A Novel Recurrent Chromosomal Inversion Implicates the Homeobox Gene Dlx5 in T-Cell Lymphomas from Lck-Akt2 Transgenic Mice

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Abstract

The oncogene v-akt was isolated from a retrovirus that induced murine thymic lymphomas. Transgenic mice expressing a constitutively activated form of the cellular homologue Akt2 specifically in immature T cells develop spontaneous thymic lymphomas. We hypothesized that tumors from these mice might exhibit oncogenic chromosomal rearrangements that cooperate with activated Akt2 in lymphomagenesis. Cytogenetic analysis revealed a recurrent clonal inversion of chromosome 6, inv(6), in thymic lymphomas from multiple transgenic founder lines, including one in which 15 of 15 primary tumors exhibited this same rearrangement. Combined fluorescence in situ hybridization, PCR, and DNA sequence analyses showed that the distal inv(6) breakpoint resides at the T-cell receptor β chain locus, Terb. The proximal breakpoint maps to a region near a locus comprising the linked homeobox/transcription factor genes Dlx5 and Dlx6. Expression analysis of genes translocated to the vicinity of the Terb enhancer revealed that Dlx5 and Dlx6 are overexpressed in tumors exhibiting the inv(6). Experimental overexpression of Dlx5 in mammalian cells resulted in enhanced cell proliferation and increased colony formation, and clonogenic assays revealed cooperativity when both Dlx5 and activated Akt2 were coexpressed. In addition, DLX5, but not DLX6, was found to be abundantly expressed in three of seven human T-cell lymphomas tested. These findings suggest that the Dlx5 can act as an oncogene by cooperating with Akt2 to promote lymphomagenesis. [Cancer Res 2008;68(5):1296–302]

Introduction

Clonal chromosome translocations and inversions are common in leukemias and lymphomas, and the characterization of such abnormalities has led to the identification of genes critical to the origin of these malignancies (1). In lymphoid malignancies, these rearrangements classically involve juxtaposition of a proto-oncogene, often encoding a transcription factor or signaling molecule (2), with a T-cell receptor (TCR) or immunoglobulin gene. As a result of these rearrangements, a proto-oncogene that is silent or expressed at low levels in T- or B-cell progenitor cells is activated when placed under the control of potent enhancer elements within the regulatory region of a TCR or immunoglobulin gene, respectively. In T-cell malignancies, activation of various transcription factor genes, including the HOX11 homeobox gene, MYC, LMO1/2, TAL1/2, and LYL1, via juxtaposition to a TCR gene is common (2). Another example is the TCL1 locus, which is activated in mature T-cell leukemias by translocations/inversions juxtaposing it to regulatory elements of TCR genes (3). Interestingly, functional analysis of TCL1 has revealed its involvement in the Akt pro-survival pathway by binding to Akt and enhancing its enzymatic activity (3).

Akt was first identified as an oncogene transduced by a murine retrovirus that induces thymic lymphomas (4), and Akt is frequently hyperactivated in human solid tumors and hematologic malignancies (5). Transgenic mouse models in which an Lck promoter was used to drive expression of constitutively activated forms of Akt1, Akt2, or Akt3 resulted in spontaneous T-cell lymphomas (6–8). The latent period for tumor development in these mice and the fact that not all T cells undergo transformation suggest that additional genetic events are required for full transformation. We hypothesized that genes that cooperate with Akt in lymphomagenesis might be identified due to their position near translocation junctions in tumors arising at the time when the T-lymphoid recombination system is active. Thus, to identify potentially cooperating oncogenic rearrangements in transgenic mice expressing activated Akt2 in the T-cell compartment, we performed cytogenetic and molecular genetic analyses on a series of lymphomas from these mice. A recurrent chromosomal rearrangement, involving the TCR β chain gene, Terb, and a locus comprising the homeobox/transcription factor genes Dlx5 and Dlx6, is reported here.

Materials and Methods

Transgenic mice. Transgenic mouse lines were generated using a previously described construct (6, 8). Mice were genotyped by PCR using primers for HA and Akt2 (Table 1).

