PAX5 is a transcription factor that is required for the development and maintenance of B cells. Promyelocytic leukemia (PML) is a tumor suppressor and proapoptotic factor. The fusion gene PAX5-PML has been identified in acute lymphoblastic leukemia with chromosomal translocation t(9;15)(p13; q24). We have reported previously that PAX5-PML dominantly negatively inhibited PAX5 transcriptional activity and impaired PML function by disrupting PML nuclear bodies (NBs). Here we demonstrated the leukemogenicity of PAX5-PML by introducing it into normal mouse pro-B cells. Arrest of differentiation was observed in PAX5-PML-introduced pro-B cells, resulting in the development of acute lymphoblastic leukemia after a long latency in mice. Among the transactivation targets of PAX5, B cell linker protein (BLNK) was repressed selectively in leukemia cells, and enforced BLNK expression abrogated the differentiation block and survival induced by PAX5-PML, indicating the importance of BLNK repression for the formation of preleukemic state. We also showed that PML NBs were intact in leukemia cells and attributed this to the low expression of PAX5-PML, indicating that the disruption of PML NBs was not required for the PAX5-PML-induced onset of leukemia. These results provide novel insights into the molecular mechanisms underlying the onset of leukemia by PAX5 mutations.

PAX5 is a member of the highly conserved paired box (PAX) domain family of transcription factors. PAX5 is expressed exclusively from the pro-B to mature B cell stage and is down-regulated during terminal differentiation into plasma cells (1). PAX5 is indispensable for B lineage commitment by the transcriptional activation of B lineage-specific genes (2), such as CD19 (3), CD79A (4), and B cell linker protein (BLNK) (5), and its target disruption has been shown to cause B lymphoid maturation arrest at the pro-B cell stage (6). Previous studies have identified the PAX5 gene as the most frequent target of somatic mutations in childhood and adult B-progenitor acute lymphoblastic leukemia (ALL), being altered in 38.9% and 34% of cases, respectively (7, 8), and these findings further emphasized the essential role of PAX5 in the proper development of B cells. Somatic mutations consist of partial or complete hemizygous deletions, homozygous deletions, partial or complete amplifications, point mutations, or fusion genes (7). These aberrations in the PAX5 gene are considered to impair PAX5 function and play a role in blocking B cell differentiation. PAX5 fusion proteins such as PAX5-TEL, PAX5-ENL, PAX5-PML, and PAX5-C20S have been shown previously to have dominant-negative effects on PAX5 transcriptional activity and have been suggested to be mainly responsible for the differentiation disorder of ALL with these fusion genes (9–12). Consistently, a previous study has reported that PAX5 haploinsufficiency cooperated with the constitutive activation of STAT5 to initiate ALL in mice (13). However, the oncogenicity of PAX5 mutations, including fusion genes, has yet to be demonstrated.

PML is a potent growth suppressor and proapoptotic factor (14, 15). In normal cells, the PML protein is localized in discrete subnuclear compartments called PML nuclear bodies (NBs) (16). In PML NBs, PML co-accumulates with more than 70 proteins that are involved in tumor suppression, apoptosis, regulation of gene expression, anti-viral responses, and DNA replication.

**The abbreviations used are:** PAX, paired box; ALL, acute lymphoblastic leukemia; NB, nuclear body; APL, acute promyelocytic leukemia; RAR, retinoic acid receptor; ATO, arsenic trioxide; PML, promyelocytic leukemia; P-PAL, PAX5-PML-induced acute lymphoblastic leukemia; BLNK, B cell linker protein.
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repair. PML has been suggested to exert its effects by regulating the functions of binding partners at the core of PML NBs (17). PML NBs have been found previously to be disrupted in human acute promyelocytic leukemia (APL) by PML–RARα, an oncogenic fusion protein of PML and retinoic acid receptor (RAR) α, which is considered to be the underlying mechanism responsible for the anti-apoptotic effects of PML–RARα (18–20). Arsenic trioxide (ATO), a chemotherapeutic agent used clinically in the treatment of APL, reportedly induced the restoration of disrupted PML NBs and apoptosis in APL cells, resulting in prolonged remission of this disease (21–24). These findings emphasize the importance of the integrity of PML NBs in tumor suppression.

