[haematologica reports]
2006;2(13):79-82

Animal models of T-cell lymphomas

Y-W. LIN*
P.D. APLAN

Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

**Currently: Department of Pediatrics, Tenri Hospital, Tenri, Nara, Japan*

We firmly believe that a better understanding of the molecular mechanisms that cause malignant transformation will lead to more effective therapies for patients with hematologic malignancy. Most hematologic malignancies have been shown to bear chromosomal aberrations such as translocations, deletions, and duplications. Therefore, we have investigated whether those genetic alterations were crucial for malignant transformation using animal, especially mouse, models. There are at least three advantages offered by animal models. First, one can investigate whether a candidate gene leads to leukemia *in vivo* by generating a transgenic mouse. If the candidate gene is truly oncogenic, then the transgenic mice should develop an increased incidence of malignancy. The second is that it is simpler to investigate the downstream target or collaborative genes of the transgenes. In clinical human samples, one often sees not only a single genetic alteration but also several aberrations. Although it is difficult to predict which is the primary event in most human cases, we know that the transgene is the primary oncogenic event in transgenic mice. Lastly, one can test the efficacy and side effects of newly developed treatments on those genetically engineered mice prior to human trials. In this meeting, the following three types of transgenic mice will be discussed in detail. The three types include the *SCL-LMO1,*[1-2] *OLIG2-LMO*1,[3] and *NUP98-HOXD1*[3] (*NHD13*) mice[4].

The *SCL* gene is also known as *TCL* or *TAL1*, and was cloned by virtue of a t(1;14)(p33;q11) chromosomal translocation in a patient with stem cell leukemia. SCL is known to act as a transcription factor that has a basic helix-loop-helix motif (bHLH). Although *SCL* is not normally expressed in mature thymocytes, more than 60% of precursor T-cell lymphoblastic lymphoma/leukemia (Pre-T LBL) patients express SCL mRNA. *SCL* is activated by either chromosomal translocation or interstitial deletion. The interstitial deletion places *SCL* coding sequences under the control of *SIL* regulatory elements. Two series of transgenic mouse that over-expressed *SCL* were generated using a vector that utilized the *SIL* promoter to drive expression of a full-length *SCL* or truncated *SCL*. Only 1.1% of these animals developed pre-T LBL in the observation period of 16 months. However, it has been shown that a subset of T-ALL patients has both an *LMO1* or *LMO2* translocation and a SIL/SCL rearrangement. Of interest, *LMO1* transgenic mice have been shown to develop pre-T LBL in later stages of life, with a low penetrance, leading to the hypothesis that *LMO1* may need additional, cooperative genetic events to produce a frank malignancy. The cross of the *SCL* mice and the *LMO1* mice uniformly developed pre-T LBL within several months of life (Figure 1). The pre-T LBL cells were characterized by positive staining for CD4 and CD8, and showed clonal or oligoclonal TCR, gene rearrangement. The *OLIG2* gene (*BHLHB1*) is located at 21q22 and encodes a transcription factor that contains the conserved bHLH motif similar to *SCL*. Although OLIG2 is normally expressed in neural tissues, overexpression of *OLIG2* has been shown in primary sample from oligodendroglioma patients as well as pre-T LBL patients. In addition, we found that *OLIG2* was overexpressed in a variety of malignant cell lines established from non-small cell lung carcinoma, breast cancer, melanoma, and leukemia, linking ectopic *OLIG2* expression to a wide spectrum of malignancies. To investigate the oncogenic potential of *OLIG2* in mice,

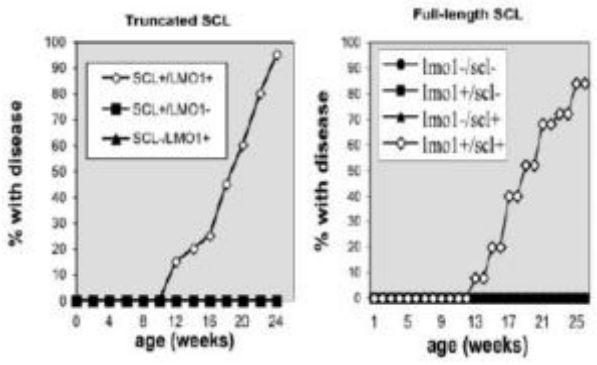

Figure 1. Cumulative incidence of T-cell lymphoma in SCL-LMO1 mice. The SCL-transgenic animals were generated with the vector that contained either truncated SCL or full-length SCL. Those SCL mice were crossed with the LMO1 mice.

