Directing oncogenic fusion genes into stem cells via an *SCL* enhancer

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***TEL-TRKC* is a fusion gene generated by chromosomal translocation and encodes an activated tyrosine kinase. Uniquely, it is found in both solid tumors and leukemia. However, a single exon difference (in *TEL*) in *TEL-TRKC* fusions is associated with the two sets of cancer phenotypes. We expressed the two *TEL-TRKC* variants *in vivo* by using the 3' regulatory element of *SCL* that is selectively active in a subset of mesodermal cell lineages, including endothelial and hematopoietic stem cells and progenitors. The leukemia form of *TEL-TRKC* (– exon 5 of *TEL*) enhanced hematopoietic stem cell renewal and initiated leukemia. In contrast, the *TEL-TRKC* solid tumor variant (+ *TEL* exon 5) elicited an embryonic lethal phenotype with impairment of both angiogenesis and hematopoiesis indicative of an effect at the level of the hemangioblasts. The ability of *TEL-TRKC* to repress expression of *Flk1*, a critical regulator of early endothelial and hematopoietic cells, depended on *TEL* exon 5. These data indicate that related oncogenic fusion proteins similarly expressed in a hierarchy of early stem cells can have selective, cell type-specific developmental impacts.**

TEL-TRKC | acute leukemia | congenital fibrosarcoma | hemangioblast

Chimeric fusion genes generated in multiple combinations are a consistent and specific feature of leukemias and some solid tumors, including sarcomas, and may arise in stem cells (1–3).

The vulnerability of stem cells and the selective association of individual fusion genes with particular subtypes of leukemia or cancer are incompletely understood but may be best explained by selection. A plausible explanation for the former is that the self-renewal competence of stem cells, coupled with their longevity, provides an opportunity for the acquisition of both initiating and necessary secondary mutations; i.e., the mutations arise at any developmental level but have selective potency in stem cells. The selectivity of fusion genes for particular cancers could reflect a restricted origin, as an illegitimate recombinant, in different stem cells but, perhaps more plausibly, by selective transforming impacts on particular stem cells or progeny. Evidence supporting the latter interpretation derives from studies on transgenic fusion genes expressed with common promoters that recapitulate *in vivo* the leukemic lineage phenotype (4) and by nonobese diabetic/severe combined immunodeficient mice transplantation experiments in which different subtypes of acute myeloid leukemia (5) were found to be driven by a common population of early stem cells.

We have sought direct evidence that oncogenic fusion proteins expressed in a hierarchy of early stem cells can have selective, cell-type-specific developmental impacts that depend on their intrinsic molecular properties. To achieve this objective, we adopted a strategy of directing the expression *in vivo* of fusion oncogenes with a regulatory element that is itself selectively active in stem cells. We also elected to conduct these studies with *TEL-TRKC* (*ETV6-NTRK3*) fusions, which, uniquely, are associated with both solid tumors and leukemias. *TEL-TRKC* encodes activated TRKC kinase (6, 7); one variant of *TEL-TRKC*, “CFS,” is a consistent genetic abnormality in congenital fibrosarcoma (CFS) (8) and congenital mesoblastic nephroma (9, 10), tumors of possible embryonic mesodermal, or mesenchymal stem cell origin. Another variant, “L,”

is a rare fusion gene in acute myeloid leukemia (11). The L differs from the CFS fusion gene solely by the lack of *TEL* exon 5.

We made transgenic constructs in which the two cancer variants of *TEL-TRKC* were under the control of the 3' regulatory elements of the *SCL* gene as defined in ref. 12 (Fig. 1). These constructs as transgenes would then be expected to be expressed selectively during development in hemangioblastic bipotential precursors of endothelial cells and blood cells (13–15), in hematopoietic stem cells (12, 16), and in some other/mesodermal progenitors (13).

