[CANCER RESEARCH 54, 2865–2868, June 1, 1994]

Advances in Brief

An RNA-binding Protein Gene, *TLS/FUS*, Is Fused to *ERG* in Human Myeloid Leukemia with t(16;21) Chromosomal Translocation[1]

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Abstract

The t(16;21)(p11;q22) translocation is a recurrent chromosomal abnormality found in several types of myeloid leukemia. We have previously demonstrated that the breakpoints of this translocation are clustered in a specific intron of the *ERG* gene on chromosome 21, which has recently been reported to be involved in Ewing's sarcoma. We show here that the *TLS/FUS* gene on chromosome 16 is fused with the *ERG* gene to produce the *TLS/FUS-ERG* chimeric transcript by this translocation. The *TLS/FUS* gene has been identified as a translocated gene in myxoid liposarcoma by the t(12;16)(q13;p11) translocation and encodes an RNA-binding protein that is highly homologous to the product of the *EWS* gene involved in Ewing's sarcoma. Thus, the *TLS/FUS-ERG* gene fusion in t(16;21) leukemia is predicted to produce a protein that is very similar to the EWS-ERG chimeric protein responsible for Ewing's sarcoma.

Introduction

Chromosomal translocations have been recognized as important events in the pathogenesis of hematopoietic malignancies. By molecular analysis of these translocations, many protooncogenes involved in neoplastic processes have been identified (1, 2). The t(16;21)(p11;q22) translocation is a recurrent chromosomal abnormality found in human AML[3] (3–5), blastic crisis of chronic myeloid leukemia (6), and myelodysplastic syndrome that has evolved to AML.[4] We have previously mapped the breakpoint on the *Not*I restriction map of chromosome 21 and shown that the *ERG* gene, a member of the *ets* protooncogene family on chromosome 21, is rearranged by this translocation (7).

Recently, some specific chromosomal translocations in solid tumors have also been analyzed and characteristic gene fusions have been reported. In Ewing's sarcoma and related peripheral neuroectodermal tumors, the *EWS* gene encoding an RNA-binding protein is fused to an *ets* family gene *FLI1* or *ERG* by t(11;22) or t(21;22) translocation, respectively (8–10). *EWS* has also been shown to fuse with the *ATF1* gene encoding a transcription factor in malignant melanoma of soft parts with t(12;22) translocation (11). In myxoid liposarcoma, the *TLS/FUS* gene encoding an RNA-binding protein similar to EWS is fused to the *CHOP* gene encoding a transcription regulator of the C/EBP family by t(12;16) translocation (12, 13). In these solid tumors, the RNA-binding domain of EWS or TLS/FUS is replaced with the DNA-binding domain of transcription factors by the respective translocation.

In this paper, we report that the t(16;21) translocation in myeloid leukemia fuses *TLS/FUS* to *ERG* and causes the replacement of the RNA-binding domain of TLS/FUS with the DNA-binding domain of ERG.

Materials and Methods

Cells and Cell Lines. Peripheral blood or bone marrow cells were obtained from four myeloid leukemia patients with t(16;21)(p11;q22) either at diagnosis (patients 4 and 6) or in relapse (patients 5 and 7) and from two healthy volunteers. Patients 4 and 5 have been described previously (7). Patients 6 and 7 were diagnosed as having AML, M2 and M5 subtypes of the French-American-British classification, respectively. A t(16;21) leukemia cell line, UTP-L12, was established by culturing bone marrow cells obtained from patient 4 in relapse in a granulocyte-macrophage colony-stimulating factor-containing medium. A Burkitt's lymphoma cell line, Namalwa, was also used as a control.

Southern Blot Analysis. Genomic DNA prepared from peripheral blood or bone marrow cells was digested with the restriction enzyme *Bam*HI, separated by electrophoresis, and transferred to a nylon membrane (Hybond-N; Amersham) using standard methods. Hybridization and autoradiography were performed as described previously (14).