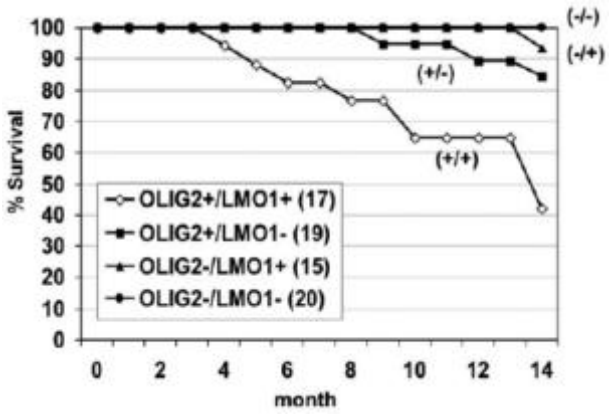

Figure 2. Survival curve in OLIG2-LMO1 mice. Both the OLIG2 and LMO1 mice were generated using Lck promoter. 60% of the OLIG2 - LMO1 mice developed T-cell lymphoma by the age of 14 months.

Table 1. Cause of death in NHD13 mice.

Disease	*FVB*		*C57B16****	
	n	*Age (mo)*	*n*	*Age (mo)*
MDS	4 (19.0%)	5-11	1 (3.8%)	10
Non-Lymphoblastic leukemia	6* (28.6%)	4-13	15 (57.7%)	4-14
Lymphoblastic leukemia	7* (33.3%)	4-13	3 (11.5%)	9-10
Undetermined	4 (19.0%)	4-12	7 (26.9/%)	8-13
total	21**		29	

*Initial observation period was 14-months. *A mouse developed both erythroid leukemia and Pre-T LBL. Another mouse with concurrent pre-T LBL and myeloid leukemia was found in additional cohort study. **A mouse that servived beyond 14 month was found dead in 15 month of age because of Pre-T LBL. ***Two mice that survived.*

we generated *OLIG2* transgenic mice using the lck promoter and found that only a few *OLIG2* mice developed pre-T LBL. We crossed *OLIG2* mice with *LMO1* mice, and noted that 60% of the double transgenic mice developed pre-T LBL by the age of 14 months (Figure 2). All affected mice had a large thymic tumor and hepatosplenomegaly. Those pre-T LBL cells were highly invasive as infiltrating into lung, liver, and kidney. The lymphoma cells were morphologically characterized by prominent nucleoli and high nuclear cytoplasmic ratio, showed clonal *TCRβ* gene rearrangement, and were positive for CD4 and CD8 (Figure 3).

The *NUP98* gene is fused to at least 15 different partner genes by chromosomal translocation in a wide spectrum of hematologic malignancies. *NUP98* fused to either *HOXD11* or *HOXD13* has been identified in the malignant cells of patients with myelodysplastic syndrome (MDS), acute myeloid leukemia (AML)-M4, and AML-M6 bearing a t(2; 11)(q31; p15). We generated NHD13 transgenic mice using vav regulatory elements. Almost all of the *NHD13* mice developed a MDS. More than 95% of *NHD13* mice died of MDS, AML, or lymphoblastic malignancies by 14 months of age. The lymphoblastic malignancies in the NHD13 mice were pre-T LBL except for a single case with pre-B LBL. The incidence of pre-T LBL was approximately 11% and 30% in NHD13 mice generated on the C57Bl6 and FVB background respectively (Table 1). We also found there were two cases of concurrent pre-T LBL and myeloid leukemias suggesting that myeloid leukemia evolved from MDS and pre-T LBL occurred independently (Figure 4).

Thus, those findings demonstrate that the concurrent overexpression of *SCL* and *LMO1*, of *OLIG2* and *LMO1*, and *NHD13* were oncogenic. However, the delayed onset of some tumors and incomplete penetrance of the pre-T LBL phenotype suggested that additional, cooperative mutations were required to generate a frank malignancy. Furthermore, it is of interest to learn what genes might be the downstream targets of these genes, given that they are known or putative transcription factors. It has been shown that *E2A* knockout mouse developed pre-T LBL. We have shown that *SCL* and *OLIG2* could bind to E2A and suppress its ability to transactivate down-