Materials and Methods

Vectors. Mouse *SCL* promoter (+6E5) and 3' enhancer fragment (3'Enh), hemangioblast/hematopoietic-specific regulatory elements of the mouse *SCL* gene, are described in ref. 12. The two variants of *TEL-TRKC* fusion were cloned from acute myeloid leukemia (11) and congenital fibrosarcoma (8) and were inserted within the +6E5 *SCL* fragment as in Fig. 1A. *TEL-TRKC* coding sequence was inserted into exon 4 within +6E5 fragment, replacing the first ATG codon of *SCL* exon 4 with the first ATG of *TEL-TRKC*. The 3' terminal of +6E5 fragment (within exon 5 of *SCL*) was directly linked to the 3' enhancer fragment (3' UTR of *SCL* exon 6) (Fig. 1). Further details of construction of these transgenic vectors are available upon request. The mouse *Flk1* promoter (17) and enhancer (18), which, in combination, confer endothelial cell-specific expression, were amplified by PCR and cloned into pGL3-Basic vector. For transient transfection of *TEL-TRKC* fusion in the luciferase assay, coding sequences of both types of *TEL-TRKC* fusion gene were subcloned into a pcDNA3 expression vector. All constructs were checked by direct sequencing to exclude mutations introduced by PCR.

Generation of *TEL-TRKC* Transgenic Mice. Transgenic mice were generated by microinjection of fertilized mouse oocytes isolated from superovulated B6/CBAF1 mice. Genomic DNA was prepared from the tails of liveborn mice or placenta of embryos, and genotyping was performed by PCR analysis with the primers specific for *TEL-TRKC* transgenic constructs. Expression of *TEL-TRKC* transgene was confirmed by RT-PCR by using peripheral blood or bone marrow cells.

N-Ethyl-N-Nitrosourea (ENU) Mutagenesis. Four- to 16-week-old L-type *TEL-TRKC* transgenic mice were injected intraperitoneally with a single sublethal dose of 100 mg/kg ENU (Sigma) as described in ref. 19.

Whole-Mount Immunostaining. The embryos were fixed overnight in 4% paraformaldehyde, and immunohistochemical analysis on

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Abbreviations: CFS, congenital fibrosarcoma; dpc, days post coitum; ENU, N-ethyl-N-nitrosourea; AGM, aorta–gonad–mesonephros.

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Fig. 1. Structure of the *TEL-TRKC* transgene. (A) Both L- and CFS-type of *TEL-TRKC* fusion gene were inserted into murine *SCL* exon 4 within +6E5 *SCL* promoter fragment, replacing first ATG codon of *SCL* with ATG of *TEL-TRKC*. The 3' terminal of +6E5 fragment was directly connected to the 3' enhancer fragment of *SCL* (3' Enh). (B) The structure of L- and CFS-type *TEL-TRKC* chimeric protein is indicated. Hatched area of TEL indicates pointed domain, and shaded area of TRKC indicates protein tyrosin kinase domain. (C) Detection of *TEL-TRKC* fusion protein expression in 293T cells by Western blot analysis.

sections or on whole embryos was performed as described in ref. 20 by using anti-PECAM1 (CD31) antibody (Pharmingen).

Flow Cytometry and Cell Sorting. Single-cell suspensions were prepared from fetal liver, bone marrow, or cultured cells and then analyzed by flow cytometry by using antibodies against c-kit, CD34, Flk1, Mac-1, Gr-1, B220, TER119, and anti-CD3 (Pharmingen). The stained cells were analyzed by FACSscan or FACSVantage (Becton Dickinson).

In Vitro Methylcellulose Hematopoietic Colony Assays and Colony Replating Assay. Fetal liver from 12.5 days post coitum (dpc) embryos was dispersed into single-cell suspensions, and the number of viable cells was counted by trypan blue staining and plated in triplicate in Methocult M3231 (StemCell Technologies, Vancouver), supplemented with 1.8 ng/ml IL-3/10 ng/ml stem cell factor/10 ng/ml granulocyte-macrophage colony-stimulating factor/2 units/ml erythropoietin. The numbers of colonies comprising >40 cells were scored after 7 days, and myeloid, erythroid, and mixed colonies were defined based on their morphology. Fetal liver cells were also sorted for CD34⁺ and c-kit⁺ and plated into methylcellulose cultures as described above. Colonies were plated in triplicate, and at least five embryos were analyzed for each group.

Clonogenic growth of the hematopoietic progenitor cells with L-type *TEL-TRKC* transgene was assayed by methylcellulose colony replating assay as described in ref. 21 with some modifications. Bone marrow cells were harvested from femurs of mice, and 1×10^4 sorted CD34⁺/c-kit⁺ cells were plated in triplicate in Methocult M3231, supplemented with 10 ng/ml murine IL-3, IL-6, granulocyte-macrophage colony-stimulating factor, and 20 ng/ml stem cell factor. After 7 days of culture, colonies were counted and 1×10^4 recovered cells were replated into methylcellulose with the same growth factors.

