Intersections between Blood Cell Development and Leukemia Genes

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Abstract

Hematopoietic development is regulated in large part by transcription factors that control cell fate decisions and cellular differentiation. Several genes first discovered in the context of chromosomal translocations in leukemia also serve important functions in blood cell development. Gene-targeting experiments related to two of these factors, SCL/tal-1 and translocation-ets-leukemia (TEL), are reviewed here. SCL/tal-1, a T-cell basic helix-loop-helix oncoprotein, is required for the formation of all hematopoietic lineages. In addition, it is essential for angiogenesis in the yolk sac, indicating a dual function in blood and vessel development. TEL, an ets-related factor which is translocated to a variety of other genes in leukemias, is also required for proper angiogenesis in the yolk sac. Additional studies, however, demonstrate that TEL function is necessary for hematopoiesis to be established in the bone marrow microenvironment. These studies emphasize the intrinsic roles of leukemia-associated transcription factors in normal blood cell and vessel development.

Introduction

Hematopoiesis is the process by which mature blood cells are generated from rare stem cells (HSCs) residing in the bone marrow of the adult. Recent findings suggest that the program of blood cell development is dictated in large part by transcriptional regulatory proteins that serve to activate or repress sets of critical target genes (1). Leukemias represent disruptions of normal hematopoiesis and are seen frequently in association with chromosomal translocations that involve loci encoding transcription factors (2). Whether these leukemia-associated genes normally participate in critical aspects of blood cell development or merely interfere with a separate regulatory network is of interest. Although it need not have been the case, recent experience teaches us that leukemia-associated genes are, indeed, typically important in establishing the program of hematopoiesis. This intersection of normal development and leukemia is compatible with models in which selection and maturation of individual hematopoietic lineages are achieved by the combinatorial actions of multiple regulatory factors.

Loci involved in chromosomal rearrangements in leukemias may be activated ectopically due to altered regulatory sequences or expressed as protein chimeras with a variety of other polypeptides (2). In this brief review, an example of each will be discussed with a particular focus on the roles they play in normal development. The SCL/tal-1 locus, which is associated with acute T-cell leukemia, is representative of those genes whose expression is deregulated by the chromosomal event (3). The TEL gene, which is fused to several different partners in different forms of leukemia, provides an example of the second class (4). Both are required in novel and distinct ways for aspects of normal development. In the studies to be reviewed, the use of gene-targeted mice and embryonic stem cells has figured prominently in the analysis. These new approaches to deciphering in vivo requirements have provided valuable insights into the roles of these and other leukemia-associated factors in hematopoiesis and other developmental pathways.

Results

SCL/tal-1: A T-Cell Leukemia Oncoprotein. The SCL/tal-1 gene encodes a basic helix-loop-helix polypeptide that is expressed selectively in hematopoietic lineages and progenitors, vascular cells, and the nervous system (3, 5–7). Rearrangements involving the locus include chromosomal translocations, as well as upstream interstitial deletions that bring lymphoid regulatory sequences in proximity to the SCL/tal-1 gene. Normally, SCL/tal-1 is not expressed in developing lymphoid cells. Its expression pattern within the hematopoietic system approximates that of GATA-1, a zinc-finger transcription factor found in multipotent progenitors and erythroid, megakaryocytic, eosinophilic, and mast cell lineages (1). As a member of the basic helix-loop-helix family, SCL/tal-1 is related to other factors with established roles in developmental fate decisions, including the myogenetic myoD-related proteins (8). Expression of SCL/tal-1 in developing T-cells under the control of the lck promoter leads to lymphomas in mice after a long latency (9, 10). Enhanced oncogenicity is observed in mice expressing both SCL/tal-1 and Rb1/LMO2, another T-cell leukemia oncoprotein of the LIM-only family of nuclear proteins (11). These findings demonstrate that deregulated expression of SCL/tal-1 can initiate leukemogenesis. Whether this results from activation of genetic programs maintained by SCL/tal-1 or through sequestration of heterodimeric partners, such as products of the E2A gene, is uncertain.

