DOI:10.1158/0008-5472.CAN-05-1400

Cancer Research

OLIG2 (BHLHB1), a bHLH Transcription Factor, Contributes to Leukemogenesis in Concert with LMO1

Ying-Wei Lin, Ramona Deveney, Mary Barbara, et al.

Cancer Res 2005;65:7151-7158. Published online August 15, 2005.

DOI:10.1158/0008-5472.CAN-05-1400

OLIG2 (BHLHB1), a bHLH Transcription Factor, Contributes to Leukemogenesis in Concert with LMO1

Ying-Wei Lin,[1] Ramona Deveney,[1] Mary Barbara,[2] Norman N. Iscove,[2] Stephen D. Nimer,[3] Christopher Slape,[1] and Peter D. Aplan[1]

[1]Genetics Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland; [2]Department of Medical Biophysics, University of Toronto, Ontario, Canada; and [3]Department of Medicine, Hematology Service, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York

Abstract

***OLIG2* (originally designated *BHLHB1*) encodes a transcription factor that contains the basic helix-loop-helix motif. Although expression of *OLIG2* is normally restricted to neural tissues, overexpression of *OLIG2* has been shown in patients with precursor T-cell lymphoblastic lymphoma/leukemia (pre-T LBL). In the current study, we found that overexpression of *OLIG2* was not only found in oligodendroglioma samples and normal neural tissue but also in a wide spectrum of malignant cell lines including leukemia, non–small cell lung carcinoma, melanoma, and breast cancer cell lines. To investigate whether enforced expression of *OLIG2* is oncogenic, we generated transgenic mice that overexpressed *OLIG2* in the thymus. Ectopic *OLIG2* expression in the thymus was only weakly oncogenic as only 2 of 85 mice developed pre-T LBL. However, almost 60% of transgenic mice that overexpressed both *OLIG2* and *LMO1* developed pre-T LBL with large thymic tumor masses. Gene expression profiling of thymic tumors that developed in *OLIG2/LMO1* mice revealed up-regulation of *Notch1* as well as Deltex1 (*Dtx1*) and pre T-cell antigen receptor α (*Ptcra*), two genes that are considered to be downstream of *Notch1*. Of note, we found mutations in the *Notch1* heterodimerization or proline-, glutamic acid-, serine-, and threonine-rich domain in three of six primary thymic tumors. In addition, growth of leukemic cell lines established from *OLIG2/LMO1* transgenic mice was suppressed by a γ-secretase inhibitor, suggesting that *Notch1* up-regulation is important for the proliferation of *OLIG2-LMO1* leukemic cells.** (Cancer Res 2005; 65(16): 7151-8)

Introduction

The *OLIG2* gene, originally designated *BHLHB1* (for basic helix-loop-helix class B, 1), is located at 21q22 and encodes a transcription factor that contains the conserved bHLH motif (1–3). Several studies have suggested that *OLIG2* expression is normally restricted to neural tissues, especially those of the mature oligodendrocyte lineage as well as embryonic oligodendrocyte precursors (4). Targeted deletion ("knockout") and overexpression studies have shown that *OLIG2* and the closely related *OLIG1* normally play an important role in oligodendrocyte differentiation and specification (5–9). More recently, it has been shown that human *OLIG* genes, including *OLIG1* (*BHLHB2*) and *OLIG2* (*BHLHB1*), are overexpressed in oligodendroglioma, in contrast to the low level or absent expression seen in astrocytoma samples (10, 11). This is an important clinical finding because there has been a lack of molecular markers that histologically clearly distinguish oligodendroglioma from astrocytoma.

The human *OLIG2* gene was originally identified by virtue of its activation by chromosomal translocation in a precursor T-cell lymphoblastic lymphoma/leukemia (pre-T LBL) patient with a t(14; 21)(q11.2; q22) chromosomal translocation present in the leukemic cells (3). In this case, *OLIG2* was overexpressed due to relocation of the *TCRA* Cα enhancer upstream of the *OLIG2* locus on the translocated chromosome. Subsequently, overexpression of *OLIG2* has been detected in additional pre-T LBL patients without known t(14;21) translocations (12, 13). Because there are no reports investigating a direct role for overexpression of *OLIG2* in malignant transformation, we began a series of studies designed to determine whether overexpression of *OLIG2* contributes to oncogenesis, especially leukemogenesis.

Materials and Methods

Northern blot and reverse transcription-PCR analysis. Expression of *OLIG2* in human malignant cell lines and human normal tissues was determined by both Northern blot analysis and reverse transcription-PCR (RT-PCR). Total RNA and poly(A) RNA were isolated from cell pellets using Trizol reagent (Invitrogen, Carlsbad, CA) or Poly(A) Pure RNA extraction kit (Ambion, Austin, TX). Ten micrograms of total RNA or one microgram of poly(A) RNA was size fractionated on a 1% agarose/formaldehyde gel, transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH), and hybridized to a ^{32}P-labeled human *OLIG2* probe. For RT-PCR experiments, after contaminating genomic DNA was removed from total RNA, 1 μg of total RNA was reverse transcribed with Superscript II reverse transcriptase and an oligo (dT) primer (Invitrogen). Parallel reactions were done without the addition of Superscript II reverse transcriptase as controls. First strand cDNAs were amplified with human *OLIG2* (form A, see text) primers (21PCR01: 5′-CCCTGAGGCTTTTCGGAGCG-3′ and 21PCR02: 5′-GCGGCTGTTGATCTTAGACGC-3′), human *OLIG2* form B primers (BB312249F: 5′-GTGGGGACTTTGTGCCTGGGCATCG-3′ and 21PCR01R: 5′-CGCTCCGAAAAGCCTCAGGG-3′), or human *β-actin* primers (hActin-F1: 5′-AGGCCGGCTTCGCGGGCGAC-3′ and hActin-R1: 5′-CTCGGGAGCCACACGCAGCTC-3′) in a volume of 20 μL. After a "hot start" at 94°C for 3 minutes, 35 cycles of 94°C for 1 minutes, 64°C for 45 seconds, and 72°C for 1 minutes were used, followed by a terminal 10-minute extension at 72°C. To amplify *LMO1*, first strand cDNAs were amplified with human *LMO1* primers (hLMO1Fw: 5′-GCGAAGCAGTCGAGGTGATA-3′ and hLMO1Rv: 5′-AAGTGTGCGTGCTGTGACTG-3′) in A volume of 20 μL. After a hot start at 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds were used, followed by a terminal 10-minute extension at 72°C. PCR products were analyzed by agarose gel electrophoresis.

