To the editor:

**SCL/Tal1 and lymphoid versus myeloid lineage assignment**

In their recent paper, Kunisato et al1 describe the role of stem cell leukemia gene (SCL) in regulating lineage fate in hematopoietic stem cells. Their experiments involve retroviral expression of SCL and a “dominant-negative” mutant of SCL (DN-SCL) in hematopoietic stem cells and their progeny. They propose that levels of SCL regulate lineage commitment: enforced expression of SCL favored myeloid differentiation, while expression of the DN-SCL favored lymphoid differentiation. We query the interpretation of the results obtained with the DN-SCL mutant, as its design and effects are not suggestive of a specific dominant-negative function. The authors cite Aplan et al2 and Krosl et al3 for the design of the dominant-negative SCL. In these papers the basic domain of SCL was deleted. This mutant is unable to bind to DNA, however, heterodimerization with E2A proteins remains intact through the presence of the helix-loop-helix (HLH) domain. The DN-SCL mutant used by Kunisato et al1 lacks both the basic and HLH domains. Such a mutant would be predicted to abrogate not only DNA binding, but also the ability to interact with E2A proteins. The remaining N- and C-terminal portions of SCL have no known function—indeed, a truncation mutant comprising only the basic and HLH domains could rescue hematopoiesis of SCL-null embryonic stem cells,4 suggesting that the N- and C-terminal amino acids are not essential. Since a dominant-negative mutant usually relies on deletion of specific functional domains while retaining vital protein interactions, it is difficult to understand how this mutant could act as a dominant negative. Moreover, enforced expression of the DN-SCL only mildly affects erythroid cell production in vitro or in vivo (Figures 3 and 7), whereas loss of SCL by conditional deletion has demonstrated that SCL is essential for erythroid burst-forming units (BFU-E) and production of red cells in vivo.5-7 Thus, there is no available data to positively suggest that the DN-SCL used by Kunisato and colleagues1 inhibits the function of SCL. Nonetheless, it is possible that the N- and C-terminal portions of SCL have an unknown function that causes the observed effects on lineage specification. However, without the correct controls, such as rescue of the DN-SCL effect with wild-type SCL, it is impossible to discriminate specific from nonspecific effects. In light of this and since the effects on myeloid and lymphoid lineage output are subtle and transient, it is important to regard with caution the assertion that the effects are due to a dominant-negative effect on SCL.

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**References**


Response:

**Dominant-negative activity of stem cell leukemia (SCL) lacking bHLH domain**

Queries from Hall and Curtis on our paper1 in *Blood* include some important issues. As they argue, the construct of interest (∆bHLH SCL) may not have an ability to interact with E2A proteins. Indeed, our experiment showed that it does not interact with wild-type (WT) stem cell leukemia (SCL) (data not shown). However, this does not imply that ∆bHLH SCL consisting only of the N- and C-terminal portions of SCL does not have any function. Contrary to the argument by Porcher et al.,1 their results could indicate that the N- and C-terminal portions of SCL have some roles, since it appears that the bHLH domain alone does not completely rescue the SCL-null phenotype. In addition, as was described in our paper (Figure 7), we found maturation arrest in the erythroid progenitors by introducing ∆bHLH SCL. This observation is considered to be biologic evidence of dominant-negative effect of ∆bHLH SCL on wild-type SCL, given the phenotype of SCL conditional knockout mice.1 In this regard, we are afraid that the questioners may misunderstand our description in the paper.

To explore the proteins that interact with ∆bHLH SCL, we have performed a coprecipitation analysis (Figure 1). We transfected HEK293 peak cells with plasmids containing FLAG-tagged WT SCL and ∆bHLH SCL under the cytomegalovirus (CMV) promoter. Two days after the transfection, lysates were prepared and immunoprecipitated with the anti-FLAG antibody-coated beads (Sigma, St Louis, MO). The samples then were resolved through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the gel was silver-stained (Dai-iichi Kagaku, Tokyo, Japan). We found that some proteins coprecipitated commonly with WT SCL and ∆bHLH SCL (solid arrows), and others coprecipitated with WT SCL alone (dotted arrows). It is possible that the commonly precipitated proteins

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**References**

We disagree with the comment by the questioners that we should be able to show the rescue of the effect of ΔbHLH SCL with WT SCL. This is not an appropriate experiment to show the dominant-negative effect of ΔbHLH SCL.

Although the biochemical mechanisms need to be further disclosed, clear are our findings on the distinct biologic functions of WT SCL and ΔbHLH SCL on the commitment fate determination of hematopoietic stem cells. We hope that our ongoing study will give a clear answer to the mechanisms for how ΔbHLH SCL functions in a dominant-negative fashion against WT SCL.

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References

To the editor:

Potential curability of newly diagnosed acute promyelocytic leukemia without use of chemotherapy: the example of liposomal all-trans retinoic acid

Several years ago we reported that liposomal all-trans retinoic acid (L-ATRA) used alone might cure some patients with untreated acute promyelocytic leukemia (APL). The median follow-up was 1.5 years from complete remission (CR) date. Because the risk of relapse does not decrease appreciably until considerably later, we herein update the study. The L-ATRA dose was 90 mg/m² every other day until CR, after which this dose was given 3 times weekly for 9 months. Using a sensitivity dose was 90 mg/m² every other day until CR, after which this dose was given 3 times weekly for 9 months. Using a sensitivity dose of 3.2 years (range, 1.4-5.5 years) years from CR date. The 10 patients who never received treatment other than L-ATRA each had a presenting WBC count less than 10 000/μL, as did 13 of the 14 who required idarubicin. The 2 groups also had similar distributions of initial WBC count, platelet count, and type of PML–RAR (isoform and age). The PCR status at CR was of no discriminatory value since all patients but one were PCR positive at CR, with 24 of 26 becoming negative by 3 months. Of the 10 patients whose first indication of failure was molecular, 9 received idarubicin in (hematologic) CR. Of those, 6 remain in hematologic CR, while 3 had hematologic relapse, which occurred within 1 year of molecular failure.

The immediate significance of our results is limited. L-ATRA is unavailable commercially. The 3 of 8 CR rate in high-risk patients seems extraordinarily low. Furthermore, while possibly sparing two fifths (ie, 10 of 26) of low-risk patients the need for chemotherapy, L-ATRA was not free of toxicity and required 3 intravenous infusions weekly. Nonetheless, the observation that patients can be potentially cured of APL without use of chemotherapy should encourage further attempts in the same direction as, for example, in our current trial using ATRA and arsenic trioxide.
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