Leukemia Translocation Gene, PLZF, Is Expressed With a Speckled Nuclear Pattern in Early Hematopoietic Progenitors

By A. Reid, A. Gould, N. Brand, M. Cook, P. Strutt, J. Li, J. Licht, S. Waxman, R. Krumlauf, and A. Zelent

The PLZF gene was discovered by studying a rearrangement of the RARα locus in a patient with acute promyelocytic leukemia and a t(11;17) chromosomal translocation. To understand further the potential role(s) of the PLZF gene product in hematopoiesis, we have examined its expression levels in a variety of murine tissues and in established cell lines that are representative of various stages of myeloid and lymphoid development. We show that murine PLZF (mPLZF) is expressed at the highest levels in undifferentiated, multipotential hematopoietic progenitor cells and that its expression declines as cells become more mature and committed to various hematopoietic lineages. Data obtained with established cell lines are corroborated by results showing the lack of human PLZF protein expression in mature peripheral blood mononuclear cells and high PLZF levels in the nuclei of CD34+ human bone marrow progenitor cells. Interestingly, unlike many transcription factors, PLZF protein in these cells possesses distinct punctate nuclear distribution, suggesting its compartmentalization in the nucleus. Taken together, our data suggest a role for PLZF protein in early hematopoiesis and the requirement of downregulation of its expression for proper differentiation of most hematopoietic lineages. © 1995 by The American Society of Hematology.

STUDIES OF SPECIFIC chromosomal translocations in various hematopoietic malignancies have lead to the identification of a number of genes whose products, which are often transcription factors, are important in normal development of blood cells. The majority of acute promyelocytic leukemia (APL) cases are associated with a t(15;17) chromosomal translocation, which fuses the retinoic acid receptor-α (RARα) locus with a gene called PML (see Warrell et al., Grignani et al., and Zelent for reviews and the references therein). PML is a member of a recently defined gene family whose products are characterized by the presence of an N-terminal cysteine/histidine-rich structural motif (called the ring-finger) and an adjacent coiled-coil domain that probably function in nucleic acid binding and protein dimerization, respectively. Although the function of the wild-type PML protein is not known, it has recently been shown that it colocalizes in a speckled nuclear pattern with several other proteins that together constitute structures known as nuclear bodies. Recent results suggest that disruption of the function of the wild-type PML protein may be important for the cellular transformation observed in APL. The involvement of retinoid signalling pathway(s) and the RARα in the molecular pathogenesis of APL, on the other hand, is strongly suggested by the requirement of retinoids and the RARα for proper myeloid maturation in vitro and by the recent demonstration that a small proportion of patients with APL do not have the classical t(15;17) but possess a variant t(11;17) or t(5;17) reciprocal chromosomal translocation that also involves the RARα gene. Molecular characterization of the t(11;17) translocation showed that, in this case, the RARα gene is fused to a Krüppel-related C2-H2 zinc-finger (Zn-finger) gene, called PLZF (promyelocytic leukemia zinc finger) and, in analogy with the t(15;17), reciprocal RARα-PLZF and PLZF-RARα fusion genes are expressed in leukemic cells. Interestingly, two different PLZF mRNA isoforms [PLZF(A) and (B)] have been identified in which the predicted hPLZF(B) protein would possess an extra 123 amino acid proline-rich region that presumably is encoded by an alternatively spliced exon.

Other genes that encode transcription factors with C2-H2 Zn-finger motifs have been shown to function in a variety of developmental systems, including hematopoiesis. For example, expression of Krox-24 and MZF-1 has been shown to be necessary for macrophage and granulocyte differentiation, respectively. Furthermore, the Ikaros and EKLF transcription factors have been shown to be essential for the development of all lymphoid lineages and activation of adult β-globin gene expression, whereas overexpression of Blimp-1 has been shown to drive the maturation of B-cell lymphoma lines to Ig-secreting cells.

To gain insight into the possible roles that the PLZF gene product may play during hematopoietic development, we have studied its expression in established murine cell lines, adult tissues, mouse embryos, human peripheral blood (PB) mononuclear cells, and in CD34+ progenitor cells purified from normal human bone marrow (BM). In the hematopoietic system, levels of mPLZF mRNAs are high in early multipotential progenitor cells, but they decrease as cells become differentiated and lineage committed. Our results suggest that the mPLZF gene product may have a specific role to play in early hematopoiesis, perhaps in the maintenance of the phenotype of uncommitted hematopoietic progenitors and/or in controlling the commitment of these cells to differentiation.