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

Requests for reprints: Peter D. Aplan, Genetics Branch, Center for Cancer Research, National Cancer Institute, NIH, 8901 Wisconsin Avenue, Bethesda, MD 20889-5105. Phone: 301-435-5005; Fax: 301-496-0047; E-mail: aplanp@mail.nih.gov.

doi:10.1158/0008-5472.CAN-05-1400

DOI:10.1158/0008-5472.CAN-05-1400

Expression of *OLIG2* in normal murine hematopoietic cells and tissues. Expression of *OLIG2* in normal murine hematopoietic precursors was determined by dot blot analysis. Thirty-seven globally amplified cDNA samples from a wide spectrum of normal murine hematopoietic colonies (14, 15) and normal murine tissues (16) were deposited, 100 ng/spot, on Hybond N+ membranes (17). The sample set comprised #1: pentapotent erythroid/megakaryocytic/macrophage/neutrophil/mast cell; #2: tetrapotent erythroid/megakaryocytic/macrophage/neutrophil; #3: erythroid/megakaryocytic/macrophage; #4: erythroid/megakaryocytic; #5: macrophage/neutrophil; #6: blast-forming unit-erythroid; #7: colony-forming unit-erythroid; #8: precursor macrophage; #9: precursor neutrophil; #10: precursor megakaryocytic; #11: erythroid; #12: neutrophil; #13: mast cell; #14: megakaryocytic; #15: B-cell; #16: macrophage; #17: T-cell; #18-21: fibroblasts; #22: thymus; #23: aorta; #24: kidney; #25: testis; #26: muscle; #27: lung; #28: brain; #29: skin; #30: lymph nodes; #31: bone marrow; #32: heart; #33: ovary; #34: spleen; and #35-37: purified hematopoietic stem cells (18). The membrane was hybridized with ^{32}P-labeled mouse *OLIG2* and *OLIG1* probes both containing sequence within 300 bases of the 3′ transcript termini. The mouse *OLIG2* probe was a 0.3 kb PCR fragment that was amplified using mouse *OLIG2* primers (mBB1-F1: 5′-GGTGATAGATACACTCTGCAGG-3′ and mBB1-R1: 5′-GTTGCTGTGAATATAGTTTGAGC-3′). The mouse *OLIG1* probe was a 0.6 kb fragment isolated from an EST clone (AW494459) with *Not*I and *Eco*RI.

Generation of *OLIG2* transgenic mice and *OLIG2-LMO1* double transgenic mice. An *OLIG2* cDNA containing the A isoform (see text) was digested with *Bam*HI and cloned into the *Bam*HI site of the pLIT2 vector that has the lck upstream enhancer, immunoglobulin H enhancer, and TCRVβ promoter (19). All cloning junctions were sequenced to verify the construct. The construct was microinjected into zygotes obtained from C57Bl6 mice. Lines were maintained by mating with wild-type C57Bl6. *OLIG2* transgenic mice were subsequently crossed with *LMO1* transgenic mice that were obtained from Dr. Korsmeyer (20). The *LMO1* transgenic mice were originally generated on F1 of a cross between C57Bl6 and C3H mice, and were backcrossed with C57Bl6 for five to six generations. The genotype of both the *OLIG2* transgene and the *LMO1* transgene was determined by PCR.

Immunohistochemistry and immunophenotyping. H&E, periodic acid Schiff, CD3 (DAKO, Carpinteria, CA), B220 (CD45R, PharMingen, San Diego, CA), anti-myeloperoxidase (DAKO), and F4/80 (CALTAG, Burlingame, CA) stainings of sections from tissues such as thymus, lymph nodes, spleen, liver, kidney, lung, and tibia were evaluated using conventional staining techniques. Bone marrow cells were harvested from both femur and tibia by flushing with Iscove's modified Dulbecco's medium (IMDM), and assessed by May-Grünwald-Giemsa stained cytospin. Two-color flow cytometry was used to determine the immunophenotype of single-cell suspension prepared from thymus, spleen, and bone marrow. The cells were stained with FITC-conjugated anti-mouse CD8 and phycoerythrin-conjugated anti-mouse CD4 (PharMingen). Diseases were classified according to the Bethesda proposals (21, 22).

Southern blot analysis for *TCRB* gene rearrangements. Genomic DNA was isolated as previously described (23) and digested with either *Hin*dIII or *Sst*I. Digested DNA was size fractionated on a 0.8% agarose gel, denatured, neutralized, and transferred to a nitrocellulose membrane. The nitrocellulose membrane was hybridized to a ^{32}P-labeled 0.4 kb *TCRB* probe that detects the constant region of the mouse *TCRB* gene.

Establishment of leukemic cell lines. Bone marrow cells were cultured in IMDM supplemented with 10% fetal bovine serum (FBS). Half of the media was replaced with fresh media once a week until adherent cells covered entire bottom of culture flasks. After that time, 70% of the media was replaced once a week until suspension cells appeared. Suspension cells were then transferred to RPMI 1640 with 10% FBS.

Gene profiling of tumors. RNA was isolated from thymic tumor and compared with pooled RNA obtained from the thymi of 21 healthy nontransgenic littermates. RNAs were isolated using the Trizol reagent and purified using the Qiagen (Valencia, CA) RNeasy mini kit. First strand cDNA was synthesized and dye coupled using a FairPlay Microarray labeling kit (Stratagene, La Jolla, CA). The experimental cDNA probe (thymic tumor) was labeled with Cy3 and the reference cDNA probe (normal thymus) was labeled with Cy5. Purification of the dye-coupled cDNA was done using a Qiagen Mini Elute PCR purification kit. The Cy3-labeled experimental probe was combined with the Cy5-labeled reference probe and the mixture was hybridized to a National Cancer Institute (NCI) production oligo DNA microarray containing 22,272 long oligo (70-mer) features (Compugen, San Jose, CA). The microarray was scanned using an Axon (Union City, CA) GenePix scanner. The fluorescence ratio was quantified for each transcript and reflected the relative abundance of the transcript in experimental mRNA sample compared with the reference mRNA.

Cell growth assay. Cell lines #1928 and #1931 were established from bone marrow of *OLIG2-LMO1* double transgenic mice with pre-T LBL and maintained in RPMI 1640 supplemented with 10% FBS. Cell line #1901 was established from bone marrow of a *NUP98-HOXD13* (*NHD13*) transgenic mouse with pre-T LBL and maintained in IMDM media supplemented with 10% FBS (24). F4-6 is a Friend virus–induced erythroleukemia cell line (25) cultured in IMDM media with 10% FBS. Cell lines seeded at a concentration of 2×10^5 cells/mL were treated with 10 μmol/L of Z-IL-CHO (γ-secretase inhibitor XII, Calbiochem, La Jolla, CA) for 48 hours (26). Viable cell counts were done by trypan blue exclusion. Expression of *Notch1* was determined by RT-PCR, as previously described (27). To determine whether G1 arrest and apoptosis were induced by Z-IL-CHO, the cells were stained with propidium iodide (Molecular Probes, Eugene, OR) and DNA content was determined by flow cytometry.

Sequence analysis of *Notch1* heterodimerization and proline-, glutamic acid-, serine-, and threonine-rich domains. The heterodimerization (HD) and proline-, glutamic acid-, serine-, and threonine-rich (PEST) domains of *Notch1* were sequenced to determine whether there is a mutation in these domains. The primers used to amplify exons 26 and 27 of the HD domain were i25F1 (5′-GGCTGAGTTTCTTTAGAGTC-3′) and i26R1 (5′-CCTCCCCTGAGGTTACACCT-3′), and i26F1 (5′-GAGTGTCCCATTGCGGGGCT-3′) and i27R1 (5′-TGCAGAGGTCAGAAAGTGTT-3′), respectively. To amplify the PEST domain, the primers PEST1 (5′-TACCAGGGCCTGCCCAACAC-3′) and PEST2 (5′-GCCTCTGGAATGTGGGTGAT-3′) were used. The PCR was done using the same protocol as the RT-PCR (27). Subsequently, the PCR products were isolated from agarose gels, and the sequence was compared with that of the wild-type murine *Notch1* genomic sequence (GenBank accession no. AL732541).