The fusion gene PAX5-PML has been detected in two cases of B-progenitor ALL with chromosomal translocation t(9;15)(p13;q24) (25). We have demonstrated previously that PAX5-PML dominant-negatively inhibited PAX5 transcriptional activity in a luciferase reporter assay and suppressed the expression of PAX5 transactivation targets when expressed in a B lymphoid cell line. Furthermore, we have shown that the expression of PAX5-PML in a non-hematological tumor cell line induced the disruption of PML NBs and resistance to apoptosis and that ATO treatment induced the reconstitution of PML NBs and abrogation of apoptosis resistance. These findings suggested the possible involvement of this fusion protein in the leukemogenesis of B-ALL in a dual dominant-negative manner and the potential of ATO therapy for this type of ALL (11).

In this study, we demonstrated the leukemogenicity of PAX5-PML by introducing it into normal mouse pro-B cells and showed selective BLNK repression among the transactivation targets of PAX5 in leukemia cells. We also showed that PML NBs were intact in leukemia cells, indicating that the disruption of PML NBs was not required for the PAX5-PML-induced onset of leukemia. These results provide novel insights into the molecular mechanisms underlying the onset of leukemia by PAX5 mutations.

Experimental Procedures

Antibodies and Reagents—The anti-PML antibody (H-238); anti-PAX5 N antibody (N-19); anti-CD19 antibody (4G7), phycoerythrin-conjugated; and arsenic trioxide have been described previously (11). The anti-CD43 antibody, phycoerythrin-conjugated; and anti-human CD8 antibody, V450-conjugated were purchased from BD Biosciences, BioLegend (San Diego, CA), and Beckman Coulter (Miami, FL), respectively. The anti-mouse PML antibody for immunostaining was from LSBio (Seattle, WA).

Plasmids—PAX5-PML/pCDNA has been described previously (11). PAX5-PML/MigRI was constructed by subcloning PAX5-PML cDNA fragments into MigRI. MigRI is a bicistronic retroviral vector using GFP as a transfection marker and was a gift from R. A. Van Etten, Tufts-New England Medical Center, Boston, MA). Another bicistronic retroviral vector using the extracellular domain of human CD8 (hCD8), MSCV-hCD8, has been described previously (26). BLNK/MSCV-hCD8 was constructed by subcloning mouse BLNK cDNA obtained from Addgene (Cambridge, MA). PAX5-PML/pBGJR, a lentivirus expression vector, has been described previously (11). pBGJR was provided by Dr. Stefano Rivella (Memorial Sloan-Kettering Cancer Center).

Cell Culture—OP9 cells were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 15% FBS. S17 cells were cultured in DMEM (Invitrogen) supplemented with 10% FBS. P-PAL cells were cultured in 10% FBS and 2 μM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) containing RPMI 1640 (Invitrogen).

Lentiviral Infection and Transplantation of Cells—Bone marrow cells of C57BL/6 mice were transfected with an empty vector and a PAX5-PML expression vector were transplanted into sublethally irradiated syngenic mice. Mice were designated control/BL6 and PAX5-PML/BL6, respectively. Three months later, bone marrow cells in the indicated mice were analyzed using a flow cytometer. GFP-positive cells in the DAPI-negative lymphoid fraction of bone marrow cells were gated, and the expression of B220 was examined. GFP-positive cells were gated in the red square, and their ratio (percent) is shown in the left panel. The expression of B220 was plotted on histograms, and the ratio (percent) of B220-positive cells is shown in the right panel. These experiments were performed using two mice for each group, and similar results were observed. Representative data are shown. SSC, side scatter.
ments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee at Nagoya University.

**Transient Transfection, Immunoprecipitation, Immunoblotting, Immunofluorescence Staining, EMSA, and Luciferase Assay**—These methods were performed as described previously (11, 29, 30). The probes of 5'-GAATGGGGCACTGAGGCG-TGACCACC-GC-3' and 5'-AACTTGGCGATGCGCTCCAG-CGAGTTTT-3', high-affinity PAX5-binding site sequences in the CD19 promoter and BLNK promoter (5), respectively, were used for EMSA. CD19-luc/pGL4 and BLNK-luc/pGL4 were used as the reporter genes for the luciferase assay. CD19-luc/pGL4 has been described previously (11). BLNK-luc/pGL4 was constructed by inserting a nucleotide fragment containing two copies of the high-affinity PAX5-binding site sequence in the BLNK promoter into the pGL4.20 vector (Promega, Madison, WI). The Renilla luciferase expression vector phRG-TK was from Promega.

