An *scl* gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice

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The product of the *scl* (also called *tal-1* or *TCL5*) gene is a basic domain, helix–loop–helix (bHLH) transcription factor required for the development of hematopoietic cells. Additionally, *scl* gene disruption and dysregulation, by either chromosomal translocations or a site-specific interstitial deletion whereby 5′ regulatory elements of the *sil* gene become juxtaposed to the body of the *scl* gene, is associated with T-cell acute lymphoblastic leukemia (ALL) and T-cell lymphoblastic lymphoma. Here we show that an inappropriately expressed *scl* protein, driven by *sil* regulatory elements, can cause aggressive T-cell malignancies in collaboration with a misexpressed LMO1 protein, thus recapitulating the situation seen in a subset of human T-cell ALL. Moreover, we show that inappropriately expressed *scl* can interfere with the development of other tissues derived from mesoderm. Lastly, we show that an *scl* construct lacking the *scl* transactivation domain collaborates with misexpressed LMO1, demonstrating that the *scl* transactivation domain is dispensable for oncogenesis, and supporting the hypothesis that the *scl* gene product exerts its oncogenic action through a dominant-negative mechanism.

*Keywords*: acute lymphoblastic leukemia/LMO1/scl/T cells/transgene

**Introduction**

The *scl* gene (also known as *TCL5* or *tal-1*) was first identified by virtue of its involvement in a t(1;14)(p33;q11) translocation present in the multipotential DU528 stem cell leukemia cell line (Begley et al., 1989; Finger et al., 1989; Chen et al., 1990). The *scl* gene product contains the ‘bHLH’ (basic domain, amphipathic α-helix, loop, amphipathic α-helix) motif, conserved in a wide array of eukaryotic transcription factors in organisms ranging from baker’s yeast to man (Murre et al., 1989; Olson and Klein, 1994). A transcription activation domain has been identified at the amino-terminal portion of the *scl* protein (Hsu et al., 1994b; Sanchez-Garcia and Rabbits, 1994; Wadman et al., 1994a), and a preferred DNA-binding sequence (CAGATG) has been identified (Hsu et al., 1994a), although no natural target genes for *scl* have been firmly established. Several forms of the *scl* protein have been detected in mammalian cells; a 43 kDa full-length protein and shorter proteins of 39 and 22 kDa (Cheng et al., 1993; Pulford et al., 1995). The 22 kDa protein can be produced by translation of an alternately spliced transcript which lacks exons 2, 3 and 4; this shorter protein lacks the *scl* transcription activation domain (Bernard et al., 1991; Cheng et al., 1993). Similarly to other bHLH proteins, *scl* has been shown to bind to the E-proteins E2-2 and E2A (Hsu et al., 1991; Voronova and Lee, 1994; Goldfarb et al., 1996). By analogy with the myogenic bHLH proteins, it has been speculated that *scl* exerts its biochemical function by binding DNA in a sequence-specific fashion and activating transcription of target genes (Green, 1996; Porcher et al., 1996; Robb et al., 1996).

Several reports have demonstrated that *scl* is expressed primarily in hematopoietic and vascular endothelial cells (Green et al., 1992; Hwang et al., 1993; Kallianpur et al., 1994; Pulford et al., 1995), although fetal brain tissue also expresses easily detectable levels of *scl* mRNA (Green et al., 1992). Recent experiments with mice harboring a targeted deletion of *scl* have demonstrated that the *scl* gene product is needed for all hematopoietic development, including T and B lymphocytes (Robb et al., 1995b; Shvidasani et al., 1995; Porcher et al., 1996). These findings have led to the speculation that *scl* expression is required for commitment of ventral mesoderm to hematopoietic progenitor cells (Porcher et al., 1996).

Non-random chromosomal translocations are a recurrent theme in hematopoietic malignancies; in T-cell acute lymphoblastic leukemia (T-cell ALL) and lymphoblastic lymphoma of T-cell phenotype, the translocations often serve to activate transcription factors inappropriately through the juxtaposition of these transcription factors with the enhancer elements of the T-cell receptor (TCR) loci (for review, see Rabbits 1994). Several of the most common translocations associated with T-cell ALL activate either HOX11 (Hatano et al., 1991), LMO1 (formerly known as *TTG1* or *RBTN1*) (McGuire et al., 1989; Boehm et al., 1991), LMO2 (formerly known as *TTG2* or *RBTN2*) (Boehm et al., 1991; Ruyer-Pokora et al., 1991) or *scl* (Begley et al., 1989; Chen et al., 1990; Finger et al., 1990). In general, these genes are not highly expressed in normal T cells, but T cells which have undergone a translocation involving one of these genes express the translocated gene at relatively high levels (Rabbits, 1994). It has been proposed that inappropriate expression of these transcription factors, in a T-cell milieu, leads to malignant transformation, possibly through direct transcriptional activation of target genes. While *scl* translocations are present in only 3% of T-cell ALL patients (Carroll et al.,...
1990), ~25% of T-cell ALL patients activate scl by a site-specific interstitial deletion which replaces the scl 5’ regulatory sequences with those of an upstream gene, sil (Aplan et al., 1990b; Brown et al., 1990). In addition, T-cell ALL cell lines and patient samples with no detectable scl gene rearrangements often express high levels of scl mRNA; one report has demonstrated ectopic scl mRNA expression in a majority (60%) of T-cell ALL patients (Bash et al., 1995).