Results

Expressions of *OLIG* genes in normal hematopoietic tissues, leukemic cell lines, and other malignant cell lines. Although expression of *OLIG2* has been thought to be restricted to neural tissues, we previously found overexpression of *OLIG2* in a patient with pre-T LBL (1–3). To determine whether other leukemic cells expressed *OLIG2*, we examined *OLIG2* expression in five leukemic cell lines (HEL, HSB-2, CEM, Jurkat, and HL60) and two glioblastoma cell lines (A172 and U87) by Northern blot analysis. Strikingly, two of those leukemic cell lines (HEL and HL60) expressed higher level of *OLIG2* mRNA than the glioblastoma cell lines (A172 and U87) used as positive controls. Furthermore, HL60 also expressed *OLIG1*, which was not detected in glioblastoma cell lines (Fig. 1*A*).

We considered the possibility that *OLIG2* is normally expressed in a subset of normal hematopoietic cells. To investigate this possibility, we determined expression of *OLIG1* and *OLIG2* using a dot blot analysis of amplified cDNA from a wide spectrum of mouse hematopoietic cells (14, 15), as well as other tissues including thymus, aorta, kidney, testis, muscle, lung, brain, skin, lymph nodes, bone marrow, heart, ovary, and spleen (16). Significant hybridization of the *OLIG1* and *OLIG2* probes was detected on brain cDNA but not on any of the normal hematopoietic cell samples (Fig. 1*B*). Because *OLIG2* was not expressed in normal hematopoietic cells, and previously was

DOI:10.1158/0008-5472.CAN-05-1400

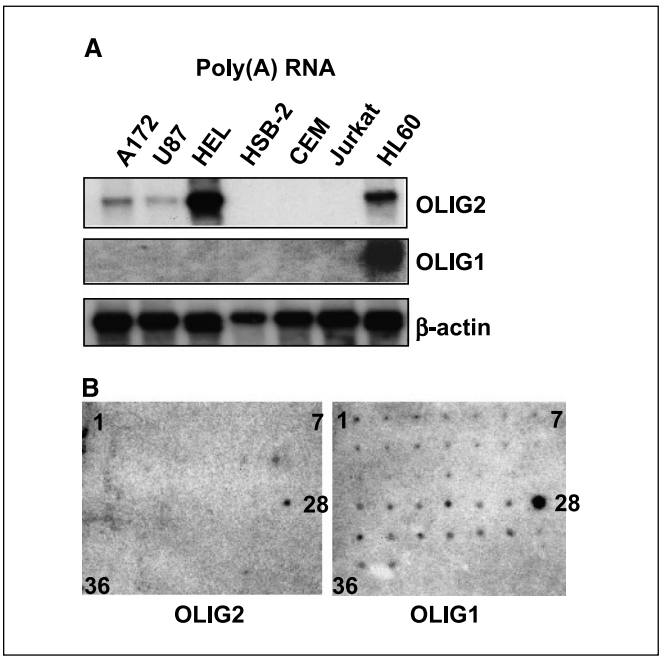


Figure 1. *OLIG1* and *OLIG2* expression in leukemic cell lines and normal hematopoietic cells. *A,* Northern blot analysis of *OLIG1* and *OLIG2* expression in leukemic cell lines and glioblastoma cell lines (A172 and U87). *B,* dot blot analysis of *OLIG1* and *OLIG2* expression in normal murine hematopoietic precursors. The membrane contains cDNA from 37 distinct normal hematopoietic colonies and tissues (see Materials and Methods). Dot #1 denotes the position of the first dot on the top left corner. There was only a single hybridization signal detectable in each membrane, which was cDNA from whole brain at position #28. Hybridization intensities on the hematopoietic cell samples (#1-17) did not exceed the background intensity on the negative control spot at position #18 (PCR primer concatamers amplified in the absence of cellular RNA template).

found to be overexpressed in the leukemic cells from patients with pre-T LBL, we considered the possibility that *OLIG2* might be expressed only in malignant hematopoietic cells. We expanded the survey to 41 leukemic cell lines using RT-PCR. The survey revealed that *OLIG2* was strongly expressed in HL60 (promyelocytic), KBM5, THP-1 (erythroleukemic), HI-MEG (megakaryocytic), and MEG-01 (megakaryocytic), and weakly expressed in MO-91 (undifferentiated), HEL, LAMA84, KMOE-1, TF-1 (erythroleukemic), and CHRF-288 (megakaryocytic; Table 1; Supplementary Fig. S1). To determine whether ectopic *OLIG2* expression was restricted to hematopoietic malignancies, we determined *OLIG2* expression in the "NCI-60" panel of malignant cell lines (28, 29). Thirteen of the 60 cell lines in this panel expressed *OLIG2*: three central nervous system (CNS) tumor cell lines (SF-295, SNB-19, and U251) and 10 non-CNS malignant cell lines. These 10 cell lines included five melanoma cell lines (UACC-257, SK-MEL2, SK-MEL28, M14, and SK-MEL5), three non–small cell lung carcinoma cell lines (NCI-H522, HOP-62, and NCI-H23), one breast cancer cell line (MDA-MB-435), and the HL60 leukemic cell line. Thus, *OLIG2* expression was detected not only in normal and malignant neural tissues but also in malignant cell lines derived from skin, lung, breast, and hematopoietic tissues (Table 1; Supplementary Fig. S2).

***OLIG2* transgenic mice.** To investigate the oncogenic potential of misexpressed *OLIG2 in vivo*, we generated transgenic mice that overexpressed *OLIG2* in the thymus using an lck promoter (19). Because the original description of human *OLIG2*, as well as the mouse and rat homologues, did not identify the *OLIG2* initiation codon (1–3), we used 5′ rapid amplification of cDNA ends to identify the initiation codon. We identified two alternative upstream exons, designated 1B and 1A. We then used RT-PCR to determine which 5′ exon was most commonly used. Although we detected exon 1A spliced to exon 2 (Form A) in a wide spectrum of samples, including normal brain and the aforementioned malignant cell lines (see Fig. 2 and Supplementary Figs. S1 and S2), we detected exon 1B spliced to the 3′ portion of exon 1A and subsequently exon 2 (form B) only in the HEL cell line. Therefore, we used an *OLIG2* form A cDNA to generate the transgenic mice.

Transgenic mice were generated on a C57Bl6 background by pronuclear injection and three independent founders (B1, D5, and K4), all male, were identified. High levels of *OLIG2* mRNA were detected in the thymus, and lower levels in the bone marrow (Fig. 2*C*) in RNA from the offspring of mouse D5; similar levels were detected in F1 mice from the other two founders (data not shown). We followed a cohort of 69 transgenic and 76 nontransgenic control littermates from these three lines for 24 months; none of the transgenic mice developed a malignant tumor.