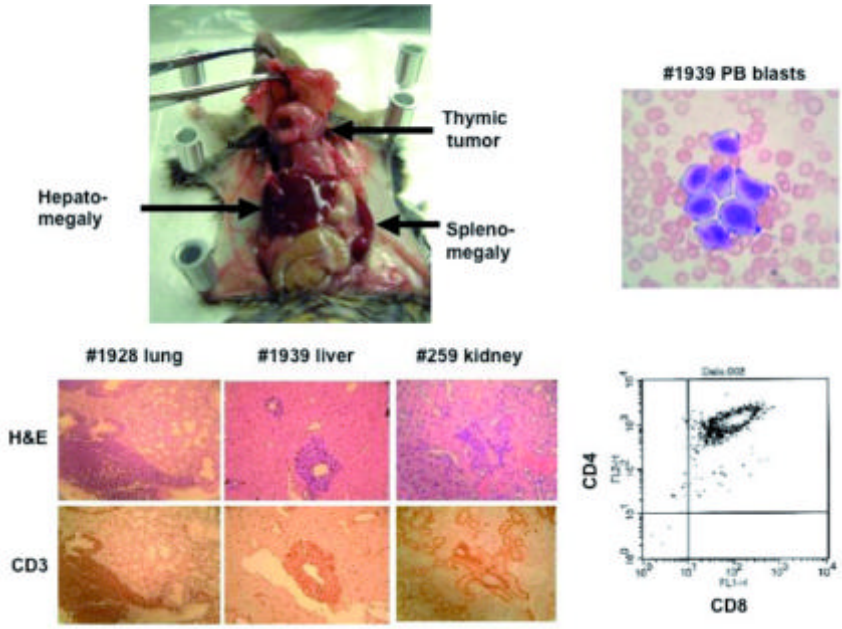

Figure 3. Characteristics of T-cell lymphoma in *OLIG2-LMO1* double transgenic mice. The disease was highly invasive. Thymic tumor, hepatosplenomegaly, and lymphadenopathy were common in the affected mice. Pleural effusion and lung infiltration of malignant cells were also noticed in some cases. The leukemic blasts emerging in the peripheral blood showed prominent nucleoli and high nuclear-cytoplasmic ratio. The surface phenotype of the leukemic blasts was positive for CD4 and CD8.

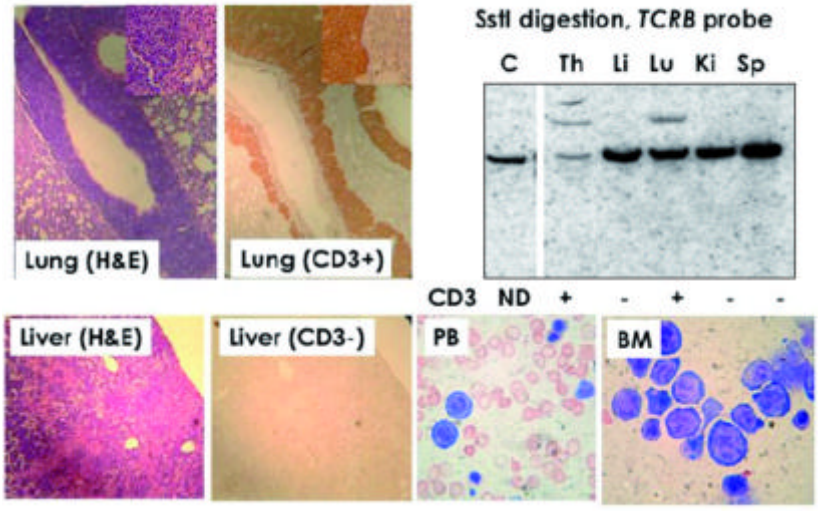

Figure 4. Concurrent pre-T LBL and erythroid leukemia in *NHD13* mice. The lung was massively infiltrated with CD3-positive leukemic blasts. The liver was also severely infiltrated with leukemic blasts that were negative for CD3. Southern blot analysis of *TCRβ* gene indicated clonal rearrangement in thymus and lung. The leukemic blasts in liver, kidney, and spleen showed typical morphologic feature of erythroid cells, which was confirmed by gene profiling that showed up-regulated erythroid-associated genes. *Notch1* mutation was noted in the thymic tumor but not in the liver.

stream reporter genes suggesting that E2A can be functionally inactivated by bHLH transcription factors including SCL and OLIG2.[2,5] Gene expression profiling of pre-T LBL thymic tumors using a microarray with 21,000 features indicated that *Notch1* was approximately 3-fold up-regulated compared with normal thymi. This was of interest since it has been shown that activating mutations in *NOTCH1* were found in 50% of pre-T LBL patients.[6] Similarly, we found *Notch1* mutations were very common events in the mouse pre-T LBL models including *SCL-LMO1* mice.[7] Only 18.5% of mutations that we identified were found in the HD domain, and all of those mutations were missence mutations. Half of the mutations in the HD domain were in positions mutated in human pre-T LBL, and the remaining half were at positions conserved in human, mouse, chicken, frog, and fish. All mutations within the PEST domain involved nucleotide insertions and/or deletions, leading to frameshift mutations and premature stop codons, as opposed to single base substitutions. We found that 50% of the mutations in the PEST domain are located in two *hot spots*. Of note, even though thymocytes from healthy *SCL-LMO1* mice aged 5 weeks showed oligo-clonal rearrangements of *TCRβ*, indicating massive expansion of one or a few clones, they did not form lymphoma upon injection into immunodeficient mice. On the other hand, thymocytes from *SCL-LMO1* mice aged 12 weeks invariably formed T-cell lymphoma upon injection into nude mice, suggesting that additional genetic events which collaborate with *SCL-*

Table 2. Notch1 mutations in pre-leukemic thymocytes from clinically healthy SCL-LMO1 Tx-mice.