RT-PCR and Primers. Total RNA of cultured cells and peripheral blood or bone marrow cells was isolated by the acid guanidinium thiocyanate/phenol/chloroform method (15). From 1 μg of this RNA, cDNA was synthesized in 20 μl of the reaction mixture using a SuperScript preamplification system (GIBCO-BRL). Using 0.4 μl of the cDNA product, PCR amplification was carried out in 20 μl of the reaction mixture by 35 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, as described previously (7). The amplified products were analyzed by electrophoresis in 4% NuSieve 3:1 agarose (FMC BioProducts) gel. For use as a hybridization probe, the PCR product was electrophoresed in 2% SeaPlaque GTG agarose (FMC BioProducts) gel and extracted from the agarose gel. PCR primers for the *TLS/FUS* and *ERG* genes (Fig. 1) were designed according to the known sequences (12, 13, 16) as follows: T1, GGTGGCTATGAACCCAGAGG; T2, CATTCTCACCCAGGCCTTGC; T3, CTCTCCCTTCAGCTTGCCAG; E1, GGAGATCAGCCTGGACCGGT; and E2, CCTCGTCGGGATCCGTCATC.

Sequencing of RT-PCR Products. RT-PCR products were cloned into a plasmid vector using a TA cloning kit (Invitrogen). Plasmid DNA prepared by a standard method was used for sequencing with an AutoRead sequencing kit (Pharmacia) and an ALF DNA sequencer (Pharmacia).

Results

Rearrangement of the *TLS/FUS* Gene on Chromosome 16 in t(16;21) Leukemia. The breakpoint on chromosome 16 of the t(16;21) translocation in myeloid leukemia is cytogenetically mapped at the same band q11.2 as the breakpoint of the t(12;16) translocation in myxoid liposarcoma. Thus, the *TLS/FUS* gene located at the breakpoint in myxoid liposarcoma is a candidate for the translocated gene in t(16;21) leukemia. Therefore, we prepared several cDNA probes

Received 3/10/94; accepted 4/21/94.

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[1] This work was supported in part by a Grant-in-Aid for Comprehensive 10-year Strategy for Cancer Control, a Research Grant on Aging and Health from the Ministry of Health and Welfare; Grants-in-Aid for Creative Basic Research (Human Genome Program), Cancer Research, and Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture; and a grant from the Special Coordination Funds for Promotion of Science and Technology from the Science and Technology Agency of Japan.

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[3] The abbreviations used are: AML, acute myeloid leukemia; RT-PCR, reverse transcription-polymerase chain reaction; cDNA, complementary DNA.

[4] Y. Hayashi, unpublished observations.

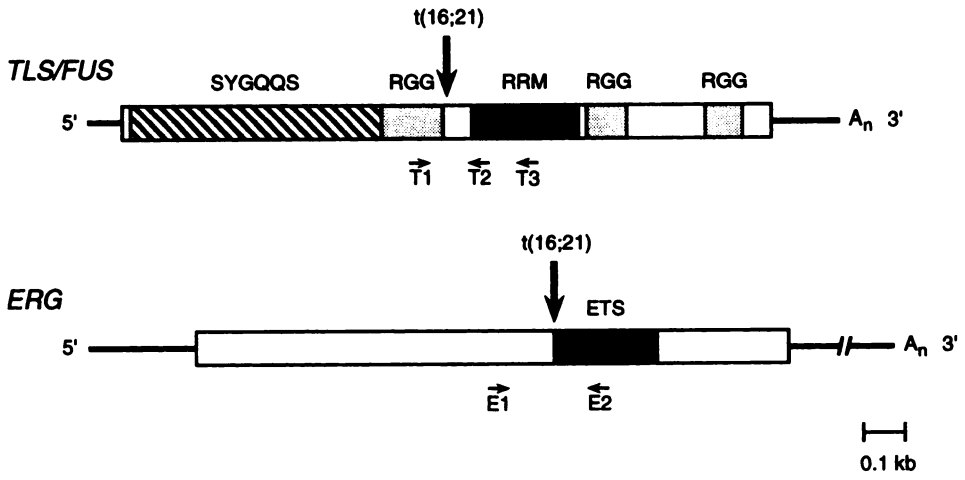

Fig. 1. Structure of the *TLS/FUS* and *ERG* transcripts previously reported (12, 13, 16). □, coding regions. Regions which correspond to the structural or functional domains of their gene products are also shown: *SYGQQS*, degenerated S–Y–G–Q–Q–S repeats; *RGG*, R–G–G repeat-containing sequence; *RRM*, RNA recognition motif homologous sequence; *ETS*, the ETS DNA-binding domain. *Vertical arrows*, breakpoints by the t(16;21) translocation; *small horizontal arrows* below the *transcripts*, primers used for RT-PCR; *kb*, kilobase.