***OLIG2-LMO1* double transgenic mice.** We considered the possibility that *OLIG2* might not be sufficient for leukemia and might require collaborative events to induce leukemia. For instance, the *SCL* (*Tal1*) gene, which encodes a transcription factor bearing the bHLH motif, was not sufficient to induce leukemia in mice when *SCL* was misexpressed under control of the *SIL* promoter (30), but was able to induce leukemia in concert with overexpression of *LMO1. SCL-LMO1* double transgenic mice developed aggressive pre-T LBL within 6 months with nearly 100% penetrance (23, 30). Because *OLIG2*, like *SCL*, encodes a class B bHLH transcription factor, we crossed the *OLIG2* transgenic mice to *LMO1* transgenic mice to investigate whether *OLIG2* overexpression might collaborate with *LMO1* overexpression to induce leukemia. lck-OLIG2 (line D5) mice were mated to lck-LMO1 mice, and the offspring were observed for signs of leukemia. Fifty-seven percent (8 of 14) of *OLIG2-LMO1* double transgenic mice died by the age of 14 months, whereas all of the wild-type control littermates survived. In addition, 8% (1 of 13) of *LMO1* only transgenic mice and 12% (2 of 16) of *OLIG2* only transgenic mice developed signs of pre-T LBL during the 14-month study period (Fig. 3*A*). Necropsy findings from these mice revealed an aggressive pre-T LBL. All *OLIG2-LMO1* double transgenic mice with pre-T LBL had markedly enlarged thymic tumors and pleural effusion (Fig. 3*B1*). Additional gross findings included hepatosplenomegaly and localized (inguinal) or generalized lymphadenopathy (Fig. 3*B2*). Histologic examination revealed a perivascular infiltration of CD3-positive lymphoblasts in the lung, as well as interstitial and perivascular infiltration of CD3-positive lymphoblasts in the kidney and liver, respectively (Fig. 3*B3-8*). Examination of peripheral blood and bone marrow revealed lymphoblasts characterized by a high nuclear-cytoplasmic ratio and variably condensed chromatin (Fig. 3*B9-10*). The malignant blasts were positive for CD3, CD4, and CD8 (Fig. 3*C1*). Clonal rearrangements of the *TCRB* gene were identified by Southern blot analysis of tumors from *OLIG2-LMO1* double transgenic mice (Fig. 3*C2*). These findings were consistent with a diagnosis of pre-T LBL. Moreover, pre-T LBL cell lines were established from the bone marrow of three independent *OLIG2-LMO1* double transgenic

DOI:10.1158/0008-5472.CAN-05-1400

Table 1. *OLIG2* expression in leukemic/lymphoma and NCI-60 cell line panel

Cell	Origin	*OLIG2*	Cell	Origin	*OLIG2*
BV173	CML-lyBC	−	SAM-1	AML-M6	−
Jurkat	Pre-T LBL	−	TF-1	AML-M6	+
Molt-4	Pre-T LBL	−	JK-1	CML-eryBC	−
CEM	Pre-T LBL	−	KU-812	CML-eryBC	−
MO-91	AML-M0	+	K562	CML-eryBC	−
EM-2	CML-myBC	−	LAMA84	CML-eryBC	+
KCL22	CML-myBC	−	CHRF-288	AML-M7	+
KBM5	CML-myBC	++	CMK	AML-M7	−
KBM7	CML-myBC	−	HU-3	AML-M7	−
KYO-1	CML-myBC	−	RS-1	AML-M7	−
THP-1	CML-myBC	++	MKPL-1	AML-M7	−
KA-1	AML-M2	−	M-MOK	AML-M7	−
KG-1	AML-M2	−	UT-1	AML-M7	−
HL60	AML-M2	++	MC-3	CML-meg/myBC	−
ML1	AML-M4	−	MEG-01	CML-megBC	++
MONO-MAC-1	AML-M5	−	NS-MEG	CML-megBC	−
U937	AML-M4?	−	MOLM-1	CML-megBC	−
AP217	AML-M6	−	T33	CML-megBC	−
AS-E2	AML-M6	−	HI-MEG	CML-megBC	++
HEL	AML-M6	+	RPMI-8226	Myeloma	−
KMOE-1	AML-M6	+	SR	Immun-Lym	−

Cell line	Tumor	*OLIG2*	Cell line	Tumor	*OLIG2*	Cell line	Tumor	*OLIG2*
A549	NSCLC	−	SF-268	CNS (AC)	−	786-0	Renal	−
EKVX	NSCLC	−	SF-295	CNS (OG)	+	A498	Renal	−
HOP62	NSCLC	+	SF-539	CNS (AC)	−	ACHN	Renal	−
HOP92	NSCLC	−	SNB-19	CNS (OG)	+	SN12C	Renal	−
NCI-H226	NSCLC	−	SNB-75	CNS (AS)	−	TK-10	Renal	−
NCI-H23	NSCLC	+	U251	CNS (OG)	+	UO-31	Renal	−
NCI-H322	NSCLC	−	LOX IMVI	Melanoma	−	MCF7	Breast	−
NCI-H460	NSCLC	−	MALME-3M	Melanoma	−	NCI/ADR-RES	Breast	−
NCI-H522	NSCLC	++	M14	Melanoma	+	MDA-MB-231	Breast	−
COLO205	Colon	−	SK-MEL-2	Melanoma	++	HS 578T	Breast	−
HCC-2998	Colon	−	SK-MEL-28	Melanoma	+	MDA-MB-435	Breast	++
HCT-116	Colon	−	SK-MEL-5	Melanoma	++	BT-549	Breast	−
HCT-15	Colon	−	UACC-257	Melanoma	++	T-47D	Breast	−
HT29	Colon	−	UACC-62	Melanoma	−	IGROV1	Ovarian	−
KM12	Colon	−	PC-3	Prostate	−	OVCAR-3	Ovarian	−
SW620	Colon	−	DU-145	Prostate	−	OVCAR-8	Ovarian	−
						SK-OV-3	Ovarian	−

Abbreviations: CML, chronic myelogeneous leukemia; lyBC, lymphoblastic crisis; myBC, myeloblastic crisis; eryBC, erythroblastic crisis; megBC, megakaryoblastic crisis; AML, acute myelocytic leukemia; Immun-Lym, immunoblastic lymphoma; NSCLC, non–small cell lung carcinoma; AC, astrocytoma; OG, oligodendroglioma.

mice and have been maintained in RPMI 1640 supplemented with 10% FBS for longer than 14 months. These cell lines continue to express *OLIG2* as well as *LMO1*, similar to findings seen with the primary thymic tumors (Fig. 4*A*).

The pre-T LBL that developed in *OLIG2* or *LMO1* only transgenic mice was similar to that seen in the double transgenic mice. Bone marrow and peripheral blood were heavily invaded by malignant lymphoblasts, and the mice had markedly enlarged thymi (Fig. 3*B11*), hepatosplenomegaly, and generalized lymphadenopathy. The lymphoblasts were positive for CD3 and the malignant blasts showed clonal *TCRB* gene rearrangements (Fig. 3*C2*).

Expression profile of *OLIG2-LMO1* tumors. To identify genes that might collaborate with *OLIG2* and *LMO1* during leukemic transformation, or identify genes of which expression might be affected by *OLIG2* and/or *LMO1*, we compared the gene expression profiles of four thymic tumors with those of normal thymus from nontransgenic littermates by means of a two-color microarray assay. Table 2 lists genes that were up-regulated more than 3-fold in the thymic tumors compared with normal thymus. Several genes that are considered to provide growth and/or survival signals were up-regulated. These genes included *Saa3* (serum amyloid A), *Aldh1b1* (aldehyde dehydrogenase 1 family, member B1), *Tfrc* (transferrin receptor), *Eif3s9* (eukaryotic