MATERIALS AND METHODS

Northern blot and reverse transcription-polymerase chain reaction (RT-PCR) analyses. Total and polyA+ RNAs were prepared.

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
as previously described. In vitro differentiation of ES cells was described elsewhere. FDCPmixA4 cells were induced to undergo neutrophilic differentiation with 1,000 U/mL granulocyte colony-stimulating factor (G-CSF; RD Systems, Minneapolis, MN), as previously described. Northern blot hybridization was performed as described using a 32P-labeled fragment of a cloned mPLZF cDNA encoding amino acids 1-348 and 250 bp of the 5′-untranslated sequences. The blot was exposed for 6 days at −80°C using two intensifying screens and Kodak XAR-5 film (Eastman Kodak, Rochester, NY).

To control for the fidelity of both RT and PCR amplification of mPLZF mRNAs and to exclude possible false-negative results due to a failure of either reaction, we have constructed plasmid mPLZF(B)ΔΔ, which, when transcribed in vitro, would give rise to cRNA lacking 180 bp of the wild-type mPLZF(B) sequences (see Fig 2A). When 1 pg of this in vitro transcribed cRNA is co-reverse transcribed with a given RNA sample and then amplified using PCR, a control amplification product that is of an interrelated sequence between the products derived from endogenous mPLZF(B) and (A) mRNAs will be obtained. In addition, as a control for RNA integrity, we have also amplified mRARα1 sequences. The reliable semiquantitative nature of the above-mentioned method is indicated by the increasing amounts of products (Fig 2B, lanes 1 through 4) that were obtained when different amounts of mPLZF(B)ΔΔ were reverse transcribed and amplified using PCR in a background of 2.5 μg of total RNA derived from cells that do not express the mPLZF gene. All cDNAs were synthesized as before using both mPLZF- and mRARα1-specific oligonucleotide primers, 2.5 μg of a given RNA (except for ES cells, in which only 200 ng of RNA was used), and 1 pg of the mPLZF(B)ΔΔ control cRNA in the same reaction. Subsequently, 2.5-μL aliquots of the above-mentioned reverse transcription reaction were separately used either for amplification of mPLZF or mRARα1 sequences. All mRARα1-specific oligonucleotide primers and probes used in this study were homologous to the previously described human primers. The relative positions of the mPLZF-specific primers and probes are shown in Fig 2A. Sequences of all primers are available on request. PCR amplifications were performed under previously described conditions, except that one round of 25 cycles was performed using an annealing step at 54°C for 1 minutes and denaturation and extension for 25 seconds at 95°C and 3 minutes at 72°C, respectively. Amplified cDNAs were detected after transfer to nitrocellulose using 32P-labeled oligonucleotide probes previously described conditions. Exposure times were in the range of 30 minutes to 1 hour at room temperature using the Kodak XAR-5 film.

Western blotting, immunofluorescence, and confocal microscopy.

High salt (0.4 mol/L NaCl) nuclear extracts were prepared from 9.5 days postcoitum (dpc) mouse embryos using previously described methodolgy. Nuclear proteins were fractionated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred in a buffer containing 39 mmol/L glycine, 48 mmol/L Tris, pH 8.3, 0.0375% SDS, and 20% methanol onto Immobilon-P membrane (Millipore, Bedford, MA). The anti-PLZF or anti-RARα RPA(F) antisemur was used at 1/1,000 dilution. When specified, diluted anti-PLZF antisemur was preincubated in the presence of protease inhibitors for 1 hour at room temperature with 50 μg of either GST or GST-mPLZF protein. Proteins were detected with peroxiredoxase-conjugated donkey antirabbit secondary antibody and ECL reagents (Amersham, Arlington Heights, IL).