In Vitro Culture of Aorta-Gonad-Mesonephros (AGM) Cells. The method described for *in vitro* AGM culture in ref. 22 was used to expand AGM cells. Cells were obtained from 12.5 dpc transgenic or wild-type (WT) embryos and cultured in DMEM supplemented with 15% FCS in the presence of 100 ng/ml stem cell factor/1 ng/ml basic fibroblast growth factor/10 ng/ml murine oncostatin M on

gelatin-coated plates and incubated at 37°C, 5% CO₂ in air. After 7 days of culture, cells were trypsinized and subjected to FACS analysis for Flk1. At least five embryos from each group were analyzed.

In Vitro Angiogenesis Assay of AGM. *In vitro* culture of AGM explant was performed as described in refs. 23 and 24 with some modifications. The stromal cell line OP9 (25) was maintained in α -MEM (GIBCO/BRL) supplemented with 20% FCS. Explants of AGM of 12.5 dpc embryos with and without transgene were cultured on OP9 stromal cells in a slide chamber in 10% FCS and 10^{-5} M 2-mercaptoethanol (Sigma) containing RPMI medium 1640 (GIBCO/BRL) and supplemented with 20 ng/ml IL-6/20 ng/ml IL-7/50 ng/ml stem cell factor/2 units/ml Epo at 37°C in a humidified 5% CO₂ air. After 12 days of culture, cells were fixed and stained with anti-CD31 antibody.

Fragments of yolk sac from 12.5 dpc embryos were plated in Matrigel basement membrane matrix (Becton Dickinson) according to the manufacturer's instructions. Tube formation was analyzed after 12 days. More than three embryos were analyzed for each genotype. ECV304 stably expressing L- or CFS-type *TEL-TRKC* were also plated in Matrigel to assess the effect of the fusion gene for angiogenesis.

Reporter Gene Assay (Luciferase Assay). Transient transfection of human umbilical vein endothelial cells was performed with Lipofectamine 2000 (Invitrogen). The pcDNA3 expression vector with each type of *TEL-TRKC* (0.3 μ g) was cotransfected with a pGL3 luciferase reporter construct (0.1 μ g), which contained the luciferase gene driven by murine *Flk1* promoter/enhancer. Cytomegalovirus immediate early *Renilla* plasmids were included as internal controls for transfection efficiency. At 36 h after transfection, firefly and *Renilla* luciferase activities were analyzed by using dual luciferase assay kit (Promega) according to the manufacturer's instructions. Firefly luciferase activities were normalized based on *Renilla* firefly levels. Each cotransfection experiment was performed at least three times.

Western Blot Analysis. Western blot analysis was performed as described in ref. 11. Antibodies used were anti-TRK (Santa Cruz Biotechnology) and anti-actin antibodies (Santa Cruz Biotechnology).

RT-PCR. Total RNA was prepared from cultured cells by using the RNeasy kit (Qiagen, Crawley, U.K.) with DNase treatment and reverse transcribed with random hexamer by using Moloney murine leukemia virus reverse transcriptase (Stratagene). Reverse transcription products were amplified by PCR with specific primers by using standard procedures. The details of primer sequences and PCR condition are available on request. The products were electrophoresed on 2% agarose gels and stained by ethidium bromide.

Statistical Analysis. A two-tailed Student *t* test was used to determine the difference between control (WT or mock) and CFS or L.

Results

Structure and Activity of the *TEL-TRKC* Transgene. The L and CFS full-length *TEL-TRKC* fusion genes were inserted into mouse *SCL* exon 4 within +6E5, replacing the first ATG codon of *SCL* with the ATG of *TEL-TRKC*. The 5' +6E5 fragment was connected to the 3' enhancer (3' Enh) of *SCL* (Fig. 1A). The resultant two variant *TEL-TRKC* constructs were as shown schematically in Fig. 1B. Expression of the two alternative constructs in 293T cells resulted in similar levels of *TEL-TRKC* protein of the anticipated sizes (Fig. 1C).