Cell lines and reagents. T cells were isolated from thymic lymphomas by passing tumor tissue through a 100 μm nylon mesh (BD Falcon) and culturing in Iscove’s DMEM containing 20% fetal bovine serum (FBS). ExactaCruz D immunoprecipitation kits and antibodies raised against Dsx1 (N-15 and FL-70), Terb (H-197), Dlx5 (C20 and Y20), and Dlx6 (C20) were from Santa Cruz Biotechnology. Other anti-Dsx1 antibodies were generated by one of us (Q.G.). A monoclonal Tcrb antibody (H57-597) was from BD PharMingen. Other antibodies included anti-HA (16B12) from Convance, anti–phospho-Akt from Santa Cruz or Cell Signaling Technologies, and anti-Dss1 antibodies were generated by one of us (Q.G.). A monoclonal Tcrb antibody (H57-597) was from BD PharMingen. Other antibodies included anti-HA (16B12) from Convance, anti–phospho-Akt from Santa Cruz or Cell Signaling Technologies, and anti–phospho-Akt from Santa Cruz or Cell Signaling Technologies. Functional studies of Dlx5 were performed with JML-5 cells (called Sld-Tac+ cells in ref. 9), derived from a thymic lymphoma that arose spontaneously in a severe combined immunodeficient mouse, as well as with Rat-1 fibroblast cells.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Primers and PCR conditions used in this investigation

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Primer name</th>
<th>Primer sequence</th>
<th>PCR condition</th>
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<tr>
<td>Genotyping of Lck-Akt2 mice</td>
<td>HA tag forward</td>
<td>AGGCACTGCCCCCTTTGAAGGC</td>
<td>94°C for 5 min; 40 cycles of</td>
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<td>Akt2 reverse</td>
<td>TTTGGGTTCTGATGTGAG</td>
<td>94°C for 1 min; 52°C for</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1 min, and 72°C for 1 min;</td>
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<td></td>
<td></td>
<td></td>
<td>and 72°C for 10 min</td>
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<td>Cloning of inversion breakpoints</td>
<td>Dss1 forward</td>
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<tr>
<td></td>
<td>TCRb reverse</td>
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<td>95°C for 20 sec, 59°C for 20 sec,</td>
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<td></td>
<td></td>
<td></td>
<td>and 72°C for 1 min; and 72°C for 10 min</td>
</tr>
<tr>
<td>Multiplex RT-PCR</td>
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<td>ACAAACCCGCCTCCCCGAGTGGCC</td>
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<tr>
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<td>Dlx5 reverse</td>
<td>CCCATCTAAATTAAAGGTCCCGGG</td>
<td>94°C for 30 sec, 58°C for 30 sec,</td>
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<tr>
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<td>Dlx6 forward</td>
<td>CCAGGCTTTAAAAACATCGCTTTC</td>
<td>and 72°C for 2 min; and 72°C for 10 min</td>
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<tr>
<td></td>
<td>Ldb1 forward</td>
<td>GGAACCCGGGTATGGTAACCTGGA</td>
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<tr>
<td></td>
<td>Ldb1 reverse</td>
<td>CACCCCCCGAGCTCATGTTG</td>
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Karyotypic analysis and fluorescence in situ hybridization. Preparation of metaphase spreads and trypsin-Giemsa (G) banding were performed according to standard procedures. Chromosome identification and karyotype designations were in accordance with the University of Washington guidelines. Bacterial artificial chromosome (BAC) clones used for fluorescence in situ hybridization (FISH) were purchased from Children’s Hospital Oakland Research Institute. BAC DNA was extracted using Psi clone BIGBAC DNA kit (Princeton Separations). For each probe, 500 ng of BAC DNA were labeled with either Spectrum Orange or Spectrum Green (Vysis) by random priming, using a BioPrime DNA Labeling kit (Invitrogen). Labeled probe was purified using sephadex G-50 columns (GE Healthcare). Probe was mixed with mouse Cot-1 DNA (Invitrogen) and precipitated with 3 mol/L sodium acetate and 100% ethanol. Precipitated probe was washed and resuspended in Hybrisol VII (MP Biomedicals) before hybridization. Smaller FISH probes were labeled by nick translation using DNA polymerase I/Dnase I in Hybrisol. Hybridization of probes to metaphase spreads and detection of FISH signals were performed according to standard procedures.