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**FIGURE 2.** Introduction of PAX5-PML into pro-B cells caused differentiation arrest in mice. A, a B cell differentiation analysis of control cells. Bone marrow (BM) and spleen (Sp) cells were collected on the indicated days after the transplantation of pro-B cells transfected with an empty vector expressing only GFP. The expression of CD43 and B220 in cells was analyzed by FACS after gating for the GFP⁺, DAPI⁻, and lymphoid fraction (scatter). The rate of GFP⁺ cells in the DAPI-negative lymphoid fraction is presented at the top. Blue, green, and red squares indicated the pro-B, pre-B and immature B, and mature B cell fractions, respectively. Numbers are the rate (percent) of each fraction in GFP⁺ cells. B, differentiation arrest of PAX5-PML-introduced cells. Mice transplanted with pro-B cells transfected with an expression vector for PAX5-PML and GFP were analyzed as in A. C, the rate of GFP⁺ positive cells. The averages of the GFP⁺ cell rates in A and B are plotted. Two mice per group were analyzed. The bar graphs are classified by color according to the rates of pro-B, pre-B and immature B, and mature B cells. Control pro-B cells differentiated into mature B cells, moved to the spleen (Sp) on day 14, and disappeared until day 21, whereas PAX5-PML-introduced (P-P-introduced) pro-B cells maintained the pro-B cell phenotype and remained in the bone marrow (BM) 56 days after transplantation.
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Results

Introduction of PAX5-PML into Pro-B cells Caused the Arrest of Differentiation in Mice—To investigate the leukemogenicity of PAX5-PML, we first attempted to transfect it into the hematopoietic progenitors of mice. Bone marrow cells were collected from BALB/c mice, transfected with a lentivirus vector expressing PAX5-PML and GFP, and transplanted into lethally irradiated BALB/c mice. B cells were not generated from GFP-positive cells in these mice, whereas ~20% of GFP-positive cells expressed B220 in control mice (Fig. 1). Although the expression of PAX5-PML was expected to start at the pro-B cell stage, similar to PAX5, it appeared to begin in earlier hematopoietic progenitors in this system. We speculated that PAX5-PML prevented the development of or was toxic to lymphoid progenitors. Therefore, we established a new system to introduce PAX5-PML into pro-B cells. We transfected the bicistronic retroviral vector expressing PAX5-PML and GFP into B220+/kit+ pro-B cells sorted from the fetal livers of BALB/c mice and transplanted these cells into half-lethally irradiated NOD/SCID mice. Control pro-B cells expressing GFP alone existed predominantly in the bone marrow until day 7 and differentiated into mature B cells, moved to the spleen until day 14, and then almost disappeared, possibly because of cell death, by day 21 (Fig. 2, A and C). On the other hand, PAX5-PML-introduced pro-B cells remained in the pro-B cell stage and stayed in the bone marrow for over 56 days, indicating the arrest of differentiation because of the introduction of PAX5-PML (Fig. 2, B and C).

PAX5-PML-introduced Pro-B cells Caused ALL in Mice—All eight mice transplanted with PAX5-PML-introduced pro-B cells died between days 63 and 158. A pathological examination revealed severe splenomegaly and a marked infiltration of leukocytes in the bone marrow and spleen (Fig. 3A). A flow cytometry analysis showed that these infiltrated leukocytes were GFP-positive and mainly had the pro-B cell phenotype (Fig. 3B), suggesting that these mice died because of ALL that developed from PAX5-PML-introduced cells. The leukemia cells obtained were transplanted serially into BALB/c mice. The mice died more rapidly with repetitions of transplantation; that is, mice that received second and third transplantations died between days 48 and 55 and between days 14 and 20, respectively (Fig. 3C). These results indicated that the introduction of PAX5-PML into pro-B cells caused ALL in mice.

Establishment of PAX5-PML-induced Leukemia Cell Lines—GFP-positive cells were collected from mice that received a fourth transplantation and then cultured with S17, a bone marrow stromal cell line, in 10% FBS and 2 μM 2-mercaptoethanol-containing DMEM. After a few repetitions to sort GFP-positive cells, the cells showed stable and exponential growth without the S17 co-culture (Fig. 4A). Cells were GFP-positive and had the pro-B cell phenotype (Fig. 4B). We designated this cell line P-PAL (PAX5-PML-induced ALL).