Table 1. CFS transgenic embryo die of hemorrhage at 12.5 dpc

Stage	No. of alive embryos (litters)	Transgenic offspring	Hemorrhage
	L-type transgenic		
12.5 dpc	137 (30)	23	0
Postnatal	27 (5)	4	—
	CFS-type transgenic		
11.5 dpc	20 (5)	8	0
12.5 dpc	99 (26)	22*	12
14.5 dpc	30 (7)	0	0
Postnatal	29 (7)	0	—

*Abnormal structure of yolk sac vessels was observed in three and retardation was observed in two embryos that did not show apparent bleeding.

Distinct Phenotypes of L- and CFS-Type *TEL-TRKC* Transgenic Mice.

CFS-type *TEL-TRKC* was embryonically lethal when present, and no offspring were born that were positive for the CFS transgene (Table 1). Viable offspring were all negative for the transgene. Detailed analysis showed that no CFS transgenic embryos survived beyond stage of 14.5 dpc. The embryos that did survive to this stage were transgene-negative (Table 1). Examination of earlier transgenic embryos at 12.5 dpc revealed extensive vascular leakage on the head and back along the intersomite vessels (Fig. 2B). This phenotype was observed in 12 CFS embryos (Table 1). Earlier embryos (11.5 dpc) with the CFS transgene appeared normal (Table 1). In contrast, 11.5–12.5 dpc L-type transgenic mice appeared identical to WT embryos in size and developmental stage and showed no bleeding phenotype (Table 1).

Disorganization of Vascular System in CFS-Type *TEL-TRKC* Transgenic Embryo and Its Yolk Sac.

12.5-dpc embryos transgenic for CFS *TEL-TRKC* indicated considerable architectural disruption in peripheral vessels. The appearance of yolk sacs of 12.5-dpc CFS embryos was quite distinct from WT and L-type. These yolk sacs were pale and “avascular” in appearance, and vitelline vessels could not be observed clearly (Fig. 2 *C* and *D*). When stained with CD31, the vasculature of the yolk sac of a 12.5-dpc CFS embryo showed few large vitelline vessels, and the regular branching from large to thin vessels forming fine honeycomb-like structures observed in WT yolk sacs was not present (Fig. 2 *E* and *F*).

Whole-mount staining with an anti-endothelial CD31 antibody showed disturbances of the vascular network structure in the body of CFS transgenic embryos as well as in the yolk sac (Fig. 2*H* and *J*). The vascular network of the tail of the 12.5-dpc WT embryo showed a fine regular network of vessels consisting of many thin vessels connecting to each other (Fig. 2*G*). In CFS embryos, at the same developmental stage, the vascular network was very irregular, loose, and randomly formed (Fig. 2*H*). The vascular network on the back of the embryo body also showed significant differences between CFS and WT. In WT embryos, the diameter of surface vessels were similar and regular in shape forming a uniform vasculature (Fig. 2*I*), whereas vessels in CFS transgenics were irregular with some narrow in diameter and some dilated (Fig. 2*J*).

In contrast to CFS embryos, all L-type transgenic embryos examined showed no abnormality in vascular networks.

Defect of Endothelial Progenitor Function in AGM and Yolk Sacs of CFS-Type *TEL-TRKC* Transgenic Embryos. Disruption of the vascular

architecture in CFS embryos indicated negative effects of the CFS-type transgene on vascular network formation (or angiogenesis) and, possibly, on differentiation/proliferation of endothelial progenitors (angioblasts). To clarify these possibilities, *in vitro* angiogenesis assays were performed by using the yolk sac and AGM region as sources of angioblasts. Yolk sac cells from WT embryos displayed vessel-like structures after 12 days of culture on Matrigel

Fig. 2. Disorganization of the vascular system in CFS-type *TEL-TRKC* transgenic embryos. Phenotypes of 12.5-dpc WT (A, C, E, G, and I) and CFS (B, D, F, H, and J) embryos are shown. The CFS mutant embryo is distinguished by the presence of hemorrhage in the intervertebral space and in peripheral areas (B). Vitelline vessels are thin and irregular in the CFS yolk sac (D). Whole-mount anti-PECAM1 (CD31) antibody staining shows irregular shape, less branching of the vitelline vessels (arrowhead), and lack of fine honeycomb-like vascular structure in the CFS yolk sac (F). Whole-mount anti-CD31 antibody staining shows abnormal, irregular vessel distribution in the tail (H) and body (J) of CFS embryos.

basement membrane matrix, whereas the yolk sacs from CFS embryos failed to form similar structures (Fig. 3*A* and *B*). Vascular networks composed of CD31-positive cells developed successfully when AGM from WT embryos was cultured on OP9 cells, whereas only vascular beds without proper sprouting were generated from the AGM from CFS embryos (Fig. 3*C* and *D*). A similar result was reproduced with the endothelial cell line ECV304, known to form vessel-like structures in Matrigel, which failed to form such structures when CFS *TEL-TRKC* was expressed (data not shown). These results suggest that there is a major defect in endothelial progenitor function to form vascular networks in CFS-type *TEL-TRKC* embryos.