Despite the striking association of scl gene activation with T-cell ALL, until recently, there had been little evidence presented to demonstrate direct proof that scl gene activation leads to malignant transformation. Two groups demonstrated that transgenic mice overexpressing scl did not develop T-cell malignancies (Robb et al., 1995a, Larson et al., 1996), nor did lethally irradiated mice reconstituted with bone marrow engineered to overexpress scl (Elwood and Begley, 1995). However, when scl transgenic mice were crossed with mice engineered to overexpress LMO2, the offspring positive for both scl and LMO2 developed T-cell malignancies 3 months earlier than did mice transgenic only for LMO2, indicating a collaborative effect of scl and LMO2 (Larson et al., 1996). More recently, 28% of mice transgenic for a construct which expressed a full-length scl protein driven by an lck promoter developed T-cell malignancies within 350 days, producing clear evidence that scl was a proto-oncogene (Kelliher et al., 1996). The onset and penetrance of disease was significantly increased when these mice were crossed with transgenic mice which overexpress casein kinase IIα (Kelliher et al., 1996). Several investigators have speculated that scl may play a role in oncogenic action either by inappropriately activating target genes through its transactivation domain, or through a dominant-negative mechanism, where scl may bind and sequester other HLH proteins, making them unavailable to form complexes with additional HLH proteins (Goldfarb and Greenberg, 1994; Green, 1996; Kelliher et al., 1996; Porcher et al., 1996).

Here we describe generation of transgenic mice using constructs which express scl mRNA driven by a sil promoter, thus mimicking the most common type of scl gene dysregulation associated with human T-cell ALL. We demonstrate that mice transgenic for either a full-length or an amino-terminal truncated scl driven by a sil promoter surprisingly display bony abnormalities and growth retardation, but do not develop T-cell malignancies. However, when crossed with mice that overexpress LMO1 in the thymus, enforced expression of an amino-terminal truncated scl, which lacks the scl transactivation domain, leads to aggressive T-cell malignancies at an early age. These experiments demonstrate that the transactivation domain of scl is dispensable for leukemogenesis, and suggest that scl is not acting by activating transcription of its normal target genes, but instead is more likely to act through a dominant-negative mechanism.

Results

Generation of constructs recapitulating sil and scl recombination

Since we were aware of reports demonstrating a lack of oncogenicity in transgenic mouse models using the CD2 promoter to target scl expression to thymocytes (Robb et al., 1995a), we decided to use sil regulatory elements to drive scl expression, thus recapitulating the most common situation seen in human T-cell ALL patients. In order to accomplish this goal, we isolated a human sil promoter, demonstrated it was functional when integrated in the mouse genome, and searched for alternate forms of sil–scl fusion mRNA species in malignant T cells which had undergone a sil–scl rearrangement. A 2.4 kb human genomic sil fragment which encompassed the predicted sil transcript initiation site (Aplan et al., 1991) was isolated and shown to activate a CAT reporter gene in transient transfection assays using human and murine cell lines. This promoter fragment was then used to produce the pSil/SCL and pSil/TsCL vectors (Figure 1A). Since this promoter had not been used previously in transgenic experiments, we verified that the sil promoter fragment

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**Figure 1.** SIL/SCL vectors. (a) Diagram of the SIL/SCL vectors. The pSil/SCL vector contains human sil promoter sequences, an scl cDNA encoding a full-length human scl protein, a human β-globin genomic fragment encompassing exon 2 and 3 sequences including the polyadenylation site, and a neomycin resistance cassette. The pSil/TsCL contains human sil promoter sequences and sil exon 1 sequences fused to scl exon 5 and 6 sequences, encoding an amino-terminal truncated scl protein. (b) Expression of the transfected scl. Northern blot analysis of four independent stable transfectants of the F4-6 murine erythroleukemia cell line hybridized to a human scl cDNA probe (1.0SX). The 28S and 18S rRNA bands are indicated; exogenous scl mRNA is indicated with an arrow. The human scl probe cross-hybridizes with the endogenous murine scl, and is seen as a band which co-migrates with the 28S rRNA. The transfectants in lanes 1 and 2 express transfected scl; those in lanes 3 and 4 express little, if any, exogenous scl.
Cells with sil–scl rearrangements generate several related mRNA species.