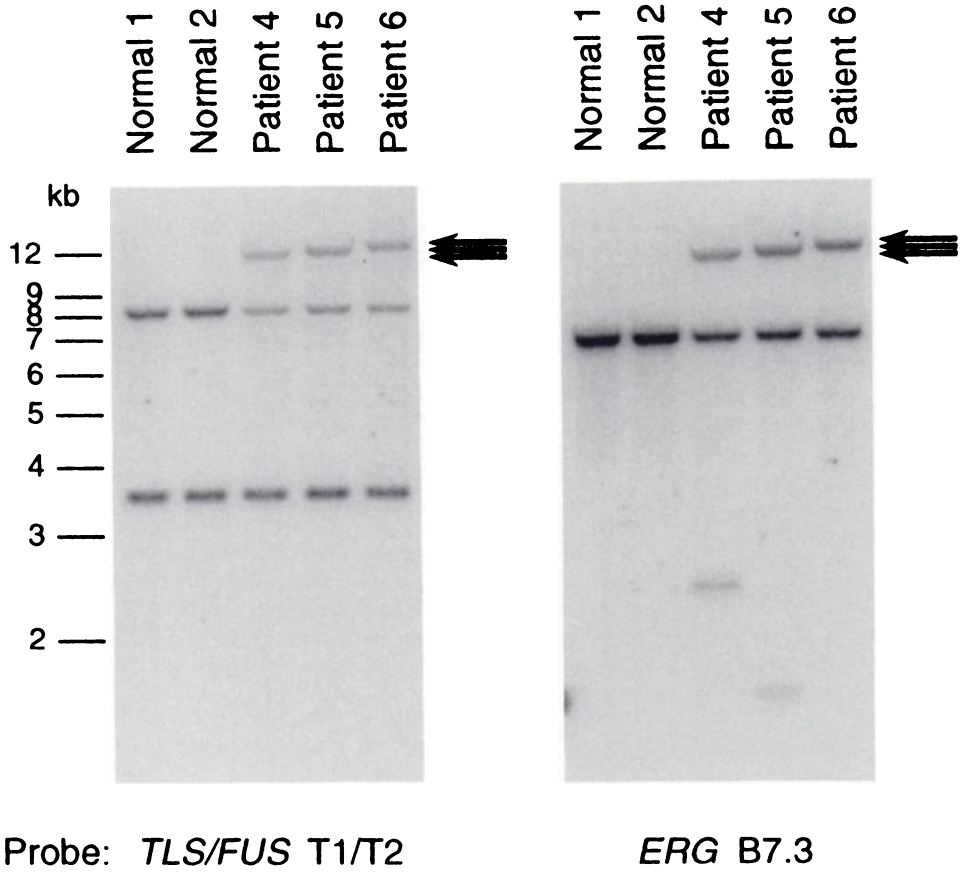

Fig. 2. Southern blot analysis of genomic DNA from t(16;21) patients with the *TLS/FUS* and *ERG* probes. *Bam*HI-digested genomic DNA from peripheral blood or bone marrow cells of two healthy volunteers (Normal 1 and 2) and three t(16;21) leukemia patients (Patients 4–6) was electrophoresed, transferred to a nylon membrane, and hybridized successively with a *TLS/FUS* RT-PCR product probe, T1/T2, and an *ERG* genomic probe, B7.3. Common rearranged bands were detected by the *TLS/FUS* and *ERG* probes, as indicated by *arrows*. *kb*, kilobases.

for *TLS/FUS* by RT-PCR and performed Southern blot analysis of *Bam*HI-digested genomic DNA from three t(16;21) patients (patients 4–6). A rearranged band was detected in addition to two germ line bands in each of the three patients (Fig. 2) when one of these probes (T1/T2) was used, which was amplified with primers T1 and T2 (see Fig. 1). The same rearranged band was also detected with the *ERG* B7.3 probe (Fig. 2), which we had previously used to identify the rearrangement of *ERG* (7). These results indicated that *TLS/FUS* is rearranged and juxtaposed to *ERG* in t(16;21) leukemia and suggested that a fusion gene(s) is formed between *TLS/FUS* and *ERG*.