DOI:10.1158/0008-5472.CAN-05-1400

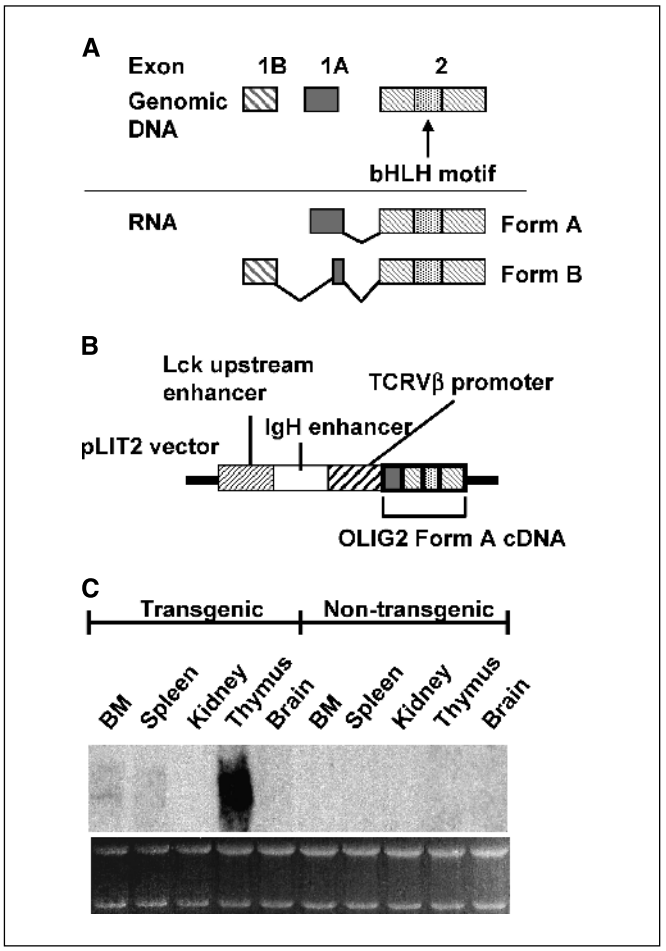


Figure 2. Generation of *OLIG2* transgenic mice. *A, OLIG2* splice forms A and B are generated by alternate splicing as indicated. *B,* splice form A of the *OLIG2* cDNA was cloned into the *Bam*HI site of the pLIT2 vector (19). *C,* expression of the transgene was determined by Northern blot analysis. *Top,* hybridization of *OLIG2; bottom,* EtBr staining of the gel as a loading control. A clear *OLIG2* signal is seen in thymus and a faint signal in bone marrow (*BM*).

translation initiation factor), and *Eef2* (eukaryotic translation elongation factor). Interestingly, we also found that *Notch1* was up-regulated an average of 3.5-fold in *OLIG2-LMO1* thymic tumors, and two genes that are thought to be downstream targets of *Notch1* [*Dtx1* (Deltex1) and *Ptcra* (pre T-cell antigen receptor α)] were up-regulated 10.2-fold and 3.1-fold, respectively.

Growth of *OLIG2/LMO1* tumor cell lines is inhibited by γ-secretase inhibitors. Because it has been shown that aberrant expression of *Notch1* is associated with the pathogenesis of pre-T LBL (31–33), we investigated whether *Notch1* up-regulation was important for the continued growth of *OLIG2-LMO1* thymic tumors. Notch1 is a transmembrane receptor of which signaling normally requires γ-secretase–mediated proteolytic processing. On cleavage by γ-secretase, the intracellular domain of Notch1 (ICN1) gains access to the nucleus where it functions as a regulator of transcription. To determine if the up-regulated *Notch1* was functionally important for the growth of *OLIG2-LMO1* cells, pre-T LBL cell lines (#1928 and #1931) established from *OLIG2-LMO1* double transgenic mice were cultured in the presence of the γ-secretase inhibitor Z-IL-CHO. Growth of *OLIG2-LMO1* double transgenic cells was markedly suppressed by Z-IL-CHO, whereas the growth of control cell lines that expressed little (#1901, a pre-T LBL cell line established from a NHD13 transgenic mouse) or no (the erythroleukemia cell line F4-6) *Notch1* was not suppressed (Fig. 4*B*). The *OLIG2-LMO1* cell line #1931 showed a decreased number of cells in S-G_2-M phase and an increased number of sub-G_1 apoptotic cells compared with the F4-6 control cell line following Z-IL-CHO treatment (Supplementary Fig. 3).

Mutations of *Notch1* in *OLIG2-LMO1* double transgenic thymic tumors. Because it has been shown that 50% of human

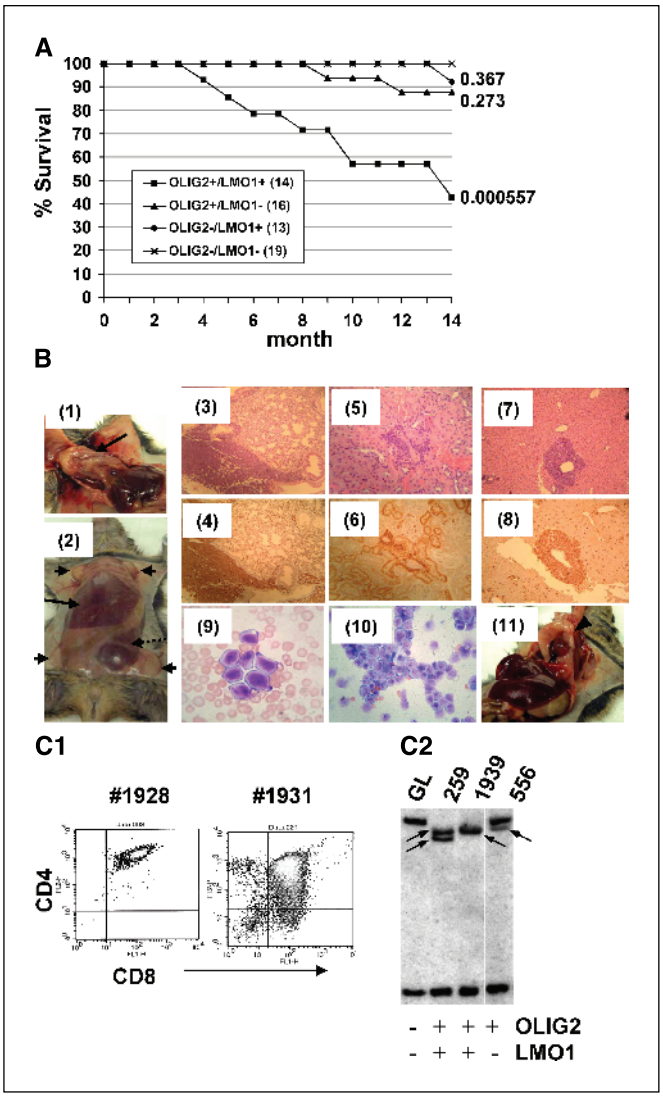
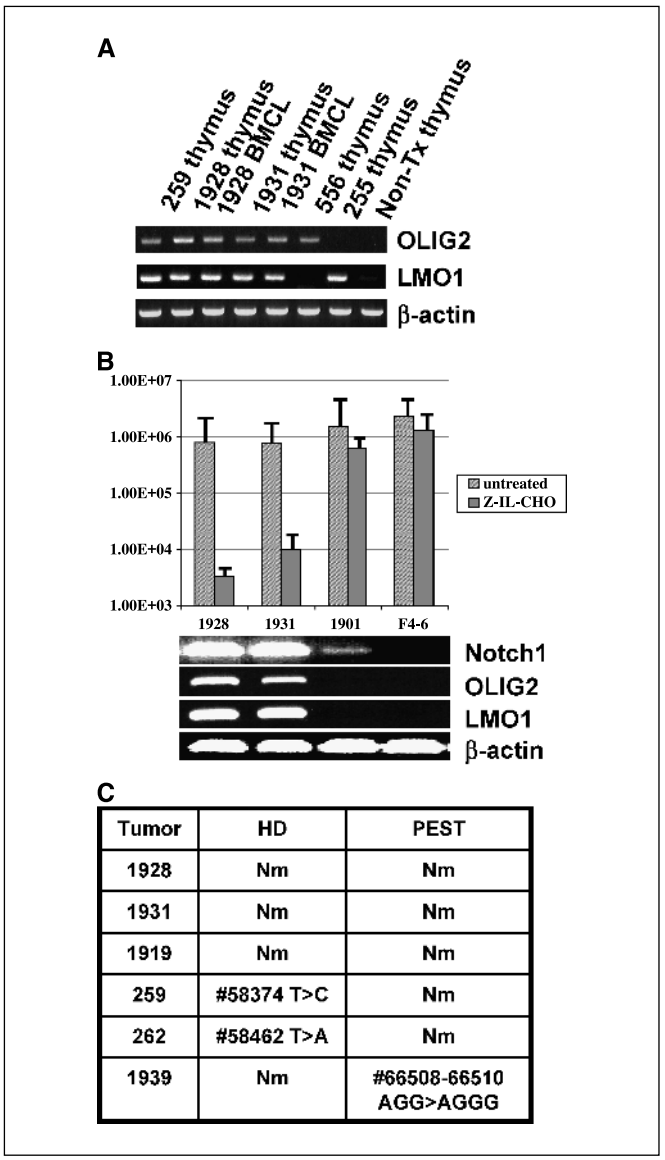