(a) Schematic representation of sil–scl fusion mRNA species. The sil locus is indicated by open boxes (A), the scl locus by filled boxes (B). Exon numbers are indicated; sil exon 1 (A), scl exon 3 (B) and scl exon 6 (C) primers are shown. Three alternately spliced transcripts are shown.

(b) Nucleotide sequence at splice junctions (brackets) of four different sil–scl fusion mRNA species.

remained functional when incorporated into the mouse genome by transfecting the pSIL/SCL vector into murine erythroleukemia cells and selecting stable transfectants. Fourteen of 20 randomly selected clones expressed easily detectable amounts of exogenous scl mRNA (Figure 1b), demonstrating that the human sil promoter was functional when integrated into the mouse genome.

Our initial experiments used the pSIL/SCL construct, which produced the full-length scl protein. While the initial cohort of mice generated with this construct was under observation, we investigated the possibility that alternately spliced forms of the fusion sil–scl mRNA may exist. We were especially interested in this possibility since the intact scl locus normally generates a complex pattern of alternately spliced transcripts, including one which produces an amino-terminal truncated form of the scl protein (Aplan et al., 1990a). We had previously used RNase protection probes to demonstrate the production of sil exon 1–scl exon 3 fusion mRNA species in leukemic cells which displayed a sil–scl recombination (Aplan et al., 1990b). Since this fusion occurs in the 5′-untranslated region (UTR) of both genes, the net effect of the sil exon 1–scl exon 3 fusion is the production of a fusion mRNA encoding a full-length scl protein.

We employed an RT–PCR assay that utilized an scl exon 6 primer and a sil exon 1 primer. Several amplification products were obtained as depicted in Figure 2; the predominant amplification product corresponded to a sil exon 1–scl exon 5 fusion. Although a sil exon 1–scl exon 3 product could theoretically have been amplified with these primers, we could not detect an amplification product corresponding to a sil exon 1–scl exon 3 fusion using these primers. This may be due to competition with the smaller sil–scl fusion mRNAs, or to difficulty in reverse transcribing through GC-rich scl exon 4 sequences, since amplification of the same mRNA templates used in Figure 2 with sil exon 1 and scl exon 3 primers generated quantities of a sil exon 1–scl exon 3 PCR product that were roughly equivalent to the amount of sil exon 1–scl exon 5 PCR products generated by sil exon 1 and scl exon 6 primers. The pSIL/TSCl vector (Figure 1a) recapitulates the sil exon 1–scl exon 5 fusion, and encodes a truncated 22 kDa scl protein that lacks the scl transactivation domain and initiates transcription at scl residue 176, similar to the protein product produced by scl exon 1–exon 5 transcripts in normal cells (Bernard et al., 1991; Cheng et al., 1993).

**Phenotype of mice generated with the pSIL/SCL vector**

Four founder animals were obtained by injection of the pSIL/SCL construct. Founder A1 and A4 both incorporated one copy of the transgene and expressed relatively low levels of the transgene in thymus, liver and spleen (Figure 3). Founder A2 incorporated two copies of the transgene and expressed 5-fold higher levels of exogenous scl mRNA. Founder A3 incorporated three copies of the transgene, but never transmitted the transgene, and mRNA expression was never assayed. Only one of 180 positive progeny from lines A1 and A4 had obviously kinked tails (Figure 4a), while being otherwise healthy. All but one (5/6) of the positive progeny of line A2, the line with the highest scl mRNA expression, displayed kinked tails in conjunction with more severe abnormalities, including growth retardation, sparse hair
Dominant-negative action of the scl gene product

Fig. 3. RNase protection assay demonstrating expression of the scl transgene. An antisense human scl probe was hybridized to total RNA extracted from the indicated tissues. Transgenic offspring from three founder lines (A2/+, A1/+, A4+/) demonstrate expression in bone marrow and thymus (indicated by an arrow); a non-transgenic littermate (A1−) is negative. Parallel samples were hybridized to a murine β-actin probe as a control. A faint band seen in bone marrow from the non-transgenic animal is due to cross-hybridization with the endogenous murine scl.

and infertility (Figure 4a). The females all had an atretic vaginal opening that could not be probed, while the only male was unable to impregnate any females despite numerous attempts at mating. It is of note that Southern blot analysis of genomic DNA from the only transgenic offspring of line A2 which did not display this unusual phenotype demonstrated that the transgene had been rearranged and largely deleted. None of >300 non-transgenic offspring observed during this period had tail kinks. Alizarin red/alcan blue staining showed the only obvious bony abnormality of these animals to be within the tail vertebrae (Figure 4a). In addition, while lines A1 and A4 transmitted the transgenic allele in expected Mendelian fashion, line A2, which expressed the highest level of exogenous scl mRNA, transmitted the transgene to only six of 45 progeny.

