S7). In agreement with a higher *Pax5* expression level, the *Ik^{Pax5/+}* mice develop aggressive T-cell lymphomas with a shorter latency than *IgH^{P5ki/P5ki}* mice. T cells of heterozygous *Ik^{+/-}* mice are known to be hyperproliferative,³⁸ and homozygous *Ik^{-/-}* mice develop T-cell lymphomas, which identified *Ikaros* as a tumor suppressor gene of the T-lymphoid lineage.³⁹ Hence, the *Ik^{Pax5}* allele provides 2 oncogenic hits, that is, ectopic expression of *Pax5* and inactivation of one *Ikaros* gene, which together facilitate the more rapid development of T-cell lymphomas in *Ik^{Pax5/+}* mice.

Molecular analyses previously revealed that the *Ik^{Pax5/+}* pro-T (DN) cells activate the B-lymphoid transcription factor gene *EBF* in addition to *Pax5*, which leads to the expression of multiple B-cell-specific genes in early thymocytes.²² Consistent with this finding, B-lymphoid genes, as exemplified by *CD19*, are also expressed to varying degrees in *IgH^{P5ki}* mutant thymocytes. An important aspect of B-cell commitment is the Pax5-dependent repression of B-lineage inappropriate genes, some of which are normally expressed in the T-lymphoid lineage.^{2,7} Here, we have shown that Pax5 represses several of these T-lymphoid genes with particularly high efficiency in *Ik^{Pax5/+}* thymocytes. Some of the repressed genes are essential for normal T-cell development or function because they code for the costimulatory receptor CD28,⁴⁰ the integrin receptor LFA-1 (Itgal),⁴¹ the integrin-associated protein CD47,⁴² the signaling adaptor protein Grap2 (Gads),⁴³ and the nuclear architectural protein Satb1.⁴⁴ Paradoxically, Pax5 is known to interfere with the entry of lymphoid progenitors into the T-cell lineage by down-regulating *Notch1* transcription⁴ and, yet within the T-lymphoid lineage, Pax5 may enhance Notch signaling as evidenced by increased *Deltex1* expression. Activating mutations of *NOTCH1* are known to be a frequent cause for human T-lymphoblastic leukemia.⁴⁵ Hence, Pax5 appears to contribute to T-cell lymphoma development by activating B-cell-specific genes, repressing T-lymphoid genes, and possibly enhancing Notch signaling.

The *BCL6* gene, coding for a transcriptional repressor involved in germinal center B-cell development, is activated as an oncogene by translocation to the *IgH* locus in human diffuse large B-cell lymphoma (DLBCL) similar to *PAX5* in B-NHL.⁴⁶ A mouse model recapitulating the *IgH-BCL6* translocation has recently been generated by insertion of a *BCL6* minigene downstream of the $I\mu$ promoter of the endogenous *IgH* locus.⁴⁷ Contrary to the *IgH^{P5ki}* allele, the *I μ BCL6* allele appears to be expressed only in B cells, where it results in a lymphoproliferative disease that progresses to DLBCL with a latency period of more than 1 year.⁴⁷ We have monitored the *IgH^{P5ki/+}* mice colony maximally for 1 year and identified only a single GFP⁺CD19⁺ plasmacytoma in addition to many T-cell lymphomas (data not shown). B-cell tumors may either develop after 1 year in *IgH^{P5ki/+}* mice or may require normal functional T-helper cells to promote germinal center formation in contrast to the aberrant T-cell development observed in *IgH^{P5ki/+}* mice. To bypass this problem and to prevent T-cell lymphoma formation, we have recently generated a conditional *IgH^{P5ki}* allele, which additionally contains a floxed transcription stop cassette in *PAX5* intron 1. Deletion of this stop cassette by an *Aid-cre* transgene activates *PAX5* expression in germinal center B cells, where the *IgH-PAX5* translocation normally arises in human patients. Hence, the conditional *IgH^{P5ki/+}* mouse has the potential to more faithfully recapitulate the *IgH-PAX5* translocation characteristic of human B-NHLs.

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Authorship

Contribution: A.S. generated and characterized the *IgH^{P5ki}* knock-in mouse, W.J. performed the histologic analyses of the tumors, and M.B. contributed to the planning of the experiments and wrote the manuscript.

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Correspondence: Meinrad Busslinger, Research Institute of Molecular Pathology, Vienna Biocenter, Dr Bohr-Gasse 7, A-1030 Vienna, Austria; e-mail: busslinger@imp.univie.ac.at.