Normal human BM CD34+ cells were isolated using monoclonal anti-CD34 IgG1 antibody attached to MACS microbeads as directed by the supplier (Miltenyi Biotec Inc, Sunnyvale, CA). To obtain mononuclear cells, whole peripheral blood was taken from normal healthy volunteers, layered onto Lymphoprep (Nycomed, Oslo, Norway), and centrifuged at 2,000 rpm for 30 minutes. The mononuclear fraction band was acquired, and the monocytes were removed by adherence to plastic for 2 hours at 37°C and 5% CO2. The adherent fraction was composed predominantly of monocytic cells (>90%) and the nonadherent fraction was composed predominantly of lymphoid cells (>90%), as determined by morphology. For immunofluorescence, previously frozen cytopsins of the above cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature. Slides were then washed in Ca2+- and Mg2+-free phosphate-buffered saline (PBS) solution and incubated for 1.5 hour at room temperature in PBS containing 0.1% Triton X-100, 0.1% bovine serum albumin (BSA), and 10 mmol/L NaAzide (PBTX buffer) or, alternatively, cells were incubated in 0.2% Triton X 100 for 10 minutes at room temperature, washed, and then incubated in 10% human serum in PBS for 30 minutes. Subsequently, cells were washed with PBS and incubated for 2 hours at room temperature with rabbit anti-PLZF antiserum, rabbit antiserum against the F-region of the RARα (see above), or normal rabbit serum control, diluted 1/200 in PBTX buffer. Cells were again washed with PBS and incubated for 45 minutes at room temperature with fluorescein isothiocyanate (FITC)-conjugated goat antirabbit Ig (Europath, Stratton, UK) or FITC-conjugated donkey antirabbit Ig (Jackson Immunoresearch, West Grove, PA), washed again in PBS, and mounted under cover slips using Moviol (Calbiochem, San Diego, CA) and antiquenching agent p-phenylenediamine (Sigma, St Louis, MO). Stained cells were visualized and photographed using a BioRad MRC600 confocal imaging system (BioRad, Hercules, CA).

Whole-mount in situ hybridization and immunohistochostomy.

Mouse embryos were dissected at various stages postcoitus, fixed in 4% paraformaldehyde in PBS for 30 minutes, and subjected to whole-mount immunohistochemistry with the anti-PLZF polyclonal antiserum at 1/100 dilution, essentially as described, except that Triton X-100 was used at 0.2%.

Whole-mount in situ hybridization was performed as previously described. mPLZF antisense cRNA probes were synthesized in vitro using cloned mouse cDNA as template, rUTP-digoxigenin, and T3 RNA polymerase as directed by the supplier (Promega, Madison, WI).

RESULTS

Expression of the PLZF gene in adult mouse tissues and cell lines.

Northern blot analysis using polyA+ RNAs (mRNAs) derived from a number of adult mouse tissues and cell lines and a 32P-labeled mPLZF(B) cDNA probe showed that mPLZF was expressed in an interleukin-3 (IL-3)-dependent myeloid progenitor cell line (32D), but not in more differentiated myeloid cell lines (WEHI3B) or in pre-B-cell line myeloid cell line MEL (Fig 1, lanes 2 through 4). Low levels of expression were also observed in erythroid precursor cell line MEL (Fig 1, lane 1; see also below). The highest levels of expression were found in adult mouse heart and lower levels were also seen in spleen, brain, muscle, lung, and testes.

To facilitate the analysis of mPLZF(B) and/or (A) mRNA levels in total RNAs isolated from a wide range of samples, we have developed a semi quantitative RT-PCR procedure (see the Materials and Methods and Fig 2A). This analysis showed further that mPLZF is expressed at highest levels in early multipotential progenitor cell lines such as FDCPmixA4 and B6SUa (Fig 2B, lanes 15 and 16). Lower levels of mPLZF(B) mRNAs were also detected in an early lymphoid pro-B–cell line LYM9 and in erythroid progenitor MEL cells (Fig 2B, lane 17 and 22). Examination of other hematopoietic cell lines showed that mPLZF expression is progressively lower with increasing stages of differentiation.
mPLZF expression in murine cell lines and adult tissues. Northern blot analysis of mPLZF mRNAs derived from MEL, 18.8, 32D, and WEHI3B cell lines (lanes 1 through 4), as well as selected adult tissues (lanes 5 through 13). Molecular markers (in kilobases) are shown on the left. Hybridization with 32P-labeled actin cDNA probe (lower panel) was used as a control for mRNA integrity. Equal amounts of mRNA (4 μg as measured by optical density) were loaded into each lane. Note that we and others have, in the past, observed variability in actin mRNA levels in different tissues, which reflects its true pattern of expression. The low level of PUF(B) expression in MEL cells was confirmed by the more sensitive RT-PCR analysis (see Fig 2B); this can be compared with the corresponding data for 18.8 cells in which no expression was seen with either technique.