To evaluate a potential dosage effect of scl transgene expression on the generation of this phenotype, we crossed two line A4 animals. Ten of 11, seven of 39, and none of 18 animals with two, one or zero copies of the transgene, respectively, displayed tail kinks. Additionally, four of the animals with two copies of the transgene were growth retarded and had sparse hair (Figure 4b). None of these animals developed a T-cell malignancy over a 16 month observation period. Some of these double-positive (A4+/ A4+) animals were fertile; one of these was crossed with a line A1-positive animal (A1+/A1−). Of 46 offspring from this cross, 31 were A4+/A1−; two of these had kinked tails; 15 offspring (only half of the expected number) were A4+/A1−, nine of these had kinked tails; one of these 15 developed an aggressive T-cell malignancy at age 15 months. Taken together, these data suggest that ectopic scl expression causes bony abnormalities in a dose-dependent fashion, and that higher levels of ectopic scl expression are associated with embryonic lethality.

Phenotype of mice generated with an scl construct lacking the transactivation domain

The fact that mice transgenic for a full-length scl were not developing T-cell malignancies led us to investigate the possibility that the amino-terminal truncated p22 scl isoform was the oncogenic form. In addition to being formed by a sil exon1–scl exon 5 fusion mRNA, the p22 scl protein can also be produced from T-cell ALL cells by a sil exon1–scl exon 5 fusion mRNA, the p22 scl protein can also be produced from T-cell ALL cells through the use of an alternate scl promoter, which initiates transcription from within scl exon 4 (Aplan et al., 1990a; Bernard et al., 1992, 1996). Therefore, we generated four additional founder lines using the pSIL/Tscl construct. Line A5(3) expressed the highest level of exogenous scl and had incorporated 5–7 copies of the transgene. Similar to the pSIL/SCL founders, 0/33, 3/29, 4/18 and 2/27 positive offspring of lines A5(1), A5(2), A5(3) and A5(4), respectively, demonstrated kinked tails. Also, while lines A5(1), A5(2) and A5(4) transmitted the transgene within the range expected for Mendelian inheritance, only 18/86 (21%) of the A5(3) offspring were transgenic, again suggesting the possibility of embryonic lethality. None of the 107 positive progeny of these founders have developed T-cell malignancies over a 12 month observation period.
Fig. 4. Phenotype of sil–scl transgenic mice. (a) sil–scl transgenic mice show kinked tails and hair loss. (A) Shows a line A4-positive animal with tail kink, (B) shows a line A2-positive animal with tail kink and hair loss, (C) and (D) each show alizarin stains of kinked tails on the left, with normal controls on the right. The misformed vertebrae are indicated with arrows; note that the tail in (D) has defects in opposite orientations, leading to a less marked kink. (b) Dose effect of the sil–scl transgene. Two offspring from a line A4×A4 cross are shown. The animal on the left has two copies of the transgene (A4+/A4+), his littermate on the right has one copy (A4+/A4-). Both animals have kinked tails; note the growth retardation and sparse hair of the animal on the left.
Fig. 5. Mice transgenic for both a sil–scl construct and an lck–LMO1 construct develop T-cell leukemia/lymphoma at a young age. (a) Cumulative incidence of leukemia/lymphoma in scl/LMO1-positive mice. Nine litters from an scl/LMO1 cross were observed for clinical signs of lymphoma/leukemia over a 6 month study period. Genotypes were scl/H11001/LMO1/H11001 (n = 20), scl/H11001/LMO1-H11005 (n = 8), scl-LMO1/-H11001 (n = 14). (b) Gross appearance of tumors in a double transgenic mouse. Note the enlarged thymus (T), liver (L), spleen (S) and lymph nodes (LN). (c) Microscopic appearance of leukemia/lymphoma. (A) Thymus (×720); (B) lung (×120); (C) bone marrow (×1200); (D) kidney (×120). Note the appearance of typical lymphoblasts with a high nuclear/cytoplasm ratio, and the perivascular infiltration of the lung and kidney.
**scl does not collaborate with pim-1 to induce T-cell malignancies**

In order to determine if *scl* was able to cooperate with other known transforming genes, we crossed offspring of the A4 line with mice transgenic for an Eμ-*pim-1* construct. *pim-1* was chosen as a candidate gene since it was identified initially by virtue of its frequent activation at MuLV insertion sites in murine T-cell lymphomas (Cuypers et al., 1984), and has been shown to accelerate dramatically lymphoid malignancies produced by the bHLH proteins c-Myc and N-Myc (van Lohuizen et al., 1989). We have now followed a cohort of mice positive for both *scl* and *pim-1* for >1 year, and compared them with control groups positive for either *scl* or *pim-1* only, or negative for both transgenes. Two of eight *scl*/+/*pim-1*+ animals have developed non-T-cell malignancies over a 15 month observation period; three of 17 *scl*/*pim-1*+ mice have developed malignancies (one spindle cell sarcoma of unknown primary, two T-cell lymphomas), and none of 15 *scl*+/*pim-1*− mice have developed T-cell malignancies. We conclude that ectopically expressed *scl* does not accelerate the rate at which *pim-1* transgenic mice develop malignancies.