Cloning and sequencing of breakpoints. A PCR-based strategy was used to clone the genomic breakpoints of the inv(6), using primers near the breakpoints in Tcrb and Dss1 (Table 1). PCR products were cloned using TOPO-TA (Invitrogen).

RNA extraction and Northern blotting. RNA was isolated using a Totally RNA kit (Ambion), following the manufacturer’s recommendations. Northern blotting was performed using a Northern Max Ambion kit, per recommended procedures. The Dlx5 probe was a full-length cDNA, a gift from Giorgio Merlo (Dulbecco Telethon Institute, Milan, Italy). The Dss1 probe was a sequence corresponding to base numbers 1 to 114 of the coding region of Dss1. The β-actin probe provided with the Northern Max Ambion kit was used as an internal control. Probe (25 ng) was labeled with α-32P-dCTP or γ-32P-dCTP by random priming, using the Prime-It II Random Primer Labeling kit (Stratagene). Labeled probes were purified using a sephadex G-50 column, mixed with mouse Cot-1 DNA, placed in hybrisol solution, and hybridized to GeneScreen Plus membrane (PerkinElmer).

Reverse transcription-PCR. Semiquantitative multiplex reverse transcription-PCR (RT-PCR) was performed using SuperScript II reverse transcriptase (Invitrogen) with oligo dT. Multiplex RT-PCR was carried out using primers, and conditions were shown in Table 1. For real-time PCR, 100 or 20 ng of DNase-treated total RNA were used in reverse transcriptase reactions. Five microliters of cDNA were used in a 25-μl final volume. Primers for actin and Dlx5 were purchased from Applied Biosystems. The sequence of the Tag Man fluorogenic probe for the β-actin gene was 5’-CAGGAGTACATGATCGCGGCC-3’, and the sequence for Dlx5 was 5’-CCAGCCAGGCCAGAGAAGTGCG-3’.

Figure 1. Akt activity and chromosome 6 inversion in Lck-Myr-Akt2 mice. A, Western blot analysis (top) demonstrating activated Akt in histologically normal thymus from 4-wk-old Lck-Myr-Akt2 mouse and increased levels of phospho-Akt in five inv(6)-positive thymic lymphomas. Immunoblotting was performed with a phospho-specific Akt antibody. Middle, immunoblotting with Akt2 antibody. Bottom, immunoblotting with β-actin antibody demonstrating even loading among lanes. WT, thymus from wild-type mouse; Tg, thymus from transgenic mouse. B, Giemsa banding of normal 6 and inv(6)(A2B1) chromosomes. Low-resolution idiograms illustrating the chromosomal segment that becomes inverted in the inv(6). The inversion results in the juxtaposition of most of the pale G-bands 6A2 and 6B1.

http://www.pathology.washington.edu/research/cytogpages/idiograms/mouse/

1 http://www.aacrjournals.org
**Immunoprecipitation and Western blotting.** Cells were incubated in lysis buffer on ice for 15 min. Protein quantitation was determined by the Bradford method. For immunoprecipitation, 1 mg protein lysates were incubated with immunoprecipitation matrix–Dlx6 antibody overnight at 4 °C on a rotator. Washed pellets were resuspended in 2X reducing buffer and loaded onto a Novex SDS-PAGE gel (Invitrogen). For immunoblotting, samples (50 μg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunoblots were incubated with