Figure 3. Pre-T LBL in transgenic mice. *A,* survival curve for offspring of *OLIG2* and *LMO1* transgenic mice. Survival differences from wild-type control littermates are shown using Student's *t* test. *B1,* typical large thymic tumor (*arrow*) from an *OLIG2/LMO1* transgenic mouse (mouse #1939). *B2,* hepatomegaly (*arrow*), splenomegaly (*arrow with dashed line*), and lymphadenopathy (*arrowhead*) were also common features (mouse #1939). *B3-4,* lung infiltration with CD3-positive blasts (mouse #1928). *B5-6,* kidney infiltration with CD3-positive blasts (mouse #259). *B7-8,* liver infiltration with CD3-positive blasts (mouse #1939). *B9-10,* peripheral blood (*B9*) and bone marrow (*B10*) infiltrated with lymphoblasts characterized by high-nuclear cytoplasmic ratio and variably condensed chromatin (mouse #1939). *B11,* pre-T LBL in *OLIG2* only transgenic mice characterized by massively enlarged thymus (*arrowhead*; mouse #556). *C1,* immunophenotype of leukemic blasts from bone marrow displaying immature CD4+CD8+ (mouse #1928 and #1931). *C2,* Southern blot analysis of DNA from thymic tumors show clonal *TCRB* gene rearrangements in *OLIG2-LMO1* double transgenic (#259 and #1939) and *OLIG2* only transgenic (#556) mice. *Arrows,* rearranged bands.

DOI:10.1158/0008-5472.CAN-05-1400



C

Tumor	HD	PEST
1928	Nm	Nm
1931	Nm	Nm
1919	Nm	Nm
259	#58374 T>C	Nm
262	#58462 T>A	Nm
1939	Nm	#66508-66510 AGG>AGGG

Figure 4. Growth suppression of *OLIG2/LMO1* cell lines overexpressing *Notch1* by γ-secretase inhibitor. *A,* expression of the *OLIG2* and *LMO1* transgenes was determined by RT-PCR. Primary thymic tumors and pre-T LBL cell lines established from bone marrow (*BMCL*) continued to express the expected transgenes; mice #259, #1928, and #1931 were double transgenic, #556 was *OLIG2* only, and #255 was an *LMO1* only transgenic mouse. *B,* the indicated cell lines were cultured in the presence of 10 μmol/L Z-IL-CHO (γ-secretase inhibitor) for 48 hours. Viable cells were identified by trypan blue exclusion. Expression of *Notch1, OLIG2*, and *LMO1* was determined by RT-PCR. Growth of the *OLIG2-LMO1* double transgenic cells (#1928 and #1931) was suppressed ~100-fold, whereas growth of the control cell lines (#1901 and F4-6) was not. *C,* mutation analysis in primary thymic tumors from double transgenic mice. The nucleotide numbers refer to mouse *Notch1* genomic sequence (GenBank accession no. AL732541). *HD,* heterodimerization domain; *Nm,* no mutation.

pre-T LBL had activating mutations that involved the HD and PEST domains of *NOTCH1*, we investigated whether primary *OLIG2-LMO1* double transgenic thymic tumors had *Notch1* mutations. We found that three of six primary tumors had mutations in either HD or PEST domain (Fig. 4*C*). Two of these three tumors showed single base substitutions in one allele of the HD domain that subsequently altered the proteins [mouse #259: nucleotide 58,374 T>C (W>R); mouse #262: nucleotide 58,462 T>A (L>Q)]. Another tumor had a frame-shift mutation in one allele of the PEST domain that resulted in an introduction of a premature stop codon [mouse #1939: nucleotides 66,508-66,510 AGG>AGGG].

Discussion

Overexpression of *OLIG2* (*BHLHB1*) has been shown in oligodendroglioma samples and has been proposed to be a specific diagnostic marker for oligodendroglioma (10, 11, 34). In this study, we confirmed that three glioblastoma cell lines overexpressed *OLIG2*, whereas three astrocytoma cell lines did not express *OLIG2*. Interestingly, we found *OLIG2* expression in non–small cell lung carcinoma, melanoma, breast cancer, and a wide spectrum of leukemic cell lines. In contrast, none of the colon (n = 7), prostate (n = 2), renal (n = 6), or ovarian (n = 4) cancer cell lines expressed *OLIG2*. Eleven of the 41 leukemic cell lines that we investigated expressed *OLIG2*, and we and others have detected *OLIG2* overexpression in patients with pre-T LBL (3, 12, 13). In summary, overexpression of *OLIG2* was found in a wide spectrum of malignant cells leading to the speculation that overexpression of *OLIG2* might be oncogenic.

In the current study, we focused on a leukemogenic effect of *OLIG2*. We initially found that none of 69 *OLIG2* transgenic mice developed malignancy. However, when we crossed *OLIG2* mice to *LMO1* mice, 2 of 16 mice transgenic for *OLIG2* only developed pre-T LBL at the age of 9 and 12 months. Although the cause for the discrepancy between these two cohorts is unknown, the low penetrance of disease suggested that overexpression of *OLIG2* was a relatively weak oncogenic trigger and required additional cooperative events to induce leukemia. This hypothesis is supported by the observation that *OLIG2-LMO1* double transgenic mice developed pre-T LBL. The penetrance of pre-T LBL in the double transgenic mice was remarkably elevated (57%) compared with that of *OLIG2* only transgenic (12%), *LMO1* only transgenic (8%), and nontransgenic control (0%) mice. Whereas the *OLIG2* only transgenic mice developed pre-T LBL at a relatively advanced age (9 and 12 months) and a single *LMO1* only transgenic mouse developed the disease at the age of 13 months, *OLIG2-LMO1* double transgenic mice developed pre-T LBL as early as 4 months of age. These findings (increased penetrance and early onset of disease) indicated that *OLIG2* and *LMO1* synergistically collaborated during leukemic transformation.