**The p22 isoform of scl collaborates with LMO1 to induce an aggressive T-cell leukemia/lymphoma**

In order to determine if *scl* could cooperate with *LMO1* to induce T-cell ALL, we crossed the A5(3) line with mice engineered to express *LMO1* from an *lck* promoter (McGuire et al., 1992). *LMO1* was chosen as a candidate since a subset of T-cell ALL patients and cell lines have activated both *scl* and *LMO1* (Wadman et al., 1994b). In a previous report, 50% of the progeny of *lck−/LMO1* line 11 developed T-cell leukemia/lymphoma at ages ranging from 5 to 16 months; the incomplete penetrance and relatively long latency period led to the speculation that additional genetic events were required to produce a frank malignancy (McGuire et al., 1992). We chose to cross the *lck−LMO1* mice with a line expressing the truncated form of *scl* to investigate whether the *scl* transactivation domain would be required for malignant transformation. As seen in Figure 5a, 19/20 (95%) of mice positive for both *scl* and *LMO1* developed an aggressive T-cell leukemia/lymphoma by age 6 months. In contrast, none of the mice positive for *scl* or *LMO1* alone developed T-cell malignancies during the 6 month observation period.

The mice who developed leukemia/lymphoma typically displayed labored breathing, lethargy, peripheral lymphadenopathy, thymic enlargement, splenomegaly and hepatomegaly (Figure 5b). Microscopic exam showed widespread organ infiltration, including liver, kidney, lung and bone marrow; the infiltration of solid tissues such as kidney and liver was typically in a perivascular pattern (Figure 5c). Bone marrow samples were largely replaced by lymphoblasts; complete blood counts generally showed a low red blood cell count, a low platelet count and a normal or increased white blood cell count. The immunophenotypes were typical of immature T-cell leukemia/lymphoma and are presented in Table I. The malignant cells expressed both *scl* and *LMO1* (Figure 6a) mRNA. Western blot analysis using a monoclonal *scl* antibody (Pulford et al., 1995) demonstrated the presence of a 22 kDa *scl* protein in tumor tissue (data not shown); lymphomas from the *lck−LMO1* mice have been shown previously to express *LMO1* by immunohistochemistry (McGuire et al., 1992). The presence of TCRβ gene rearrangements was evaluated on a subset of the tumors and demonstrated clonal patterns of TCRβ rearrangements (Figure 6b). As further evidence of their malignant nature, cells from a subset of these tumors have been cultured *in vitro* for several months without the addition of hematopoietic growth factors.

Since the T-cell leukemia/lymphomas were not evident until 12 weeks at the earliest, it is conceivable that additional mutational events are required for malignant transformation of the *scl/LMO1*+ cells. Given the frequent

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**Table I. Clinical and immunophenotypic characteristics**

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Abbreviations: M, male sex; F, female sex; Thy, thymus; Liv, liver; Spl, spleen; LN, lymph nodes. Age reflects the age in weeks when the animal was sacrificed. Immunophenotype is expressed as percent of malignant cells positive for the indicated antigen.
Dominant-negative action of the scl gene product

Discussion

In this study, we demonstrate a role for scl deregulation in generating aggressive T-cell malignancies. The immunophenotype and clinical manifestations of disease in the scl/LMO1 double positive mice is quite similar to that recognized in human patients with T-cell ALL. The fact that all but one of a cohort of 20 double-positive mice developed T-cell leukemia/lymphoma within a relatively short time span indicates that scl and LMO1 can cooperate quite effectively to induce T-cell malignancies. Moreover, given that both of these genes were originally identified at chromosome translocation breakpoints, and that both of these genes are activated in human T-cell leukemias, this would seem to be a valid, useful murine model of the human disease.

In order to simulate the situation seen in human T-cell ALL, we generated transgenic mice which ectopically expressed either a full-length or an amino-terminal truncated scl protein under the control of sil regulatory elements. We focused our later experiments on the sil exon1–scl exon 5 fusion splice form, since it was one of the more abundant forms, and encoded a truncated scl gene product which lacked the scl transactivation domain. This finding, together with the observation that some T-ALL patients produce a truncated scl transcript encoding a protein which lacks the scl transactivation domain (Bernard et al., 1992; Goldfarb and Greenberg, 1994), suggested that the scl transactivation domain may be dispensable for oncogenesis.