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**Figure 2.** FISH and sequence analysis of breakpoints involved in the inv(6). A, FISH mapping of lymphoma with inv(6) showing that the distal breakpoint resides in Tcrb locus. BAC clone RP23-442M8 (red) localizes to 6B1 on normal 6 (N6) but is split in inv(6). BAC RP23-442M8 encompasses part of the variable and constant regions of Tcrb, including the enhancer. B, delineation of the proximal breakpoint of inv(6) from the same tumor. In normal 6, signals obtained with BAC clone RP24-204P9 map to the centromeric region. In inv(6), the signal is split. This BAC clone encompasses the entire Dss1 locus. Insets in A and B align normal 6 (left) with inv(6) (right). C, DNA sequences demonstrating that breakpoints of inv(6) are located near the RSS site located in diversity gene D1 of the Tcrb locus and a cryptic RSS site in intron 1 of the Dss1 gene. Red arrowheads, inv(6) breakpoint locations in relation to genomic sequences in the normal chromosome 6. Heptamer (yellows) and nonamer (gray) sequences similar to RSS are found adjacent to inv(6) breakpoints. D, schematic diagram of the inv(6). Inversion breakpoints (dashed lines) are in Tcrb locus and intron 1 of Dss1. The rearrangement places the Tcrb enhancer (E) near the Dlx5 and Dlx6 genes. Orientation is from centromere (cen) to telomere (tel).
primary antibodies at 4°C overnight, followed by incubation with secondary antibody conjugated with horseradish peroxidase for 60 mins at room temperature.

**Retroviral transduction of lymphocytes.** Phoenix-E retroviral packaging cells were transfected with murine stem cell virus-based pBmiGII retroviral vectors using the calcium phosphate transfection method. Cells were maintained in complete Iscove’s medium containing 20% FBS. JML-5 cells at a concentration of 1 × 10^6/mL were infected for 12 h at 37°C with retroviral supernatant. At 30 h postinfection, cells were isolated by flow cytometry for analysis. Transduction efficiencies were evaluated by determining the percentage of green fluorescent protein (GFP)-positive cells using flow cytometry. For the proliferation assay, GFP-positive cells were sorted, and then 10^4 cells per well were suspended in 100 µL of medium in 96-well plates. Cell numbers were determined at 24 h and 48 h using a hemacytometer.

**Soft agar assay.** Rat-1 fibroblasts (2 × 10^5 cells) were seeded in 6-well plates and incubated at 37°C overnight. Cells were cotransfected with 2 µg of each plasmid by using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, the cells were selected by culturing in medium containing 100 µg/mL Hygromycin (Invitrogen) and 400 µg/mL G418 (Invitrogen). Two weeks after selection, cells were trypsinized and counted. Then, 1 × 10^5 cells from each stable cell line were suspended in 1 mL RPMI containing 10% FBS. One milliliter of 0.6% agar solution (diluted from fresh 3% Noble Agar with RPMI 1640 containing 10% FBS) was added to the cells. The mixture was poured into the 6-well plates prefilled with 2 mL of 0.6% bottom agar. The plates were left at room temperature for 20 min and then incubated at 37°C. Cells were fed weekly with 1 mL RPMI containing 10% FBS. After 14 days of incubation, cells were fixed with 10% formaldehyde in PBS for 5 min and stained with 0.5 µL of 0.005% Crystal Violet (Sigma) for 1 to 2 h. All colonies with >200 cells were counted.

**Results**

Five independently derived founder lines were generated using a previously described Lck-Myr-Akt2 vector (7) in which the Lck promoter is used to direct expression of myristylated, constitutively active Akt2 in immature T lymphocytes. Thymic lymphomas developed in 80% to 100% of animals from each line, with a mean latency of 11 to 19 weeks in different lines. Immunoblotting showed the presence of activated Akt in histologically normal thymus from 4-week-old transgenic mice, with increased levels in lymphomas (Fig. 1A).

Karyotyping revealed a novel chromosome 6 inversion, inv(6)(A2B1) (Fig. 1B), in lymphomas from three founder lines. FISH analysis using an Lck-Myr-Akt2 plasmid as probe revealed that the transgene integration site involved different chromosomes (3, 7, and 10) in the three founder lines exhibiting the inv(6). One founder line showed the inv(6) in all 15 lymphomas examined, whereas nonmalignant tissues from the same mice had normal karyotypes. To our knowledge, such consistency of a specific rearrangement in a transgenic founder line has not been previously described.