Fig 2. (A) Schematic diagram of the mPLZF(B) cDNA, as well as strategy for RT-PCR analysis. Various structural regions, such as POZ-domain, acidic (minus sign), and proline (Pro)-rich regions, as well as Zn-fingers, are as indicated. Primers used for the reverse transcription (RT), PCR (1 and 3), and hybridization (2) are shown below the diagram. To avoid confounding results due to amplification of mPLZF sequences that may be present in small amounts of contaminating genomic DNAs, the primers for PCR amplification were derived from regions encoded in separate exons (data not shown); exon/exon boundary is indicated with arrowheads. The expected size of the amplification product corresponding to mPLZF(B) mRNA is indicated below the diagram. To create the mPLZF(B)Δ control cDNA, a 180-bp MscI restriction enzyme fragment demarcated by the vertical arrows was deleted. (B) RT-PCR analysis of PUF expression (upper panel) in myeloid and multipotential hematopoietic progenitor cell lines such as 32D, FDCPmixA4,65 and B6SU1A65 (lanes 14 through 16), as well as pro-B-cell line LYD9, erythroid precursor MEL cells, promyelocytic and macrophage cell lines 416B64 and J774.2,69 and more mature transformed T- and B-lymphoid cell lines EL-468 and 18.8, respectively (lanes 17 through 22). Expression was also examined in NIH3T3 cells67; F9 embryonal carcinoma cells66; ES cells at days 0, 6, and 10 of differentiation; and BM, PB, thymocytes, and thymus (lanes 5 through 13). Lanes 23 and 24 show PUF(B) levels in FDCPmixA4 cells before (d0) and after 4 days (d4) of treatment with G-CSF. The bottom panel shows the results of amplification of mRARα1 sequences from the corresponding samples. Lanes 1 through 4 show the results of amplification of decreasing amounts of the mPLZF(B)Δ control cRNA, as indicated. The corresponding sizes of amplified cDNAs, which are identified on the left of each panel, are indicated in basepairs on the right.
and lineage restriction of the given cell type examined. For example, progressively more differentiated and lineage-restricted myeloid cells such as 416B and J774.2 expressed progressively lower levels of mPLZF(B) mRNA (Fig 2B, lanes 18 and 19). Likewise, more mature pre-B cells (18.8) and mature transformed T cells (EL4) did not express the mPLZF gene, despite the presence of low mPLZF(B) mRNA levels in a pro-B-cell line (see above) and thymocytes (Fig 2B, lane 12). Lower levels of mPLZF gene expression were also detected in BM, PB, and thymus (Fig 2B, lanes 10, 11, and 13). No expression was seen in nonhematopoietic cells such as fibroblasts (3T3) or embryonal teratocarcinoma (F9) cells. We note that, possibly because of the appearance of hematopoietic progenitors, mPLZF(B) mRNA levels were induced in differentiating embryonic stem (ES) cells (Fig 2B, lanes 7 through 9). Levels of mPLZF(B) mRNAs markedly decreased (at least 10-fold), on the other hand, during myeloid differentiation of multipotential progenitor FDCPmixA4 cells (Fig 2B, lanes 23 and 24). However, only a small decrease in mPLZF mRNA levels (at most twofold) was seen when these cells underwent erythroid differentiation in response to erythropoietin (data not shown). Interestingly, in all the samples examined, only mRNAs encoding the (B) isoform of the mPLZF protein were detected, suggesting that the mPLZF(A) isoform is expressed either at a level below detection by our RT-PCR assay or in a highly
restricted cell type and/or developmental-stage–specific manner.

The PLZF protein is expressed in the mouse embryo. To analyze the expression of the PLZF protein(s), we have raised polyclonal anti-PLZF antibodies against purified glutathione-S-transferase (GST)-hPLZF fusion protein produced in *Escherichia coli*. The preparation and thorough characterization of this antiserum will be described elsewhere. Despite the fact that this antiserum was raised against the hPLZF(B) protein, it readily detected its murine homologue that was expressed from a transiently transfected expression vector in Western blot, immunoprecipitation, and immunofluorescence experiments (data not shown). The murine protein appeared to migrate slightly lower than its human counterpart and comigrated with an in vitro translated mPLZF(B) protein at approximately 80 kD (Fig 3, lane 3). Because its observed molecular size is close to the predicted molecular weight (M,) of the mPLZF(A) protein (M, 61,514), it is tempting to speculate that it may represent the shorter mPLZF isoform. However, the possibility that this band is a degradation product of the mPLZF(B) protein or the result of alternative codon usage for translation initiation cannot be excluded.

In the 9.5- to 10.5-dpc mouse embryos, both in situ hybridization and immunohistochemical analyses (Fig 4A and B, respectively) showed coexpression of the PLZF mRNAs and proteins in the developing central nervous system, limbs, and branchial arches. Staining for PLZF mRNAs and proteins are as follows: BCL-6/LAZ-3 (Z21943/U60115), ZF-5 (L15325), kup (X16576), unpublished brain cDNA HBPCM81 (M85739), ZFJ8 (L16896), ZR (X82018), FBP-B (X79950), myxoma virus protein vm-18 (P22611), vaccinia virus protein VA55 (P24768/M58054), ectromelia virus protein vp65 (S24675), GAGA (L22295), ttk (X17126), lola (U07607), BR-C (X54864/S79446), E (var) 3-9D (X75498/ X75499), bric-a-brac (bab) (U01333), kelch (L08483), *C. elegans* putative protein c08c3 (L14433).