An unexpected finding was the observation that mice transgenic for either the full-length or truncated form of the scl gene product developed bony abnormalities and growth retardation, in what seemed to be a dose-dependent fashion. We initially would not have expected the sil promoter to be functional in non-hematopoietic tissues, based on reports (Aplan et al., 1991) that suggested sil expression was limited to hematopoietic tissues. However, additional studies have demonstrated that sil mRNA is ubiquitously expressed in proliferating cells, and seems to be an immediate early response gene (Collazo-Garcia et al., 1995; Izraeli et al., 1995). Seen in this light, one would expect the sil promoter to be active, and generate ectopic scl production, in all proliferating tissues of the transgenic mouse. The most consistent and obvious phenotypic effect of ectopic scl expression was the incomplete formation of tail vertebrae. Numerous animals from several different founder lines produced from both scl transgenic bone marrow; lane 2, non-transgenic liver; lanes 3–7, five representative tumors (tissue from bone marrow, thymus or lymph node). An arrow indicates a signal from the exogenous scl. Bottom panel: a duplicate blot was hybridized to a LMO1 (TTG1) cDNA probe. (b) Clonal TCRβ gene rearrangements in tumor tissue. Southern blot of SstI-digested tumor tissue hybridized to a human TCRβ Cβ2 probe. Lane 1, tail DNA from non-transgenic mouse used as a germline control, lanes 2–6, DNA from five different tumor samples. Clonal rearranged bands from one or both alleles are seen in all five tumor samples. Size standards are in kb.

homzygous deletions of p16 in human T-cell ALL and lymphoblastic lymphoma (Hirama and Koeffler, 1995), we probed Southern blots of tumor tissue with a PCR-generated p16 probe. No samples showed evidence of a homozygous deletion of p16 (data not shown).
p16, CDK4, D-type cyclins and pRB in T-cell leukemia/lymphoma (Hirama and Koeffler, 1995), these proteins would seem to be reasonable candidates for the proposed additional events. However, a preliminary analysis of the genes coding for p16 and pRB did not reveal any gross abnormality (deletion or rearrangement) at the Southern blot level. A more thorough analysis of these proteins as potential candidates is currently underway.

Several investigators (Green, 1996; Kelliher et al., 1996; Porcher et al., 1996) have speculated that the oncogenic potential of dysregulated scl may be conferred either through inappropriate activation of physiologic scl target genes, or through a dominant-negative mechanism, where misexpressed scl binds to and functionally inactivates those proteins to which it normally binds (Figure 7). Our data suggests that scl is unlikely to exert its oncogenic potential through inappropriate activation of target genes, since mice transgenic for a sil-scl fusion mRNA lacking the scl transactivation domain developed aggressive T-cell leukemia/lymphomas. This finding supports the notion that scl is likely to be oncogenic through a dominant-negative mechanism.

If one supports the concept that scl is oncogenic through a dominant-negative mechanism, the next obvious question focuses on binding partners for scl. The bHLH 'E-proteins' E2-2, E2A and HEB (Hsu et al., 1991; Voronova and Lee, 1994; Goldfarb et al., 1996), the LIM domain proteins LMO1 and LMO2 (Wadman et al., 1994) and the putative tumor suppressor DRG (Mahajan et al., 1996) have all been shown to bind to scl protein. DRG, a GTP-binding protein originally isolated through a subtractive hybridization approach designed to identify potential tumor suppressor genes (Schenker et al., 1994), has been recovered recently as an scl-binding protein in a yeast two-hybrid screen for scl-binding proteins (Mahajan et al., 1996). The LIM domain proteins LMO1 and LMO2 have been shown to bind scl using both co-immunoprecipitation and two-hybrid assays (Wadman et al., 1994), and mice transgenic for both scl and either LMO1 (this report) or LMO2 (Larson et al., 1996) develop aggressive T-cell leukemia/lymphomas. However, although scl and LMO1 clearly cooperate in the genesis of T-cell malignancies, it remains possible that this cooperativity does not need to take place through a direct interaction, but that scl and LMO1 may instead be acting through different pathways.