Several features of the pattern of expression of SCL/tal-1 and effects of SCL/tal-1 overexpression in cell culture implicate it in blood cell development. Expression is detected in embryonic and extraembryonic mesoderm at embryonic day 7.5 (E7.5), in blood islands of the yolk sac at E8.5, and thereafter in adult hematopoietic tissues. Moreover, enforced expression of SCL/tal-1 cDNA in mouse erythroleukemia cells stimulates erythroid maturation (12). Conversely, expression of a dominant-negative form of the protein impairs erythroid maturation. These findings suggested a potential role in blood cell development and more specifically within the erythroid compartment.

A Requirement for SCL/tal-1 in Development of All Hematopoietic Lineages. To investigate potential roles for SCL/tal-1 in development, the SCL/tal-1 locus was disrupted by gene targeting in mouse ES cells, and embryos lacking SCL/tal-1 (SCL/tal-1(-/-)) were examined (13, 14). Loss of SCL/tal-1 is embryonic lethal due to extreme anemia at E9.5. Close inspection of the yolk sac blood islands, the first site of red cell production during development, and the embryo proper revealed the absence of any hematopoietic cells or precursors. Not only were maturing red blood cells absent, but progenitors for myeloid lineages were undetectable in hematopoietic colony assays of yolk sac tissue. Further studies with in vitro differentiated SCL/tal-1(-/-) ES cells and mouse chimeras revealed that SCL/tal-1 expression is required for the formation of all hematopoietic lineages, including lymphoid lineages where it is normally not expressed (15, 16). These findings are most readily accounted for by...
the SCL/tal-1 locus to the clo mutation. These findings are most consistent with a model in which SCL/tal-1 acts downstream of the as yet unknown gene clo to specify formation of precursors for hematopoietic and vascular cells.

The genetic experiments in mice and zebrafish provide complementary yet different perspectives on the role of SCL/tal-1 in development. Both give compelling support for important functions in blood and vascular development. A requirement for SCL/tal-1 in fate determination of vascular cells is suggested by the zebrafish experiments, whereas those in mice argue for a somewhat later function in angiogenesis. Such differences are often seen in the comparison of loss-of-function versus forced expression experiments. Although the present data do not bear directly on the existence of the hemangioblast, they predict expression of SCL/tal-1 within the hemangioblast. These inferences are consistent with recent evidence establishing expression of SCL/tal-1 in endothelial progenitor cells in the developing chicken (23). In the future, it will be of great interest to identify the target genes regulated by SCL/tal-1 within the hemangioblasts and within hematopoietic and vascular cells and also define potential functions for SCL/tal-1 in angiogenesis in the adult during normal and oncogenic settings. The dual function of SCL/tal-1 in blood and vascular development is summarized in Fig. 1.

**TEL: Roles in Angiogenesis and Bone Marrow Hematopoiesis.** The TEL gene (also known as ETV6) is a member of its family of transcription factors. The gene was first discovered through its fusion in a chromosomal translocation to the platelet-derived growth factor β receptor in a case of chronic myelomonocytic leukemia (4). The involvement of the TEL gene in leukemia is particularly interesting in that different translocations lead to the production of diverse chimeric proteins, each associated with a distinct form of disease (24). Fusion of the oligomerization domain of TEL with kinases, such as c-abl, platelet-derived growth factor β receptor, or JAK2, lead to constitutive activation of signaling pathways. Among the most intriguing fusions is the union of TEL with the runt-related AML-1/CBFα2 gene, as commonly seen in childhood acute pre-B-cell lymphoblastic leukemia (25, 26). Two aspects of this fusion are notable: (a) this chromosomal translocation confers a favorable prognosis (27, 28); and (b) loss of heterozygosity accompanies the TEL/AML1 fusion gene (28–32). In patients the normal TEL allele is consistently absent. This suggests that functions of the normal TEL protein retard or block the development or progression of leukemia.