Fusion between the *TLS/FUS* and *ERG* Genes. RT-PCR analysis of RNA from three t(16;21) patients (patients 4, 6, and 7) showed that both types of fusion genes, *5′-TLS/FUS-ERG-3′* and *5′-ERG-TLS/FUS-3′*, were formed and expressed in all of the patients. When primers T1 and E2 (see Fig. 1) were used to detect the *TLS/FUS-ERG* chimeric transcript, three PCR products of different sizes were am-

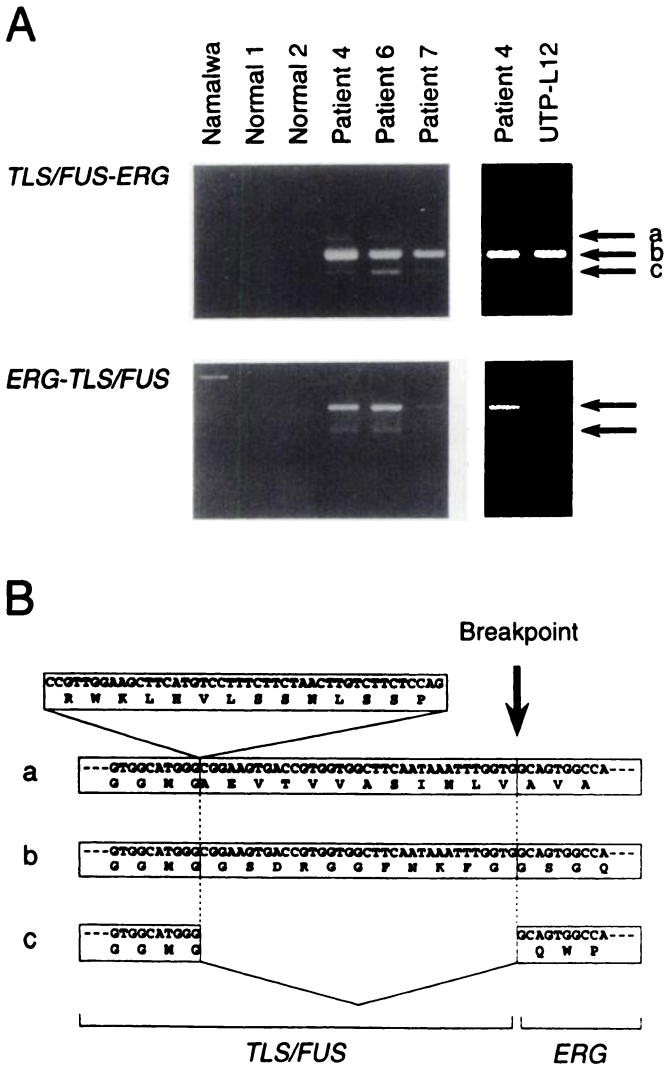

Fig. 3. RT-PCR analysis of the *TLS/FUS-ERG* and *ERG-TLS/FUS* chimeric transcripts in t(16;21) patients. (*A*) Detection of RT-PCR products by electrophoresis. The *TLS/FUS-ERG* and *ERG-TLS/FUS* chimeric transcripts were amplified with primers T1 and E2 and primers E1 and T3 (see Fig. 1), respectively, from RNA of Namalwa, peripheral blood, or bone marrow cells from two healthy volunteers (Normal 1 and 2) and three t(16;21) leukemia patients (Patients 4, 6, and 7), and also from the t(16;21) leukemia cell line UTP-L12. Three and two RT-PCR products of different sizes were generated with primers T1 and E2 (*TLS/FUS-ERG*) and primers E1 and T3 (*ERG-TLS/FUS*), respectively (*arrows*). (*B*) Sequence of the junctions of the *TLS/FUS-ERG* chimeric transcripts. Three RT-PCR products (*a*, *b*, and *c*) amplified with primers T1 and E2 were cloned and sequenced. Nucleotide sequences and predicted amino acid sequences around the junctions are shown. Among the three products, only *Product b* has an in-frame junction.

plified from each of the three patient samples, whereas no detectable products were generated from Namalwa or normal controls (Fig. 3*A*). However, when primers E1 and T3 for the *ERG-TLS/FUS* chimeric transcript were used, two PCR products specific to t(16;21) patients were also amplified, although their amounts varied among the patients (Fig. 3*A*). This variation is likely to reflect the activity of their *ERG* promoters, since the corresponding amounts of RT-PCR products for normal *ERG* transcripts were amplified (data not shown).