Scl binding with E-proteins through HLH domain interactions has been demonstrated by several laboratories; a recent report (Zhuang et al., 1996) has studied mice lacking either E2-2, E2A or HEB (Hu et al., 1992). Mice lacking E2-2 appeared to have normal T-cell development, while mice lacking HEB demonstrated abnormal fetal and neonatal T-cell development, primarily manifested by a lack of CD4 and CD5 expression in thymocytes. Interestingly, whereas CD4 and CD8 expression on thymocytes from 5-week-old mice positive for either an scl or LMO1 transgene is similar to that seen with negative control animals, thymocytes from scl/LMO1 double-positive mice also show decreased numbers of CD4-positive cells (D.S.Chernevsky and P.D.Aplan, unpublished data). It is not known whether mice lacking HEB are prone to developing T-cell malignancies as they generally die of undefined causes before weaning (Zhuang et al., 1996). In addition, a region within the scl carboxy-terminus that appears to repress the transactivation domain of the E-proteins (Hofmann and Cole, 1996) has been identified, making the E-proteins likely candidates for scl sequestration and functional inactivation in a dominant-negative model.

In summary, we have demonstrated that scl and LMO1 can cooperate to generate aggressive T-cell malignancies in a mouse model of human T-cell ALL. The oncogenic effect of scl can be produced by an amino-terminal truncated form of the scl protein which lacks the scl transactivation domain and is commonly found in T-cell ALL, demonstrating that the scl transactivation domain is dispensable for its oncogenic action. Furthermore, mice transgenic for either the full-length or truncated scl display developmental abnormalities in additional mesoderm-derived tissues. Although other models cannot be excluded, the most likely model that accounts for both malignancies and bony abnormalities using constructs which lack the scl transactivation domain is one which asserts that scl acts in a dominant-negative fashion.
Materials and methods

**Plasmid construction**

The pSil/SCL plasmid was generated as follows. A 2.4 kb SrfI–SrfI human sil genomic fragment encompassing the sil transcript initiation site was isolated from a genomic sil clone (Aplan et al., 1990). The 5′ SrfI site is derived from the pBS II (Stratagene) polylinker, the 3′ SrfI site is located within sil exon 1. This SrfI fragment was then cloned into the SrfI site of pBSII, and a HindIII–HindIII fragment from the pMSCW–WT vector (Aplan et al., 1992) containing (i) a srl cDNA encoding the full-length human sil protein; (ii) a human β-globin fragment encompassing 18 bp of β-globin exon 2 and all of exon 3 (including the polyadenylation signal); and (iii) a neomycin resistance cassette, was cloned into the HindIII site of pBSII, immediately downstream of the sil promoter fragment. The vector was sequenced to verify its orientation. The pBSI/TSCl was constructed in three steps. First, RT–PCR was used to amplify a sil exon 1–scl exon5 fusion from a cell line (CEM) known to contain a sil–scl fusion (Aplan et al., 1990b), using oligonucleotides A and B (see below). This PCR product was cloned into the PCRII (Invitrogen) vector and sequenced to verify that no PCR artefacts had been introduced. Next, a 0.3 kb insert from this plasmid containing sil exon 1, scl exon 5 and a portion of scl exon 6 was excised with SrfI [the 5′ SrfI site was derived from the PCRII polylinker, the 3′ SrfI site is within scl exon 6 (nucleotide 354 of DDBJ/EMBL/GenBank accession No. M29038)] and ligated in-frame to a 0.2 kb neomycin resistance cassette, and (iv) the pBSII plasmid backbone. Lastly, the 2.4 kb SrfI–SrfI sil promoter fragment was then cloned into the Sil site (located within sil exon 1) of this plasmid, generating the pBSI/TSCl plasmid. All cloning junctions were sequenced to verify the construct.

**Nucleic acid manipulations**

Genomic DNA was isolated from tumors and tail biopsies using conventional techniques, Southern blots were performed as described previously (Aplan et al., 1990a). Total RNA was isolated using Trizol (BRL). Northern blots were performed as described previously (Collazo-Garcia et al., 1995). Probes used in this study included a 1.2 kb HindIII–XhoI human scl cDNA fragment (678X, Aplan et al., 1992), a 1.0 kb SrfI–Xbal sCL cDNA probe (1.0SX, Begley et al., 1989), a PCR-amplified human LMO1 cDNA fragment (nucleotides 544–957 of DDBJ/EMBL/GenBank accession No. M26682), a PCR-amplified murine p61 cDNA fragment (nucleotides 206–458 of DDBJ/EMBL/GenBank accession No. L76150) and a 0.2 kb human TCR β2 probe (gift of Dr Ian Kirsch). The sil–scl fusion mRNA RT–PCR assay was accomplished using RT DNA polymerase (Perkin Elmer Cetus). Briefly, 0.5 μg of total RNA from CEM or HS62 (T-cell lines known to have undergone a sil–scl recombination) or HL60 (a myeloid cell line used as a negative control) was reverse transcribed using an scl exon 6 antisense oligonucleotide (primer C in Figure 1; 5′-ATGTTGGAATCCAGTTCG-3′) for 15 min at 70°C. A sil exon 1 sense oligonucleotide (primer A in Figure 1; 5′-GCTCTTACCTGGAGACGAAGG-3′) was added followed by 35 PCR cycles of 60 s at 95°C, 60 s at 51°C and 90 s at 72°C, and a terminal extension of 7 min at 72°C. Amplification of the sil–scl exon 1–scl exon 3 fusion mRNA was performed using an identical protocol, except an scl exon 3 antisense oligonucleotide (primer B in Figure 1; 5′-GGCATATT- TAGAGAGACCG-3′) was used in place of primer C. RNase protection assays were carried out using a RPAII kit (Ambion) and the manufacturer’s recommended protocol. The hybridization conditions were: 50°C for 16 h; the probe used was a human scl cDNA fragment encompassing scl exons 1a, 5 and 6 (Aplan et al., 1990a). Nucleotide sequencing was carried out using Sequenase (USB) enzyme and reagents.