To map the inv(6) breakpoints, FISH was performed on tumor metaphases, using numerous BAC probes corresponding to known map locations. The distal breakpoint was mapped to the TCR β chain locus (Tcrb) in band 6B1, specifically near the enhancer (Fig. 2A). The proximal breakpoint was localized to an ~100-kb region encompassing the Dss1 gene (Fig. 2B). Finer FISH mapping (data not shown), using long-range PCR-generated probes corresponding to 3' and 5' genomic sequences in Dss1, revealed that the proximal inv(6) breakpoint resides within this gene.

The proximal breakpoint junction was cloned using a PCR-based strategy, using primers corresponding to genomic sites within Dss1 and Tcrb, close to each inversion breakpoint (Supplementary Fig. S1). All primers were designed such that each would be in the sense orientation in the normal chromosome 6 and, therefore, would not result in a PCR product. However, in the inv(6), some primers would be oriented in the antisense direction and near enough to result in a PCR product. Using this strategy, a ~4.5-kb PCR product was obtained in genomic DNA from 4 of 10 inv(6)-positive lymphomas tested. PCR products from two tumors were cloned and sequenced to confirm disruption of Dss1. Sequence analysis of the breakpoints revealed the presence of a consensus recombination signal sequence (RSS) site at the breakpoint in Tcrb and a variant RSS in Dss1 (Fig. 2C), suggesting RAG-mediated variable, diversity, and joining [V(D)J] recombination. A schematic diagram of the inv(6) is shown in Fig. 2D.

Northern blot analysis showed that inv(6) lymphomas exhibit an endogenous Dss1 transcript and a larger transcript, representing a Dss1 exon1-Tcrb fusion transcript (Supplementary Fig. S2). This was confirmed by RT-PCR analysis in 20 of 20 inv(6)-positive tumors tested, and sequencing of the PCR product revealed a chimeric cDNA fragment consisting of Dss1 exon1 fused in-frame

![Figure 3. Deregulated expression of Dlx5 and Dlx6 in inv(6) lymphomas.](https://example.com/figure3.png)
to the constant region of Tcrb. Dss1 exons2/3 were fused to the Tcrb D1 segment in an arrangement that precludes the potential for mRNA or protein production based on genetic database predictions. Quantitative RT-PCR showed that exon 1 was up-regulated, whereas exons 2 and 3 were not. However, Western blot analysis with anti-Dss1 antibodies raised against either the full-length protein or amino terminus did not detect a Dss1-Tcrb fusion protein in inv(6) lymphomas. Similarly, immunoblot analysis with Tcrb antibodies, and immunoprecipitation/Westerns using various combinations of Dss1 and Tcrb antibodies, did not detect a fusion protein. Sequencing of the remaining Dss1 allele from six different inv(6) lymphomas did not reveal any point mutations.

Because oncogenic rearrangements involving TCR loci classically result in translocation of an intact gene into an abnormal regulatory context, the genomic region juxtaposed with the Tcrb enhancer was therefore explored for intact candidate genes that might be affected. Database searches revealed two genes (Dlx5 and Dlx6) within 250 to 300 kb of the Tcrb enhancer in inv(6) lymphomas. To determine if expression of Dlx5 and/or Dlx6 was up-regulated in inv(6) lymphomas, we used multiplex RT-PCR (Fig. 3A and B) and verified the findings by real-time PCR (data not shown). Northern blot analysis also confirmed up-regulation of Dlx5 (Supplementary Fig. S2). Although greatly elevated expression of both Dlx genes was observed in inv(6) lymphomas, transcripts were not detected in thymocytes from wild-type mice or other T-cell lymphoma cell lines without the inv(6). Immunoblot analysis documented consistent, greatly up-regulated expression of Dlx5 in inv(6) lymphomas and no detectable Dlx5 in normal thymocytes or lymphomas without the inv(6) (Fig. 3B). In our hands, commercially available anti-Dlx6 antibodies have not worked for direct Westerns, although we detected varying levels of up-regulation of Dlx6 protein in inv(6) lymphomas by immunoprecipitation/Western analysis (Fig. 3C). We also used real-time RT-PCR to examine expression of Evf1/2 and several imprinted genes that cluster in the Dlx5/Dlx6 region of chromosome 6 (Supplementary Fig. S3). Expression of a Dlx6 gene (Dlx5) in JML-5 cells accelerates cell proliferation. Control or retrovirally transduced GFP-positive JML-5 cells were isolated by flow sorting, and then 10^5 cells per well were seeded into 100 μL of medium. Cell numbers were determined after incubating for 24 h. Figure depicts one of three replicate experiments. Columns, mean; bars, SD. B, graphical representation of the number of cycles needed to detect control actin or Dlx5 expression in untransduced (negative) cells, cells transduced with vector (vector), and cells transduced with Dlx5 (Dlx5) for 24 h. Note that Dlx5 expression was not detectable in untransduced or vector-transduced JML-5 cells. C, overexpression of Dlx5 enhances colony formation in soft agar. Rat-1 cells were cotransfected and selected by G418 and Hygromycin for 2 wk. Cells were plated in soft agar, and colony numbers were counted after 2 wk of growth. The plasmid combinations were as follows: 1, pcDNA3.1-Hygro (vec1) + pcDNA3.0-Neo (vec2); 2, pcDNA3.1 + pcDNA3.0-HA-MycAkt2; 3, pcDNA3.1 + pcDNA3.0-HA-Akt2E299K; 4, pcDNA3.1-Dlx5 + pcDNA3.0; 5, pcDNA3.1-Dlx6 + pcDNA3.0-HA-Akt2E299K; and 6, pcDNA3.1-Dlx5 + pcDNA3.0-HA-MycAkt2.