CFS-Type *TEL-TRKC* Transgene Represses Flk1 Expression. One possible interpretation of the transgenic CFS *TEL-TRKC* phenotype is that it reflects failure of endothelial progeny of hemangioblastic stem cells to differentiate appropriately in response to developmental cues. To assess this possibility, we isolated AGM cells from 12.5 dpc transgenic or control embryos and analyzed the expression of Flk1, a major extracellular regulator of endothelial cell development (26). Because it is difficult to assess the number of Flk1-positive cells directly from each embryo, AGM cells were put

Fig. 3. Defective angiogenesis in the yolk sac and AGM of CFS-type *TEL-TRKC* transgenic embryos. Fragments of yolk sacs from 12.5-dpc WT (A) and CFS embryos (B) were plated in Matrigel basement membrane matrix. Yolk sacs from CFS embryos failed to form complex vessel-like structures as observed in yolk sacs from WT littermates after 12 days of culture. AGM from 12.5-dpc WT (C) and CFS embryos (D) were plated on OP9 cells to promote vasculo-angiogenesis and stained with anti-CD31 antibody after 12 days of culture. AGM from CFS embryos failed to form a vascular network. vb, vascular bed; vn, vascular network. (Original magnifications: $\times 50$ – 100 .)

in culture for 7 days as described in ref. 22 in the presence of oncostatin M, a growth factor for hemangioblasts. AGM cells from CFS transgenic embryos showed significantly lower Flk1 positivity compared with AGM cells from WT embryos or L transgenic embryos (Fig. 4A).

We next tested this potential level of developmental impairment more directly by evaluating the ability of CFS and L forms of TEL-TRKC to repress expression of a luciferase gene under the control of the *Flk1* promoter and enhancer in endothelial human umbilical vein endothelial cells. When cotransfected with L-type TEL-TRKC fusion gene, the luciferase activity in human umbilical vein endothelial cells was similar to mock-transfected cells. On the other hand, when CFS-type TEL-TRKC was introduced, there was a >3-fold suppression in luciferase activity (Fig. 4B). These data again indicate that CFS TEL-TRKC may repress *Flk1* expression.

Defects of Hematopoietic Progenitors in the Fetal Liver of CFS-Type TEL-TRKC Transgenic Embryos. *Flk1* expression is a marker of hemangioblasts and vascular progenitors (27) but is also present in a population of early embryonic hematopoietic precursors (28, 29). A

Fig. 4. CFS TEL-TRKC targets Flk1-positive cells. (A) Flk1-positive cells after 7 days of culture of AGM cells were significantly reduced in CFS-type transgenic mice compared with WT littermates. (B *Upper*) Selective down-regulation of *Flk1* promoter/enhancer activity by CFS TEL-TRKC chimeric protein (luciferase assay). The normalized reporter gene activity of the control transfections (Mock) was arbitrarily set at 1. A schematic of luciferase vector used in the experiment is shown in B *Lower*.

Fig. 5. Reduced hematopoietic progenitors in CFS embryos. Fetal liver cells from WT, L, and CFS embryos were analyzed to assess the status of hematopoietic progenitors. Hematopoietic colonies (A) or percentage of c-kit⁺/CD34⁺ cells (B) were significantly lower in fetal liver of CFS embryos compared with that of WT or L-type transgenic embryos. (C) When c-kit⁺/CD34⁺ cells were sorted, the number of colonies formed were similar between WT and L or CFS transgenic embryos, indicating that defects of hematopoiesis reside at the level of expansion of stem/progenitor cells rather than in downstream differentiation pathways. Results are presented as means $\pm$ SD. NS, not significant.