We investigated the events that might be downstream of or collaborate with *OLIG2* and *LMO1* using two-color microarray expression profiling of *OLIG2-LMO1* tumors. A large number of genes that contribute to cell growth and/or survival were found up-regulated in *OLIG2-LMO1* thymic tumors. We noted with interest that *Notch1* was one of the most highly and consistently up-regulated genes. Expression of *Notch1* in thymic tumors from *OLIG2-LMO1* double transgenic mice was more than 3-fold up-regulated compared with that of normal control thymus. In addition, two genes that are considered to be downstream of *Notch1* (*Dtx1* and *Ptcra*) were also up-regulated.

Notch1 is required to maintain definitive hematopoiesis (35) and induces T-cell differentiation, at least in part, by inhibiting B-cell differentiation of common lymphoid progenitors (36). It has been shown that activated *Notch1* induces pre-T LBL in a murine bone marrow transduction model with complete penetrance (33). More recently, it was reported that over 50% of pre-T LBL patients lacking *Notch1*-related chromosomal translocations showed activating

DOI:10.1158/0008-5472.CAN-05-1400

Table 2. Genes up-regulated more than 3-fold in thymic tumors

Gene	Fold increase	Gene	Fold increase	Gene	Fold increase
1700042ORik	45.5	*2610511O17Rik*	4.5	*Tagln2*	3.5
Lmo1	12.6	*2610510E10Rik*	4.5	*C330013E15Rik*	3.4
Dtx1	10.2	*Gzma*	4.4	*Gsr*	3.4
4930550C14Rik	14.4	*Phgdh*	4.3	*Fubp1*	3.4
Saa3	8.4	*Tfrc*	4.3	*Mtap*	3.3
Ccl8	8.2	*Rapgef2*	4.2	*6330415M09Rik*	3.3
2010005H15Rik	7.7	*Eef2*	4.0	*5330440M15Rik*	3.3
Aldh1b1	6.9	*2010308M01Rik*	4.0	*Ar*	3.2
7420701I03Rik	5.9	*4632417K18Rik*	3.8	*Mtdh*	3.2
2300002D11Rik	5.9	*Fkbp5*	3.7	*Ppm1g*	3.2
Igk-V	5.9	*D17H6S56E-3*	3.7	*Catnb*	3.2
Tcrb-V13	5.7	*Slc38a1*	3.7	*2010001M07Rik*	3.2
Eif3s9	5.6	*Pgd*	3.6	*Lyar*	3.1
Parvg	5.6	*Rwdd2*	3.6	*E130201H02Rik*	3.1
Bst1	5.4	*Ralgds*	3.6	*2810022L02Rik*	3.1
Tars	5.1	*Adcy3*	3.5	*2610005L07Rik*	3.1
Ddx46	4.9	*Cd53*	3.5	*Ptcra*	3.1
Ccl21b	4.8	*Stip1*	3.5	*1810003N24Rik*	3.0
Eaf1	4.6	*Notch1*	3.5	*Oact2*	3.0
Olig3	4.6	*Angpt1*	3.5		

NOTE: Average expression level in thymic tumors as fold increase compared with normal thymus.

mutations of *Notch1* (37). Thus, deregulated *Notch1* can contribute to the pathogenesis of pre-T LBL. The Notch1 protein is a transmembrane receptor that binds multiple ligands including Δ and Jagged. On ligand binding, the intracellular domain of Notch1 (ICN1) is proteolytically cleaved by γ-secretase from the transmembrane portion and transferred into the nucleus, where it activates downstream signaling pathways (38). It has previously been shown that γ-secretase inhibitors suppressed the growth of *Notch1*-transformed pre-T LBL cells (39). We investigated whether up-regulation of *Notch1* in the *OLIG2-LMO1* tumors was important for the transformed phenotype using pre-T LBL cell lines established from *OLIG2-LMO1* double transgenic bone marrow. These pre-T LBL cell lines showed both cell death and growth inhibition in the presence of a γ-secretase inhibitor (Fig. 4 and Supplementary Fig. S3), whereas two control cell lines that did not overexpress *Notch1* were unaffected by the γ-secretase inhibitor. Thus, *Notch1* expression is important for cell survival in malignant cells that overexpress *OLIG2* and *LMO1*. We also investigated whether the activation of *Notch1* was induced by activating mutation in the HD and/or PEST domain as reported for pre-T LBL patients (37). Similar to the result that has been shown in human pre-T LBL, 50% (3 of 6) of *OLIG2-LMO1* double transgenic tumors had activating mutations in either the HD or PEST domain.

SCL/Tal-1, a bHLH transcription factor like OLIG2, has been shown to bind directly to E2A (40, 41); this inhibition of E2A by SCL was shown to be enhanced by overexpression of *LMO1* (23). The inhibition of E2A function by overexpression of *SCL* and *LMO1* is likely to be important for leukemogenesis given that *E2A* null mice develop pre-T LBL (42, 43). We have previously shown that E2A function was suppressed by *OLIG2* overexpression in a transient transfection assay (3). Given that coexpression of *LMO1* and *OLIG2* was more strongly oncogenic than expression of either protein alone, it seems reasonable to suggest that *LMO1* might potentiate the *OLIG2*-mediated inhibition of E2A function, and exert its oncogenic effect through inhibition of E2A.

We noted that *PDGFRα* was not up-regulated (average 1.0 ± 0.2-fold) in thymic tumors compared with that of normal thymus. *PDGFRα* overexpression previously was detected in several oligodendroglioma samples, leading to the suggestion that *PDGFRα* is a downstream target of *OLIG2* that might contribute to the pathogenesis of oligodendroglioma (2, 44–47). However, given that *PDGFRα* was not up-regulated in pre-T LBL tumors from *OLIG2* and *OLIG2/LMO1* mice, it seems plausible that the downstream targets of *OLIG2* might be cell type specific.

In summary, we found that *OLIG2* overexpression was not restricted to malignant oligodendrocytes but was also seen in malignant cells of skin, lung, breast, and hematopoietic origin. We showed that *OLIG2* overexpression was only weakly oncogenic in thymocytes and required collaborative events to induce a highly penetrant leukemia. Putative collaborative events include up-regulation of *LMO1, Notch1*, as well as other cell proliferation signals.

Acknowledgments

Received 4/25/2005; revised 6/12/2005; accepted 6/15/2005.

Grant support: Specialized Center of Research grant from the Leukemia and Lymphoma Society (S.D. Nimer).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank R. Keith Humphries of University of British Columbia for his gift of the pLIT2 vector, Stan Korsmeyer of Howard Hughes Medical Institute for the lck-LMO1 mice, Lionel Feigenbaum of the NCI transgenic core facility for microinjection and animal husbandry, Trang Hoang of the University of Montreal for helpful discussions, Donal MacGrogan of the Memorial Sloan-Kettering Cancer Center for preparation of RNA from human leukemic cell lines, and Junji Tsurutani of the NCI for valuable discussion in cell cycle analysis.