We established a t(16;21) leukemia cell line, UTP-L12, from the bone marrow cells of patient 4 in relapse. RT-PCR analysis revealed that the *ERG-TLS/FUS* chimeric transcripts are not produced in this cell line (Fig. 3*A*). In addition, cytogenetic analysis also showed that UTP-L12 has lost the der(16) chromosome carrying the *ERG-TLS/FUS* fusion gene (data not shown). These results suggest that the *ERG-TLS/FUS* fusion gene may not be involved in the pathogenesis of t(16;21) leukemia.

Sequencing of the Junctions of the *TLS/FUS-ERG* Fusion Transcripts. In RT-PCR analysis of the *TLS/FUS-ERG* fusion gene, three PCR products of different sizes were generated. Sequence analysis of these products revealed that they were composed of three types of *TLS/FUS* sequences and the same *ERG* sequence. In the major product of 211 base pairs (*Product b* in Fig. 3*A*), a 91-base pair *TLS/FUS* sequence is fused to a 120-base pair *ERG* sequence. The *TLS/FUS* junction point is identical to that previously identified in the *TLS/FUS-CHOP* chimeric transcript of myxoid liposarcoma (12, 13). The transcript corresponding to this major PCR product is expected to produce the TLS/FUS-ERG chimeric protein since it has an in-frame junction (Fig. 3*B*). The other two minor products (*Products a* and *c*, Fig. 3*A*) either contained a previously unknown 44-base pair sequence within the *TLS/FUS* sequence or lacked the 35-base pair *TLS/FUS* sequence and fused to *ERG* with out-of-frame junctions (Fig. 3*B*). These minor transcripts may be produced by unusual splicing activated by the t(16;21) translocation since such splicing products have not been reported in normal *TLS/FUS*.

Discussion

The results of this study show that the *TLS/FUS* gene on chromosome 16 is fused with the *ERG* gene on chromosome 21 to form two types of chimeric genes, *ERG-TLS/FUS* on der(16) chromosome and *TLS/FUS-ERG* on der(21) chromosome, by the t(16;21) translocation in myeloid leukemia. Both types of fusion genes are expressed in leukemic cells obtained from t(16;21) patients. However, we found that the der(16) chromosome was lost and *ERG-TLS/FUS* was not expressed in a t(16;21) leukemia cell line, UTP-L12, established from a patient in relapse. This finding strongly suggests that *TLS/FUS-ERG* is responsible for the pathogenesis of t(16;21) leukemia, although the possibility remains that the *ERG-TLS/FUS* fusion gene may function in the process of leukemic transformation but may not affect the proliferating ability of the leukemic cells.

TLS/FUS was first identified as a translocated gene in myxoid liposarcoma (12, 13) and encodes an RNA-binding protein which has extensive amino acid sequence homology (56% identical) to the *EWS* gene involved in Ewing's sarcoma and related tumors (12). The NH_2 terminals of these two gene products consist of multiple repeats of a degenerated polypeptide motif with the consensus S–Y–G–Q–Q–S. These NH_2-terminal regions have been suggested to contain a potent transcription activation domain (17). The COOH terminals contain two types of sequences implicated in RNA binding: R–G–G tripeptide repeats (18) and a sequence of about 90 amino acids homologous to the RNA recognition motif RRM (19). In myxoid liposarcoma, most of this COOH-terminal region of TLS/FUS is replaced by the CHOP protein, a member of C/EBP family (12, 13). In Ewing's sarcoma, most of the COOH-terminal region of EWS is replaced by the ETS DNA-binding domain of FLI1 or ERG (8–10) (see Fig. 4). The present results indicate that a similar replacement also occurs in leukemia with the t(16;21) translocation. In the expected product of the *TLS/FUS-ERG* fusion gene, the NH_2-terminal sequence of TLS/FUS is fused to the ETS domain of ERG. Thus, the resulting TLS/FUS-ERG chimeric protein in t(16;21) leukemia has a similar putative transcription activation domain and the same DNA-binding domain as the EWS-ERG chimeric protein in Ewing's sarcoma (see Fig. 4). These similar proteins may activate the same genes in the neoplastic processes of these two different types of tumors. Alternatively, it is also possible that these proteins activate different genes specific to their tumor types by interaction with other tissue-specific factors.