TEL is normally quite widely expressed in different tissues and cell types. However, it is expressed at a relatively higher level in hematopoietic tissues, such as the developing fetal liver. Knockout of the **Fig. 1. Dual function of SCL/tal-1 in blood and vessel development.** Genes expressed in each progenitor cell are shown in the shaded boxes. X, the stage at which vascular or blood cell development is blocked in the absence of SCL/tal-1.
TEL gene yielded an unexpected phenotype—a failure of yolk sac angiogenesis, as well as death of selected neural and mesenchymal populations in the embryo (33). While establishing that TEL is an essential gene for mouse development, these findings did not point to a specific role in hematopoiesis or explain how TEL loss might contribute to childhood pre-B-cell leukemia leukemia.

Recent experiments in which chimeras were generated with TEL−/− ES cells, however, have provided unanticipated findings with regard to a requirement for TEL function in the hematopoietic system (34). TEL expression appears entirely dispensable for hematopoiesis within the yolk sac blood islands and in the fetal liver. In addition, TEL is not required for fetal lymphopoiesis. Nonetheless, TEL−/− ES are unable to contribute to hematopoiesis within the bone marrow microenvironment. Although the molecular basis of this abnormality is yet to be defined, these findings implicate TEL function in the migration or homing of HSCs or their progenitors to the bone marrow is yet to be defined, these findings implicate TEL function in the migration or homing of HSCs or their progenitors to the bone marrow microenvironment. From one perspective, the observation that TEL loss of function in otherwise normal hematopoietic rearrangements are undoubtedly inciting genetic events in leukemia. How, if at all, the hematopoietic defect seen upon loss of TEL function may relate to loss of heterozygosity and leukemia progression in childhood pre-B-cell leukemia is unknown. Nonetheless, a demonstrated effect of TEL loss on the behavior of HSCs or progenitors in bone marrow hematopoiesis is unlikely to be innocuous in the setting of an expressed TEL/AML1 fusion protein. Chromosomal pathways might be endowed with novel functions. A critical unknown in nearly all settings is the nature of the critical targets that are aberrantly regulated in the context of leukemias. Indeed, for nearly all of the relevant transcription factors, we are ignorant of the genes that they normally control in hematopoietic (or other) lineages.

In addition to genetic studies that link SCL/tal-1 and normal hematopoietic development, biochemical approaches implicate SCL/tal-1 in a regulatory network that includes GATA-1 and Rbnt2/LMO2. SCL/tal-1 interacts physically with Rbnt2/LMO2, a LIM-only leukemia oncoprotein that is also required for hematopoiesis (37, 38). The most parsimonious model posits that these proteins work in concert within a larger complex in a common regulatory pathway. Rbnt2/LMO2 has also been reported to interact with GATA-1 (39), a GATA-family zinc-finger protein essential for both erythroid and megakaryocytic lineages (40–42). Moreover, SCL/tal-1 (in a heterodimer with E2A products), Rbnt2/LMO2, GATA-1, and a Rbnt2/LMO2 interacting protein Ldb1 assemble on a composite GATA-E-box sequence element (43). Although such composite elements have yet to be identified within critical hematopoietic expressed genes, a convergence between genes controlled by GATA-1 and by the SCL/tal-1-Rbnt2/LMO2 axis appears quite likely in vivo. In this manner, deregulated expression of SCL/tal-1 or Rbnt2/LMO2 within developing hematopoiesis within the embryo (33). While establishing that TEL is an essential gene for mouse development, these findings did not point to a specific role in hematopoiesis or explain how TEL loss might contribute to childhood pre-B-cell leukemia.

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topoietic progenitors or early committed lymphoid cells might impinge on transcriptional regulation mediated through GATA factors. The essential roles of GATA-2 in early hematopoietic progenitors (44) and GATA-3 in T-cell development (45) suggest how expression of these leucemic oncoproteins may perturb normal developmental pathways. From these and other, studies models emerge in which lineage selection and maturation from multi-potential progenitor cells are controlled largely through the action and modulation of multicomponent hematopoietic-specific transcriptional protein complexes (1, 46). From this perspective, it becomes apparent how leukemia oncoproteins might disrupt the function of such complexes and prevent cells from completing their normal developmental programs.