Mouse	*Age (weeks)*	*Notch 1 mutation*	*Injection into nude mice*	*Nude mice Tumor*	*Nude mice Tcrb*	*Notch1 mutation*
7162/3	5	n	-	N/A	N/A	N/A
7543/3	5	n	+	-	N/A	N/A
7544/2	5	n	+	-	N/A	N/A
7286/1	8	66601-4 CCCC >CCCCC	+	+	+	66601-4 CCCC >CCCCC
7193/3	10	66569-4 GACCCC> CCATGACTCCT	-	N/A	N/A	N/A
7151/4	12	66419C>GG	+	+	+	66419C>GG
7150/3	12	66456>CC	+	+	+	66456>CC

Thymocytes from the mice aged 5 weeks, which did not show Nothch1 mutation, did not form a tumor in nude mice, whereas those from the mice aged 8-12 weeks,which had Notch1 mutations, formed a tumor in nude mice.

LMO1 to produce a frank malignancy occur between 5 and 12 weeks of age. Consistent with this hypothesis, thymocytes from 5-week-old mice did not have *Notch1* mutations, whereas those from the mice aged 8-12 weeks had *Notch1* mutations.[7] Those findings indicated that N*otch1* mutation was not the primary event in these models of T-cell malignancy, but were complementary events that occurred as the thymocytes became fully malignant (Table 2).

Of the 30 most up-regulated genes in thymic T-cell lymphomas from the *SCL-LMO1, LMO1*, and *NHD13* mice, nine genes including *Tcrb* and *Tfrc* were commonly found up-regulated in all three series of Tx-mice. The commonly up-regulated genes might confer growth advantages on the leukemic cells through activated transcription or post-transcriptional control of mRNA. On the other hand, sixteen genes including *Prss16*, *Erf1*, and *H2-Aa* were down-regulated in all three series of Tx-mice. The down-regulated genes might be either associated with the escape from elimination mediated by immune system, or tumor suppressor genes. Therefore, it is reasonable to speculate that T-cell oncogenesis is a consequence of acceleration of cell growth, acquisition of immortality, and escape from immune response. We noticed that *chemokine ligand 8 (Ccl8),* which binds to chemokine receptor 3 (Ccr3), was up-regulated in the *SCL-LMO1*, *OLIG2-LMO1,* and NHD13 lymphomas. An antagonist for Ccr3 killed T-lymphoma cell lines indicating a novel therapeutic option for T-cell lymphomas. Cofilin1 is uniquely up-regulated in the SCL-LMO1 lymphoma. Inactivation of *Cofilin1* by Okadaic acid suppressed cell growth of the *SCL-LMO1* cell lines but not the control cell lines. We also found the *Pbx3* was up-regulated specifically in NHD13 T-cell lymphomas as well as *NHD13* MDS bone marrow. Transfection of *NHD13* expression vector into *SCL-LMO1* cells induced expression of *Pbx3* suggesting that *Pbx3* was a downstream target of *NHD13*.

In conclusion, animal models for T-cell lymphoma/leukemia are a useful tool to investigate oncogenes, which could provide an insight into T-cell oncogenesis that might lead to development of novel therapeutic approaches.

References

1. 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.
2. Chervinsky DS, Zhao XF, Lam DH, et al. 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.
3. Lin YW, Deveney R, Barbara M, et al. OLIG2(BHLHB1), a bhlh transcription factor, contributes to leukemogenesis in concert with LMO1. Cancer Res 2005;65: 7151-8.
4. Lin YW, Slape C, Zhang Z, and Aplan PD, NUP98-HOXD13 transgenic mice develop a highly penetrant, severe myelodysplastic syndrome that progress to acute leukemia. Blood 2005;106: 287-95.
5. 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. Pros Natl Acad Sci USA 2000;97: 3497-502.
6. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306: 269-71.
7. Lin YW, Nichols RA, Letterio, JJ, Aplan, PD. Notch1 mutations are important for leukemic transformation in murine models of precursor-T leukemia/lymphoma. Blood 2006;107: 2540-3.