DOI:10.1158/0008-5472.CAN-05-1400

References

1. Lu QR, Yuk D, Alberta JA, et al. Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron 2000;25:317–29.
2. Zhou Q, Wang S, Anderson DJ. Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron 2000;25:331–43.
3. Wang J, Jani-Sait SN, Escalon EA, et al. The t(14;21)(q11.2;q22) chromosomal translocation associated with T-cell acute lymphoblastic leukemia activates the BHLHB1 gene. Proc Natl Acad Sci U S A 2000;97:3497–502.
4. Takebayashi H, Yoshida S, Sugimori M, et al. Dynamic expression of basic helix-loop-helix Olig family members: implication of Olig2 in neuron and oligodendrocyte differentiation and identification of a new member, Olig3. Mech Dev 2000;99:143–8.
5. Zhou Q, Choi G, Anderson DJ. The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2. Neuron 2001;31:791–807.
6. Mizuguchi R, Sugimori M, Takebayashi H, et al. Combinatorial roles of olig2 and neurogenin2 in the coordinated induction of pan-neuronal and subtype-specific properties of motoneurons. Neuron 2001;31:757–71.
7. Hack MA, Sugimori M, Lundberg C, Nakafuku M, Gotz M. Regionalization and fate specification in neurospheres: the role of Olig2 and Pax6. Mol Cell Neurosci 2004;25:664–78.
8. Lee SK, Lee B, Ruiz EC, Pfaff SL. Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. Genes Dev 2005;19:282–94.
9. Setoguchi T, Kondo T. Nuclear export of OLIG2 in neural stem cells is essential for ciliary neurotrophic factor-induced astrocyte differentiation. J Cell Biol 2004;166:963–8.
10. Marie Y, Sanson M, Mokhtari K, et al. OLIG2 as a specific marker of oligodendroglial tumour cells. Lancet 2001;358:298–300.
11. Lu QR, Park JK, Noll E, et al. Oligodendrocyte lineage genes (OLIG) as molecular markers for human glial brain tumors. Proc Natl Acad Sci U S A 2001;98:10851–6.
12. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002;1:75–87.
13. Ferrando AA, Neuberg DS, Dodge RK, et al. Prognostic importance of TLX1 (HOX11) oncogene expression in adults with T-cell acute lymphoblastic leukaemia. Lancet 2004;363:535–6.
14. Billia F, Barbara M, McEwen J, Trevisan M, Iscove NN. Resolution of pluripotential intermediates in murine hematopoietic differentiation by global complementary DNA amplification from single cells: confirmation of assignments by expression profiling of cytokine receptor transcripts. Blood 2001;97:2257–68.
15. Brady G, Billia F, Knox J, et al. Analysis of gene expression in a complex differentiation hierarchy by global amplification of cDNA from single cells. Curr Biol 1995;5:909–22.
16. Yu TS, Moctezuma-Anaya M, Kubo A, Keller G, Robertson S. The heart LIM protein gene (Hlp), expressed in the developing and adult heart, defines a new tissue-specific LIM-only protein family. Mech Dev 2002;116:187–92.
17. Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation *in vivo* without apparent exhaustion. Curr Biol 1997;7:805–8.
18. Benveniste P, Cantin C, Hyam D, Iscove NN. Hematopoietic stem cells engraft in mice with absolute efficiency. Nat Immunol 2003;4:708–13.
19. Hough MR, Takei F, Humphries RK, Kay R. Defective development of thymocytes overexpressing the costimulatory molecule, heat-stable antigen. J Exp Med 1994;179:177–84.
20. McGuire EA, Rintoul CE, Sclar GM, Korsmeyer SJ. Thymic overexpression of Ttg-1 in transgenic mice results in T-cell acute lymphoblastic leukemia/lymphoma. Mol Cell Biol 1992;12:4186–96.
21. Kogan SC, Ward JM, Anver MR, et al. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. Blood 2002;100:238–45.
22. Morse HC III, Anver MR, Fredrickson TN, et al. Bethesda proposals for classification of lymphoid neoplasms in mice. Blood 2002;100:246–58.
23. Chervinsky DS, Zhao XF, Lam DH, Ellsworth M, Gross KW, Aplan PD. Disordered T-cell development and T-cell malignancies in SCL LMO1 double-transgenic mice: parallels with E2A-deficient mice. Mol Cell Biol 1999;19:5025–35.
24. Lin YW, Slape C, Zhang Z, Aplan PD. NUP98-HOXD13 transgenic mice develop a highly penetrant, severe myelodysplastic syndrome that progresses to acute leukemia. Blood 2005;106:287–95.
25. Aplan PD, Nakahara K, Orkin SH, Kirsch IR. The SCL gene product: a positive regulator of erythroid differentiation. EMBO J 1992;11:4073–81.
26. Nie L, Xu M, Vladimirova A, Sun XH. Notch-induced E2A ubiquitination and degradation are controlled by MAP kinase activities. EMBO J 2003;22:5780–92.
27. Hitoshi S, Alexson T, Tropepe V, et al. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev 2002;16:846–58.
28. Alley MC, Scudiero DA, Monks A, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988;48:589–601.
29. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 1991;83:757–66.
30. Aplan PD, Jones CA, Chervinsky DS, et al. An scl gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice. EMBO J 1997;16:2408–19.
31. Ellisen LW, Bird J, West DC, et al. TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991;66:649–61.
32. Pear WS, Aster JC, Scott ML, et al. Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. J Exp Med 1996;183:2283–91.
33. Aster JC, Xu L, Karnell FG, Patriub V, Pui JC, Pear WS. Essential roles for ankyrin repeat and transactivation domains in induction of T-cell leukemia by notch1. Mol Cell Biol 2000;20:7505–15.
34. Hoang-Xuan K, Aguirre-Cruz L, Mokhtari K, Marie Y, Sanson M. OLIG-1 and 2 gene expression and oligodendroglial tumours. Neuropathol Appl Neurobiol 2002;28:89–94.
35. Hadland BK, Huppert SS, Kanungo J, et al. A requirement for Notch1 distinguishes 2 phases of definitive hematopoiesis during development. Blood 2004;104:3097–105.
36. Pui JC, Allman D, Xu L, et al. Notch1 expression in early lymphopoiesis influences B versus T lineage determination. Immunity 1999;11:299–308.
37. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269–71.
38. Das I, Craig C, Funahashi Y, et al. Notch oncoproteins depend on γ-secretase/presenilin activity for processing and function. J Biol Chem 2004;279:30771–80.
39. Weng AP, Nam Y, Wolfe MS, et al. Growth suppression of pre-T acute lymphoblastic leukemia cells by inhibition of notch signaling. Mol Cell Biol 2003;23:655–64.
40. Hsu HL, Cheng JT, Chen Q, Baer R. Enhancer-binding activity of the tal-1 oncoprotein in association with the E47/E12 helix-loop-helix proteins. Mol Cell Biol 1991;11:3037–42.
41. Voronova AF, Lee F. The E2A and tal-1 helix-loop-helix proteins associate *in vivo* and are modulated by Id proteins during interleukin 6-induced myeloid differentiation. Proc Natl Acad Sci U S A 1994;91:5952–6.
42. Bain G, Engel I, Robanus Maandag EC, et al. E2A deficiency leads to abnormalities in αβ T-cell development and to rapid development of T-cell lymphomas. Mol Cell Biol 1997;17:4782–91.
43. Yan W, Young AZ, Soares VC, Kelley R, Benezra R, Zhuang Y. High incidence of T-cell tumors in E2A-null mice and E2A/Id1 double-knockout mice. Mol Cell Biol 1997;17:7317–27.
44. Hall A, Giese NA, Richardson WD. Spinal cord oligodendrocytes develop from ventrally derived progenitor cells that express PDGF α-receptors. Development 1996;122:4085–94.
45. Smith JS, Wang XY, Qian J, et al. Amplification of the platelet-derived growth factor receptor-A (PDGFRA) gene occurs in oligodendrogliomas with grade IV anaplastic features. J Neuropathol Exp Neurol 2000;59:495–503.
46. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes *in vivo*. Genes Dev 2001;15:1913–25.
47. Spassky N, Heydon K, Mangatal A, et al. Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFRα signaling. Development 2001;128:4993–5004.