References

- Nutt SL, Urbánek P, Rolink A, Busslinger M. Essential functions of Pax5 (BSAP) in pro-B cell development: difference between fetal and adult B lymphopoiesis and reduced V-to-DJ recombination at the *IgH* locus. *Genes Dev*. 1997;11:476-491.
- Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature*. 1999;401:556-562.
- Mikkola I, Heavey B, Horcher M, Busslinger M. Reversion of B cell commitment upon loss of Pax5 expression. *Science*. 2002;297:110-113.
- Souabni A, Cobaleda C, Schebesta M, Busslinger M. Pax5 promotes B lymphopoiesis and blocks T cell development by repressing *Notch1*. *Immunity*. 2002;17:781-793.
- Horcher M, Souabni A, Busslinger M. Pax5/BSAP maintains the identity of B cells in late B lymphopoiesis. *Immunity*. 2001;14:779-790.
- Schebesta M, Pfeffer PL, Busslinger M. Control of pre-BCR signaling by Pax5-dependent activation of the *BLNK* gene. *Immunity*. 2002;17:473-485.
- Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M. Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. *Immunity*. 2006;24:269-281.
- Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. *Nat Rev Immunol*. 2002;2:920-932.
- Busslinger M, Klix N, Pfeffer P, Graninger PG, Kozmik Z. Deregulation of *PAX-5* by translocation of the $E\mu$ enhancer of the *IgH* locus adjacent to two alternative *PAX-5* promoters in a diffuse large-cell lymphoma. *Proc Natl Acad Sci U S A*. 1996;93:6129-6134.
- Iida S, Rao PH, Nallasivam P, et al. The t(9;14)(p13;q32) chromosomal translocation associated with lymphoplasmacytoid lymphoma involves the *PAX-5* gene. *Blood*. 1996;88:4110-4117.
- Morrison AM, Jäger U, Chott A, Schebesta M, Haas OA, Busslinger M. Deregulated *PAX-5* transcription from a translocated *IgH* promoter in marginal zone lymphoma. *Blood*. 1998;92:3865-3878.
- Sonoki T, Willis TG, Oscienc DG, Karran EL, Siebert R, Dyer MJ. Rapid amplification of immunoglobulin heavy chain switch (*IgHS*) translocation breakpoints using long-distance inverse PCR. *Leukemia*. 2004;18:2026-2031.
- Hamada T, Yonetani N, Ueda C, et al. Expression