The PML Nuclear Body Was Not Disrupted in PAX5-PML-induced Leukemia Cells—The disruption of PML NBs by PML-RARα is known to be involved in the leukemogenesis of APL. ATO has been shown to induce apoptosis in APL cells through the reconstruction of PML NBs (see “Introduction”). We have previously demonstrated the disruption of PML NBs because of the overexpression of PAX5-PML and the reconstruction of the disrupted PML NBs by ATO in HeLa cells (11). Therefore, here we determined whether PML NBs were disrupted in PAX5-PML-induced leukemia cells. The disruption of PML NBs was not detected in PAX5-PML-induced leukemia cells (Fig. 5A) or P-PAL cells (Fig. 5B). Furthermore, ATO treatment did not significantly alter PML NBs, reduce the number of tumor cells, or improve the survival of leukemic mice (Fig. 5, A and B, and 6, A and B). These results indicated that PAX5-PML did not exert its dominant-negative effect on PML function in this system and that the inhibition of PML function was not necessary for the PAX5-PML-induced onset of leukemia.

We examined the expression of PAX5-PML in P-PAL in an attempt to clarify why PAX5-PML did not disrupt PML NBs in PAX5-PML-induced leukemia cells. Although the mRNA expression of PAX5-PML was confirmed by RT-PCR (Fig. 5C), its protein expression was so weak that it could not be detected by immunoblotting using whole-cell lysates of P-PAL cells. The protein expression of PAX5-PML was confirmed by immunoblotting immunoprecipitates of the whole-cell lysate with an
anti-PAX5 antibody or anti-PML antibody (Fig. 5D), indicating that PAX5-PML did not significantly disrupt PML NBs because of its weak expression in leukemia cells.

Selective Repression of BLNK May Be Important for the Differentiation Block by PAX5-PML—
PAX5 is known to have many target genes for transcriptional activation and regulates B cell differentiation. We have demonstrated previously that PAX5-PML had a dominant-negative effect on PAX5 transcriptional activity. PAX5-PML appeared to be involved in leukemogenesis through the differentiation block caused by the inhibition of PAX5 transactivity. To elucidate the mechanism underlying the differentiation block by PAX5-PML, we quantified the mRNA expression levels of PAX5 target genes such as CD19, CD79A, BLNK, and CD72 at the pro-B cell stage in PAX5-PML-induced leukemia cells. The expression of BLNK was repressed significantly in leukemia cells, whereas the repression of CD19, CD72, and CD79A was mild, suggesting the
importance of BLNK repression for the differentiation block by PAX5-PML (Fig. 7A). We confirmed that BLNK protein expression was reduced in PAX5-PML-induced leukemia cells (Fig. 7B). In an attempt to determine whether this differential inhibition of PAX5 transactivity was intrinsic to PAX5-PML, we compared its DNA binding ability and inhibitory effects on transactivation by PAX5 between a CD19 promoter and BLNK promoter. No significant difference was observed in the DNA binding ability of PAX5-PML between the CD19 promoter and BLNK promoter in EMSA (Fig. 7C). Its DNA binding ability to both promoters was very weak. However, the DNA binding domain of PAX5 was maintained in PAX5-PML. These results were consistent with our previous findings and those of others (11, 31) and suggested that PAX5-PML had no preference of DNA binding between the two promoters. Although PAX5-PML exhibited a weak DNA binding ability, PAX5 transactivity on the BLNK promoter was inhibited dominant-negatively by PAX5-PML in the reporter gene assay, similar to that on the CD19 promoter. PAX5-PML did not show any preference of inhibition of PAX5 transactivity between the CD19 promoter and BLNK promoter (Fig. 7D). These results indicated that the selective suppression of BLNK was extrinsic to PAX5-PML. In these reporter gene assays, PAX5-PML almost completely inhibited PAX5 transactivity, even when its expression level