**Transfections**

The F4-6 murine erythroleukemia cell line was transfected with the pSil/SCL vector, using Lipofectin (BRL), as described previously (Aplan et al., 1992). Clonal stable transfecants were selected with 800 μg/ml of G418 (BRL). The stable transfecants were expanded over a 2 week period, and 5×10⁶ cells were harvested for mRNA isolation.

**Generation of transgenic lines**

The pSil/SCL and pSil/TSCl inserts were purified away from plasmid sequences by agarose gel electrophoresis and glass beads (Bio101). Transgenic mice were produced by pronuclear microinjection using established methods (Hogan et al., 1986). Constructs were microinjected into zygotes derived from a C57Bl10Ros×C3H/HeRoscross (BCF1). Founders were identified by Southern blot analysis of DNA from tail biopsy and lines established by mating with BCF1 animals. Transgenic mice were maintained on Harlan Teklad Laboratory Rodent Diet. Mice transgenic for an E-pgk-1 construct (van Lohuizen et al., 1989) were obtained from National Institute of Aging. Mice transgenic for a sil–LMO1 construct (line 11 of McGuire et al., 1992) have been described previously.

**Alizarin staining**

Mouse skeletons were stained using a modification of previously described techniques (Kimmel and Trammell, 1981). Briefly, animals were anesthetized, the skeleton disarticulated in 5% ethanol for 24 h, defatted in acetone for 5 days, and stained for 6 h at 37°C using a 70% ethanol/acidic acid/saturated alizarin red/0.14% alcian blue solution. The skeletons were cleared in 1% KOH for 2–5 days and fixed using stepwise incubations with 20, 50 and 80% glycerol in 1% KOH. The skeletons were stored in 100% glycerol.

**Immunophenotype and Western blot analysis**

Cells were immunophenotyped using monoclonal antibody conjugates and standard techniques. Briefly, 10⁶ cells were blocked with 1 μg of rat IgG for 15 min at 4°C followed by the addition of 1 μg of the indicated monoclonal antibody conjugate for 45 min at 4°C. Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and resuspended in 1% buffered formalin until analysis. Data was scored and analyzed using a FACScan analyzer (Beckton-Dickinson). Five thousand events were acquired for each sample. The antibodies used were anti-mouse CD3R-PE, CD4-FITC and CD8-Red613 (GIBCO/Invitrogen). For Western blots, ~10⁶ cultured cells were harvested, washed once in ice-cold PBS, and resuspended in RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing a cocktail of proteinase inhibitors (1 mg/ml Pefabloc SC, 1 mM EDTA, 25 μg/ml leupeptin, 10 μg/ml pepstatin, 2 μg/ml aprotinin). Cells and organelles were disrupted in a hand-held glass Dounce homogenizer, incubated on ice for 30 min and centrifuged at 14 000 r.p.m. for 30 min at 4°C microcentrifuge. The precipitates were discarded and the supernatants aliquoted and stored at −80°C. Protein concentrations were determined by the Bradford method. Cell lysates from tissues were prepared as above except that individual cells were first released and separated from tissues by brief homogenization in ice-cold PBS before they were precipitated and resuspended in RIPA buffer. For Western blots, 100 μg of cell lysate protein was resolved by 15% SDS–PAGE (4% stacking gel) and transferred electrophoretically (Towbin et al., 1979) to a Hybond nitrocellulose membrane (Amersham). Blots were incubated at 4°C overnight in 5% non-fat dry skim milk in TBS (50 mM Tris–HCl, pH 7.6, 150 mM NaCl) containing 0.05% Tween-20 (TBS-T). The blots were rinsed briefly with TBS-T and incubated at room temperature for 1 h with a human scl monoclonal antibody (antibody BTL73, kind gift from Dr Karen Pulford). Immunoreactive proteins were revealed using horseradish peroxidase-labeled anti-mouse IgG and ECL Western blotting detection reagents (Amersham) by exposure to Kodak X-ray film.

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**References**


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