- of the *PAX5*/BSAP transcription factor in hematological tumor cells and further molecular characterization of the t(9;14)(p13;q32) translocation in B-cell non-Hodgkin's lymphoma. *Br J Haematol*. 1998;102:691-700.
14. Busslinger M. Transcriptional control of early B cell development. *Annu Rev Immunol*. 2004;22:55-79.
 15. Offit K, Parsa NZ, Filipa D, Jhanwar SC, Chaganti RSK. t(9;14)(p13;q32) denotes a subset of low-grade non-Hodgkin's lymphoma with plasmacytoid differentiation. *Blood*. 1992;80:2594-2599.
 16. Cook JR, Aguilera NI, Reshmi-Skarja S, et al. Lack of *PAX5* rearrangements in lymphoplasmacytic lymphomas: reassessing the reported association with t(9;14). *Hum Pathol*. 2004;35:447-454.
 17. Poppe B, De Paepe P, Michaux L, et al. *PAX5/IGH* rearrangement is a recurrent finding in a subset of aggressive B-NHL with complex chromosomal rearrangements. *Genes Chromosomes Cancer*. 2005;44:218-223.
 18. Sonoda E, Pewzner-Jung Y, Schwes S, et al. B cell development under the condition of allelic inclusion. *Immunity*. 1997;6:225-233.
 19. Kitamura D, Roes J, Kühn R, Rajewsky K. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature*. 1991;350:423-426.
 20. Lee PP, Fitzpatrick DR, Beard C, et al. A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity*. 2001;15:763-774.
 21. Betz UAK, Voßhenrich CAJ, Rajewsky K, Müller W. Bypass of lethality with mosaic mice generated by Cre-*loxP*-mediated recombination. *Curr Biol*. 1996;6:1307-1316.
 22. Fuxa M, Skok J, Souabni A, Salvaggio G, Roldán E, Busslinger M. Pax5 induces *V-to-DJ* rearrangements and locus contraction of the *immunoglobulin heavy-chain* gene. *Genes Dev*. 2004;18:411-422.
 23. Höflinger S, Kesavan K, Fuxa M, et al. Analysis of Notch1 function by in vitro T cell differentiation of *Pax5* mutant lymphoid progenitors. *J Immunol*. 2004;173:3935-3944.
 24. Ohno H, Furukawa T, Fukuhara S, et al. Molecular analysis of a chromosomal translocation, t(9;14)(p13;q32), in a diffuse large-cell lymphoma cell line expressing the Ki-1 antigen. *Proc Natl Acad Sci U S A*. 1990;87:628-632.
 25. Kemp DJ, Wilson A, Harris AW, Shortman K. The immunoglobulin μ constant region gene is expressed in mouse thymocytes. *Nature*. 1980;286:168-170.
 26. Grosschedl R, Weaver D, Baltimore D, Costantini F. Introduction of a μ immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibodies. *Cell*. 1984;38:647-658.
 27. Godfrey DI, Kennedy J, Suda T, Zlotnik A. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3⁺CD4⁺CD8⁻ triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. *J Immunol*. 1993;150:4244-4252.
 28. Barr FG. Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. *Oncogene*. 2001;20:5736-5746.
 29. Robson EJ, He S-J, Eccles MR. A PANorama of PAX genes in cancer and development. *Nat Rev Cancer*. 2006;6:52-62.
 30. Kroll TG, Sarraf P, Pecciarini L, et al. *PAX8-PPAR γ 1* fusion oncogene in human thyroid carcinoma. *Science*. 2000;289:1357-1360.
 31. Strehl S, König M, Dworzak MN, Kalwak K, Haas OA. *PAX5/ETV6* fusion defines cytogenetic entity dic(9;12)(p13;p13). *Leukemia*. 2003;17:1121-1123.
 32. Kozmik Z, Sure U, Rüedi D, Busslinger M, Aguzzi A. Deregulated expression of *PAX-5* in medulloblastoma. *Proc Natl Acad Sci U S A*. 1995;92:5709-5713.
 33. Stuart ET, Kioussi C, Aguzzi A, Gruss P. *PAX5* expression correlates with increasing malignancy in human astrocytomas. *Clin Cancer Res*. 1995;1:207-214.
 34. Baumann Kubetzko FB, Di Paolo C, Maag C, et al. The *PAX5* oncogene is expressed in N-type neuroblastoma cells and increases tumorigenicity of a S-type cell line. *Carcinogenesis*. 2004;25:1839-1846.
 35. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. *J Clin Oncol*. 2005;23:6306-6315.
 36. Chen J, Trounstein M, Alt FW, et al. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the J_H locus. *Int Immunol*. 1993;5:647-656.
 37. Elliott JI, Festenstein R, Tolani M, Kioussis D. Random activation of a transgene under the control of a hybrid α CD2 locus control region/Ig enhancer regulatory element. *EMBO J*. 1995;14:575-584.
 38. Avitahl N, Winandy S, Friedrich C, Jones B, Ge Y, Georgopoulos K. Ikaros sets thresholds for T cell activation and regulates chromosome propagation. *Immunity*. 1999;10:333-343.
 39. Wang J-H, Nichogiannopoulou A, Wu L, et al. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. *Immunity*. 1996;5:537-549.
 40. Shahinian A, Pfeffer K, Lee KP, et al. Differential T cell costimulatory requirements in CD28-deficient mice. *Science*. 1993;261:609-612.
 41. Dustin ML, Bivona TG, Philips MR. Membranes as messengers in T cell adhesion signaling. *Nat Immunol*. 2004;5:363-372.
 42. Reinhold MI, Lindberg FP, Kersh GJ, Allen PM, Brown EJ. Costimulation of T cell activation by integrin-associated protein (CD47) is an adhesion-dependent, CD28-independent signaling pathway. *J Exp Med*. 1997;185:1-11.
 43. Yoder J, Pham C, Izuka Y-M, et al. Requirement for the SLP-76 adaptor GADS in T cell development. *Science*. 2001;291:1987-1991.
 44. Alvarez JD, Yasui DH, Niida H, Joh T, Loh DY, Kohwi-Shigematsu T. The MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development. *Genes Dev*. 2000;14:521-535.
 45. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of *NOTCH1* in human T cell acute lymphoblastic leukemia. *Science*. 2004;306:269-271.
 46. Ye BH, Chaganti S, Chang C-C, et al. Chromosomal translocations cause deregulated *BCL6* expression by promoter substitution in B cell lymphoma. *EMBO J*. 1995;14:6209-6217.
 47. Cattoretti G, Pasqualucci L, Ballon G, et al. Deregulated *BCL6* expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. *Cancer Cell*. 2005;7:445-455.