FIGURE 7. Selective repression of BLNK in PAX5-PML-induced leukemia cells. A, quantification of mRNA expression of PAX5 transcriptional target genes. The expression of the indicated genes was quantified by quantitative RT-PCR using mRNA from pro-B cells employed in the transplantation assay (control pro-B cells) and PAX5-PML-induced leukemia cells (P-P-induced LC) and plotted on bar charts. The average values relative to basal expression in control pro-B cells in two independent analyses are shown (results are mean ± S.D.). B, reduced expression of the BLNK protein in PAX5-PML-induced leukemia cells. Lysates of the indicated cells were subjected to immunoblotting (IB) with an anti-BLNK antibody (ab). C, PAX5-PML bound very weakly to both PAX5 binding sites in the CD19 and BLNK promoters. Equal amounts of PAX5 and PAX5-PML were incubated with radiolabeled oligonucleotides containing the PAX5 binding sites of the indicated promoters in the presence of a 200-fold molar excess of unlabeled oligonucleotides (Competitor), normal goat IgG (Control Ab), or an anti-PAX5 N antibody as indicated. Black and white arrowheads indicate PAX5 DNA complexes and supershifted bands, respectively. PAX5-PML DNA complexes were hardly observed and are indicated with asterisks. Similar results were obtained from two independent experiments. Representative data are shown. D, the dominant-negative transcriptional repression by PAX5-PML was similar in both promoters. The luciferase assay was performed by transfecting 125 ng of PAX5/pCDNA, increasing the amounts of PAX5-PML/pCDNA (31.25–125 ng), and the reporter genes containing the PAX5 binding sites of the indicated promoters into 293T cells. Luciferase activities in three independent transfection experiments are shown as average values relative to the basal activation of each reporter gene by PAX5 (results are mean ± S.D.). Another set of cells transfected with the same plasmids as in the luciferase assay described above were lysed for immunoblotting. The lysates were subjected to immunoblotting with an anti-PAX5 N antibody to determine the protein expression levels of PAX5 and PAX5-PML (bottom panel). PAX5 and PAX5-PML (P-P) are indicated by single and double arrowheads, respectively.
was markedly weaker than that of PAX5 (Fig. 7D, lanes 4 and 10). Taken together with the DNA binding ability of PAX5-PML being markedly weaker than that of PAX5, these results suggested that the mechanism underlying the dominant-negative inhibition of PAX5 transactivity was not the occupation of promotores by PAX5-PML.

We also determined whether the enforced expression of BLNK abrogated the differentiation block and survival to further establish the importance of BLNK repression for PAX5-PML-induced leukemia development. We transfected the bicistronic retroviral vector expressing PAX5-PML and GFP together with that expressing BLNK and hCD8 into mouse pro-B cells and transplanted them into mice. Only GFP⁺ hCD8⁻ cells, but not GFP⁻ hCD8⁺ cells, could survive in the recipient mice on day 28 (Fig. 8A). On the other hand, control pro-B cells expressing PAX5-PML, GFP, and hCD8 but not BLNK could survive in the recipient mice, regardless of hCD8 positivity, when they expressed GFP (Fig. 8A). In addition, other control pro-B cells expressing GFP, hCD8, and BLNK but not PAX5-PML could not survive in the recipient mice (Fig. 8A). The remaining GFP⁺ cells in the bone marrow were mainly pro-B cells in both mouse groups transplanted with PAX5-PML-introduced pro-B cells (Fig. 8B). These results indicated that the enforced expression of BLNK abrogated the PAX5-PML-induced differentiation block and survival in mice.

**Discussion**

Although numerous types of PAX5 fusion genes have been identified to date, their oncogenicities have not yet been confirmed. Our mouse leukemia model is the first to be induced by the PAX5 fusion gene. The introduction of PAX5-PML caused a differentiation block in pro-B cells that may have been the result of the suppression of PAX5 transactivity by PAX5-PML. Our results demonstrated that the differentiation block by PAX5-PML did not require the suppression of all PAX5 target genes. The repression of CD19, CD72, and CD79A was not required for the differentiation block. In other words, the repression of BLNK was sufficient for the differentiation block caused by PAX5-PML. Abrogation of the PAX5-PML-induced differentiation block and initial survival by enforced BLNK expression further established its involvement in the formation of the preleukemic state induced by PAX5-PML (Fig. 8). BLNK is an adaptor protein that bridges B cell receptor-associated kinases with a multitude of signaling components and is essential for B cell differentiation but not proliferation. The ablation of Blnk in mice has been shown previously to cause a B cell differentiation block at the pro-B cell stage without reducing the number of bone marrow cells (32). On the other hand, CD79A and CD19 are known to be essential for differentiation and proliferation. CD79A is the main component of B cell receptor, the signal of which is required for the survival and differentiation of B cells. CD19 is a co-stimulatory molecule
that amplifies B cell receptor signaling. Gene ablation of Cd19 and Cd79A in mice not only caused a differentiation block but also decreased the number of B cells (33, 34). These findings suggested that the escape of CD19 and CD79A from repression by PAX5-PML gave advantage to the development of leukemia by PAX5-PML and may be the reason for the selective repression of BLNK. A previous study has reported that leukemia cells of patients with PAX5-PML-positive ALL expressed CD19 and CD79A (25), implying that escape from repression by PAX5-PML also occurred in human leukemia cells.