Figure 4. Expression of Dlx5 contributes to increased cell proliferation and growth in soft agar. A, transduction of JML-5 cells with Dlx5 accelerates cell proliferation. B, real-time PCR analysis depicting expression of DLX5 and DLX6 in seven human T-cell lymphomas compared with expression in a nonmalignant lymph node (N). Up-regulation of DLX5 was observed in three lymphomas, whereas none of the clinical tumor specimens showed up-regulation of DLX6. (–), negative control; (+), positive control. GAPDH was used as a loading control.
antisense RNA, Dlx6-as, was examined by semiquantitative RT-PCR. None of the imprinted genes translocated near the proximal inv(6) breakpoint were up-regulated in inv(6) lymphomas compared with normal thymocytes. Instead, up-regulation was limited to Dlx5, Dlx6, Evf1/2, and Dlx6-as, each of which is located near the distal inv(6) breakpoint, driven by the 3′ enhancer of Tcrb. Expression of Acn9, located ~375 kb distal to Dss1, was not elevated in inv(6) lymphomas.

To determine whether Dlx5 can enhance cell proliferation, we used a retroviral vector to express Dlx5 in JML-5 cells. Overexpression of Dlx5 resulted in a 40% increase in cell number 24 h after plating (Fig. A4 and B).

Soft agar experiments indicated that exogenous overexpression of Dlx5 can promote anchorage-independent growth of Rat-1 fibroblasts, with about a 25% increase in the number of colonies formed by cells transfected with a plasmid expressing Dlx5 compared with that for cells transfected with an empty vector (Fig. 4C and D). These data provide support for our hypothesis that aberrant expression of Dlx5 is oncogenic. Furthermore, coexpression of Dlx5 and Myr-Akt2 in Rat-1 cells resulted in a 25% increase in the number of colonies compared with Rat-1 cells expressing Myr-Akt2 alone (Fig. 4C and D), suggesting that Dlx5 can cooperate with Akt2 to promote clonogenic growth in soft agar. As expected, expression of dominant-negative Akt2 (E299K) decreased the number of colonies observed. In preliminary experiments, we were able to achieve significant knockdown of Dlx5 with one shRNA, which resulted in decreased cell proliferation and decreased expression of cyclin D1 (Supplementary Fig. S4).

Finally, of potential clinical significance, Dlx5, but not DLX6, was abundantly expressed in several human T-cell lymphomas (Fig. 5). Semiquantitative RT-PCR analysis revealed up-regulated DLX5 transcript levels in three of seven patient-derived T-cell lymphomas compared with that observed in nonmalignant lymph node samples. Expression of DLX6 was not detected in any of the human T-cell lymphomas or noncancerous lymph nodes tested.