References
Discussion

Dr. Phillip Sharp: So, if I understood it correctly, SCL is required both for the angiogenesis of the tissue as well as the red blood cell generation.

Dr. Orkin: Yes.

Dr. Sharp: Differentiation step in both, if I understood that correctly. Is that same relationship common of other cell types such as myo-D in muscle?

Dr. Orkin: Not that I am aware of, not in terms of . . .

Dr. Sharp: Has it been looked for?

Dr. Orkin: I don’t. . . . I don’t know. There are probably other people who could address that more fully, but I am not aware of any. I think what this points to is really the origin of hematopoietic cells from hemangioblasts, a common hematopoietic/vascular progenitor, which I don’t think exists, that I am aware of, in the myogenic system. I think we will hear more about this commonality from Nancy Speck.

Dr. Edison Liu: Dr. Murphy?

Dr. Sharon Murphy: I’m Murphy from Chicago. In the childhood ALL translocation, TEL-AML, could you explain how it is that you think that other allele loses the function on the other . . .

Dr. Orkin: How does the other allele lose function?

Dr. Murphy: Yes.

Dr. Orkin: In most cases, it is a deletion, as far as I am aware. How does that occur? I don’t know.

Dr. Murphy: That is what I wanted to know, why is that so consistent.

Dr. Orkin: Presumably, it is selected for as a second event in leukemogenesis, but . . .

Dr. Murphy: It is consistent? It is in every case?

Dr. Orkin: It is almost every case. More than 90% of the cases involve sickle cell anemia.

Dr. Zhen-Ping Xu: I have a single question. Zheng-ping Xu. Is SCL expressed in the tumor vasculature or other body tissue?

Dr. Orkin: Yes, we haven’t looked at that ourselves, but others have reported expression of SCL within vascular cells and even in vascular beds around tumors. So, we suspect that SCL is actually involved in angiogenesis within the adult as well as a regulator of angiogenesis.

Dr. Liu: In your experiment where you reconstitute partially the animals with the SCL and find that you have capillary repopulation but not large vessel repopulation, are you implying that there are two separate populations of vasculature that goes to different places, or is it a defect in migration?

Dr. Orkin: That is a good point. The vascular cells, we believe, are the same, and what happens during the evolution of the vasculature in the yolk sac is the first step is the generation of the primary capillary bed, and then that bed is remodeled through angiogenesis to generate the larger vessels. So, this is a problem of reorganization. Presumably, it involves cellular migration response to maybe mesenchymal stimuli or growth factors and cell-cell interactions.

In fact, what we see in the chimeras is that you only need a small input of the SCL−/− cells to disrupt the vasculature, suggesting that it is almost dominant-negative at the cell-cell level, suggesting that there are cell-cell interactions.

Dr. Liu: Dr. Adams?

Dr. Jerry Adams: Stu, I wondered if you would comment on how many cases you think you can distinguish between whether a gene is actually causing commitment to a particular lineage versus simply being needed to maintain a particular cell type.

Dr. Orkin: Yes, the question is whether the genes we and others studied are involved in commitment or specification of cell types or required only for their development, I guess, and I think it depends, in a way, on how you do the assays. There are experimental systems in which you can show that genes such as GATA-1, SCL, now PU.1 experiments, as shown by Thomas Graf, suggest that you can program lineages or select lineages by these factors, but knockout experiments, by and large, have not been so demonstrative.

I think it is partly the way you slice the pie and the way you look at it. I think these factors do have influences on lineage that probably are pretty subtle and are hard to discern in the knockout situation.

Dr. Liu: Dr. Dixon?

Dr. Dixon: Your work suggests that SCL-1 is a marker for the hemangioblast and that, potentially, a GFP knockout could be used to isolate the hemangioblast. Would you like to comment on that?

Dr. Orkin: I think it might be a marker for hemangioblasts. There are experiments in the chicken by others which suggest that SCL is expressed in a pre-hemangioblast-like cell, and I think putting markers in and trying to use those markers to either fish out or identify the cells is certainly a good strategy.
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