The exact mechanism underlying selective BLNK repression has not yet been elucidated. No significant preference of the DNA binding of PAX5 was observed in the CD19 promoter and BLNK promoter, and PAX5-PML bound very weakly to both promoters (Fig. 7B). It currently remains unknown how PAX5-PML acts as dominant-negative inhibitor of PAX5 without DNA binding ability. We have demonstrated previously that PAX5-PML inhibited the transactivity of PAX5 by binding to PAX5 on the promoter (11). This model may explain the inhibition of PAX5 transactivity by PAX5-PML even when its expression was markedly lower than that of PAX5 (Fig. 7D, lanes 4 and 10) and that PAX5-PML may suppress the expression of BLNK despite its very weak expression in PAX5-PML-induced leukemia cells (Figs. 5D and 7A). No significant difference was observed in the dominant-negative effects of PAX5-PML on PAX5 transactivity between the two promoters (Fig. 7D). Therefore, the differential repression of PAX5 target genes appeared to be extrinsic to PAX5-PML. Transplanted pro-B cells usually die soon after they differentiate into mature B cells unless they are stimulated with a specific antigen, as shown in Fig. 2A. Therefore, transplanted pro-B cells remained in their recipient mice only when they succeeded in causing a differentiation block and resisted the growth-suppressive effects of PAX5-PML. Only cells that succeed in selectively suppressing BLNK may be able to pass through this selection and survive in recipient mice as preleukemia cells. Some feedback signals and/or cellular compensatory mechanisms may be involved in maintaining the expression of CD19 and CD79A.

Although we demonstrated the involvement of BLNK repression in PAX5-PML-induced formation of the preleukemic state, it is not the only mechanism underlying the development of leukemia. The long latency to develop leukemia after the differentiation block by the introduction of PAX5-PML implies the requirement of additional genetic and/or epigenetic events. BLNK repression and PAX5-PML expression appear to be insufficient to induce autonomous proliferation. Target(s) of a second hit for the development of leukemia have not yet been identified. We performed microarray analyses to compare the mRNA expression profiles between normal pro-B cells and PAX5-PML-induced primary leukemia cells but were unable to identify the gene expression change(s) responsible.

PML exerts its function by forming PML NBs and is involved in tumor suppression and stress-induced apoptosis. PML-RARα has been suggested to contribute to the development of APL through a dominant-negative effect on RARα and the disruption of PML NBs. Therefore, PAX5-PML may also have contributed to the development of leukemia through the disruption of PML NBs. However, our results clearly demonstrated that PML NBs were not disrupted in PAX5-PML-induced leukemia cells, and this may have been due to the insufficient expression of PAX5-PML (Figs. 4D and 5A). Although we showed that PAX5-PML did not require the disruption of PML NBs for the development of leukemia in mice (Fig. 5A) and that ATO was not effective in the treatment of PAX5-PML-induced ALL (Fig. 6, A and B), these results denied neither the involvement of the disruption of PML NBs in the development of APL induced by PML-RARα nor the reconstruction of PML NBs in the mechanism of action of ATO in APL cells. It currently remains unclear whether ATO is effective for patients with PAX5-PML-positive ALL because the status of PML NBs in the leukemia cells of such patients has not yet been examined (25).

In summary, here we demonstrated the leukenmogenicity of PAX5-PML and partially elucidated the molecular mechanism underlying the development of leukemia. The genetic events leading to the development of leukemia during the long latency after a differentiation block need to be identified. Our model is suitable for analyzing the multistep development of B-ALL and sheds new light on this field.

Author Contributions—F. H., N. I., S. K., and S. T. designed the research, performed experiments, and wrote the paper. T. M., Y. K., T. Y., and K. S. performed experiments. T. N. and H. K. designed the research. All authors reviewed the results and approved the final version of the manuscript.

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