**Discussion**

V(D)J recombination puts early thymocytes at risk for malignant transformation should the DNA breaks be rejoined inappropriately and cause the kind of chromosomal rearrangements associated with certain leukemias and lymphomas (2). For V(D)J recombination at the Tcrb locus, the fidelity of the rearrangement events is monitored by the β selection checkpoint, at which cells with aberrant Tcrb rearrangements are eliminated by apoptosis in the thymus (10). However, the concomitant expression of activated Akt has been shown to influence thymocyte selection and promote peripheral T-cell survival (11). Our working model of lymphomagenesis in Lck-Myr-Akt2 mice is that activation of the Akt pathway would prevent apoptosis in a T-cell progenitor that undergoes aberrant V(D)J recombination connected with the inv(6), whereas genes affected by the inversion would promote malignant transformation.

In T-lymphoid malignancies, tumor-specific translocations and inversions classically juxtapose potent enhancer elements of a TCR gene with a proto-oncogene often encoding a transcription factor such as Myc and Hox11 (2). As a result, proto-oncogenes that are silent or expressed at low levels in T-lymphoid progenitor cells become activated (2).

In inv(6) lymphomas, the rearrangement places the Tcrb enhancer in the vicinity of two homeobox genes, Dlx5 and Dlx6, resulting in up-regulated expression. Dlx5 and Dlx6 belong to a conserved superfamily of developmental regulatory genes encoding transcription factors involved in cell fate and tissue identity. Although not extensively studied in lymphoid progenitor cells, dysregulation of homeobox genes is increasingly recognized as contributing to hematologic malignancies. Expression of tightly linked Dlx genes, such as the Dlx5 and Dlx6 bigene pair, is often overlapping and partially redundant in function; and linked Dlx genes share cis-regulating sequences, i.e., intergenic regions containing enhancer elements (12). In mice, Dlx5 is expressed at very low levels in whole thymus and is not expressed in Thy1+ thymocytes; Dlx6 is not detected in the thymus (13). Interestingly, microarray studies have revealed up-regulation of DLX5 in several human cancers, including endometrial carcinomas and lung carcinomas (14, 15), suggesting that overexpression of DLX5 could be oncogenic. Further evidence suggesting an oncogenic role of DLX5 comes from our experiments illustrating that overexpression of Dlx5 in mammalian cells results in enhanced cell proliferation and increased colony formation, and that knockdown of Dlx5 in inv(6)-positive thymic lymphoma cells may result in decreased cell proliferation. Moreover, we have knocked down the expression of DLX5 in human ovarian cancer cells overexpressing DLX5, using several different shRNA constructs, and decreased expression of DLX5 coincided with a decrease in cyclin D1 expression and reduced cell proliferation.8

Recent work has shown constitutive activation of Akt in the majority of primary human lymphomas, supporting its proposed key role in lymphoma cell survival (16). Among the members of the Akt family, Akt2 may have particular importance in mediating PI3K-dependent oncogenic effects (17). Our work indicates that Akt2 activation plays an important role in the development of thymic lymphomas seen in a transgenic mouse model. However, the frequent occurrence of the inv(6) seen in tumors from these mice indicates that, in addition to Akt2, other genetic lesions are necessary for malignant transformation.

Collectively, our findings suggest that Dlx5 acts oncogenically by cooperating with Akt2 to promote T-cell lymphomagenesis. In addition to contributing to our understanding of the pathogenesis of lymphoma in this mouse model system, aberrant expression of DLX5 is also involved in some human malignancies (14, 15). Given the fact that DLX5 is up-regulated in some human endometrial and lung carcinomas, DLX5 could potentially be exploited therapeutically. Akt is often activated in human cancers, including lung and endometrial carcinomas (5). To date, use of Akt as a therapeutic target has been limited by toxicity (18), due to the fact that Akt signaling is ubiquitously involved in cell survival not only in transformed cells but also in normal tissue, and Akt2 plays a critical role in glucose metabolism (18). This problem might be mitigated, should DLX5 represent a “druggable” target that displays a more restricted expression pattern.

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References

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