null mutation of *Flk1* can affect both vasculogenesis and hematopoiesis (26, 30). We therefore evaluated the hematopoietic status of CFS embryos, anticipating a defect here also. Fetal liver cells from 12.5-dpc CFS embryos were assayed for hematopoietic colonies. As shown in Fig. 4, 12.5-dpc CFS embryos had a 6-fold deficit of progenitor cells measured by myeloid colony formation and a 3-fold deficit of CD34⁺/c-kit⁺ cells in the fetal liver (Fig. 5 A and B). On the other hand, fetal liver from CFS embryos had the capacity to form similar numbers of colonies compared with fetal liver cells from the WT or L-type transgenic embryos when CD34⁺/c-kit⁺ cells were sorted and same number of cells were plated on methylcellulose (Fig. 5C). Morphological analysis of colony forming cells showed no differentiation arrest of the hematopoietic cells from CFS fetal liver (data not shown). These data suggest that CFS *TEL-TRKC* impedes hematopoiesis at the level of stem/progenitor cell commitment and/or expansion rather than the differentiation competence of progeny cells.

Increased Proliferation and Self-Renewal of Hematopoietic Stem Cells with L-Type *TEL-TRKC* Transgene. In view of the cancer subtype

associations of the *TEL-TRKC* variants, we anticipated that the L form might have an impact on self-renewal capacity of hematopoietic stem cells. CD34⁺/c-kit⁺ sorted cells from offspring transgenic for L *TEL-TRKC* were harvested from bone marrow and assessed for self-renewal potential as described in refs. 21, 31, and 32 (Fig. 6A). In contrast to progenitors from nontransgenic littermates, whose replicative potential was exhausted by two rounds of serial plating in methylcellulose, L transgenic bone marrow stem cells maintained high level clonogenicity indicative of a transformed or preleukemic status (Fig. 6B).

Fig. 7. A speculative model for the possible stages of cell transformation by CFS and L TEL-TRKC fusion proteins. Because some mesodermal stem cells appear to express Flk1 also (44), the model postulates that some tumors with CFS-type TEL-TRKC may arise at this level (i.e., congenital fibrosarcoma and congenital mesoblastic nephroma) because of a block in further differentiation. On the other hand, the L form of TEL-TRKC enables cells to differentiate into hematopoietic stem cells (HSC), where constitutive kinase activity of the fusion protein transforms cells into leukemic cells. Cells expressing SCL are indicated by gray circles, and Flk1 expressing cells are in the gray box. Me, mesoderm; HA, hemangioblast; End, endothelial cell. Two parallel lines represent blocks in differentiation, and circular arrows represent enhanced self-renewal.

pattern can have differential impacts. This selectivity is most readily explained by *TEL-TRKC* variants having their selective impacts at different levels within the developmental hierarchy of stem cells and precursors, i.e., hemangioblasts or hematopoietic stem cells (Fig. 7). The *SCL* enhancer-driven construct used constrains the expression patterns and phenotypes that are possible, and we have no “transformed” readout for CFS *TEL-TRKC*. Nevertheless, we

speculate that the nonleukemic cancers with CFS-type *TEL-TRKC* fusions might originate from a subset of mesodermal stem cells “upstream” of the hemangioblasts (Fig. 7), generating divergent mesenchymal phenotypes that depend on the tissue site of transformation.

The selective impacts of activated TRKC kinase on either endothelial and hematopoietic differentiation versus hematopoietic stem cell renewal can be entirely ascribed to activity residing in exon 5 of the *TEL* fusion partner of *TRKC*, because these two chimeric genes have equivalent TRKC kinase activation (refs. 6 and 7 and unpublished data).

The finding that the CFS variant of TEL-TRKC but not the L form interferes with the expression of Flk1 provides a lead in how this selectivity might arise, as does the known activity of central region of TEL (encoded by exon 5) to recruit transcriptional corepressors (40–43). CFS TEL-TRKC protein mainly localizes in the cytoplasm (data not shown), and, therefore, any transcriptional deregulating activity would presumably be indirect, e.g., by sequestering components in the cytoplasm.

In summary, we have presented a transgenic model for targeting oncogenes into stem cells that may provide a useful tactic to explore other aspects of stem cell regulation and fate. The model provides the evidence that oncogenic fusion proteins expressed in a hierarchy of early stem cells can have selective, cell-type-specific developmental impacts that depend on their intrinsic molecular properties. This result in turn provides a rationale for the striking associations between different chromosome translocations, unique fusion genes, and cancer or leukemia phenotypes.

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