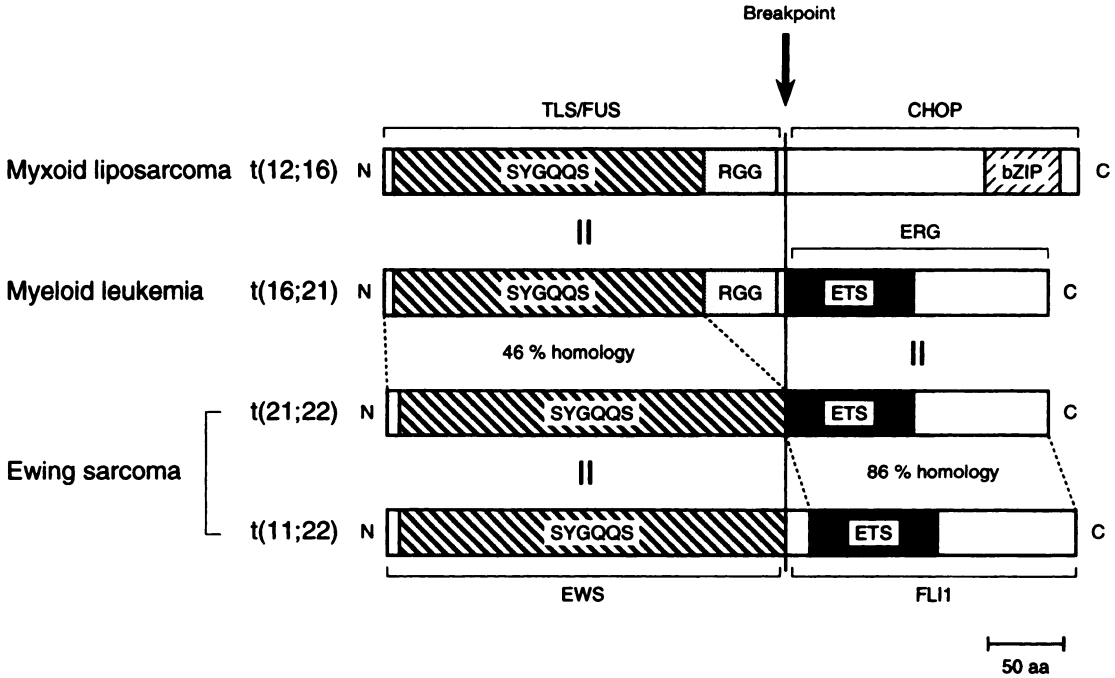

Fig. 4. Comparison of the chimeric proteins in myxoid liposarcoma with t(12;16), myeloid leukemia with t(16;21), and Ewing's sarcoma with t(21;22) and t(11;22). The structure of the expected TLS/FUS-ERG protein in t(16;21) leukemia is compared with the TLS/FUS-CHOP protein in myxoid liposarcoma and the EWS-ERG and EWS-FLI1 proteins in Ewing's sarcoma. Although several types of EWS-ERG and EWS-FLI1 proteins are known which contain different regions of EWS, ERG, and FLI1, their minimal regions are shown here. The structural or functional domains of the chimeric proteins are: *SYGQQS*, degenerated S–Y–G–Q–Q–S repeats; *bZIP*, the DNA-binding domain consisting of a basic region and a leucine zipper; *RGG*, R–G–G repeat-containing region; *ETS*, the ETS DNA-binding domain. Homology of the NH_2-terminal region between TLS/FUS and EWS was calculated according to the alignment reported by Crozat *et al.* (12), although many types of alignments are possible.

Acknowledgments

We thank Drs. Nobuo Maseki and Yasuhiko Kaneko for providing leukemia samples.

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