![Fig 5. Analysis of PLZF protein expression in human PB and BM cells. CD34+ cells purified from normal BM were analyzed by indirect immunofluorescence and confocal microscopy (A through C) after staining with PLZF antiserum (A and C) or normal rabbit serum (B). The punctate subnuclear distribution of the PLZF protein has been observed when using two different fixation procedures (data not shown) and after chemical induction of PLZF gene expression in a myelodysplastic cell line. Adherent (D through F) or nonadherent (G through I) mononuclear cells were stained with PLZF antiserum (D and G), normal rabbit serum (E and H), or RARalpha antiserum (F and I).](image-url)
### Expression of the PLZF Gene During Hematopoiesis

#### Sequence Comparison

<table>
<thead>
<tr>
<th>Consensus</th>
<th>PLZF</th>
<th>BCL-6/LAZ-3</th>
<th>zF5</th>
<th>kup</th>
<th>HFBAC81</th>
<th>ZFJeS</th>
<th>ZfD</th>
<th>FBP-B</th>
<th>vmt8</th>
<th>va55</th>
<th>vpl5</th>
<th>GAGA</th>
<th>ttt</th>
<th>Lola</th>
<th>BR-C</th>
<th>E(3-90)</th>
<th>bab</th>
<th>kecch</th>
<th>c09c3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consensus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Diagram

![Diagram](image_url)
teins could also be detected in the mesonephros (Fig 4, arrows in A and B). The developing heart was negative for mPLZF mRNAs (data not shown) and proteins (Fig 4B), indicating that the lower levels of staining, as seen, eg, in the mesonephros, represent true patterns of mPLZF expression. This latter point is corroborated further by the high degree of similarity in the patterns of mRNA and protein expression, which also underlines the specificity of the anti-PLZF antibody.

**PLZF is present in specific subnuclear domains in normal human BM CD34+ cells.** We have also used the antiserum described above in immunofluorescence studies (in combination with confocal microscopy) to investigate the expression of PLZF protein(s) in human CD34+ BM progenitor cells and in mature PB mononuclear cells (Fig 5). As predicted from results obtained with the murine hematopoietic cell lines (see above), the progenitor cells (Fig 5A and C), but not adherent (predominantly mature monocytes) and nonadherent (predominantly lymphoid) mononuclear cells (Fig 5D and G), contained high levels of the PLZF proteins. Furthermore, this analysis has shown that, like PML as well as spliceosomal small nuclear ribonucleoprotein particles35 and some factors which are associated with DNA synthesis,36 PLZF protein is localized in distinct nuclear speckles (Fig 5A and C). The PLZF containing nuclear speckles varied in size and were present in essentially every cell within a given visual field (Fig 5C). In view of these results, it will be interesting to see if some or all of the PLZF speckles also contain the PML protein and/or other factors. As in the case of PML, the functional significance of the speckled nuclear localization of the PLZF protein is not known.

It is worth noting that the PLZF protein contains a highly conserved N-terminal domain that characterizes a subfamily of krüppel-related C2-H2 Zn-finger proteins, as well as some viral proteins (Fig 6). This domain is present in another potentially oncogenic Zn-finger protein, BCL-6/LAZ-3,35,36 suggesting that this region may be important for cellular transformation. Recently, Bardwell and Treisman37 showed that this domain, which they called POZ (for Pox virus and Zn-finger), may be responsible for a specific nuclear localization of a C2-H2 Zn-finger protein called ZID and can facilitate protein-protein interactions. We show here that the endogenous PLZF protein possesses distinct punctate nuclear localization that, by analogy to ZID, may also be determined by the presence of the POZ-domain. In this respect, it is worth noting that transiently expressed PLZF(B)-RARα protein also possesses speckled nuclear localization pattern (data not shown), and it remains to be seen if it colocalizes with the wild-type PLZF and/or other related POZ-domain proteins and whether it can alter their native nuclear localization patterns.

**DISCUSSION**

Although the list of transcription factors that are thought to function in hematopoiesis is growing, the number of proteins that may control gene expression in self-renewing hematopoietic progenitor cells and/or in the hematopoietic stem cells is uncertain. Mutations of genes encoding key hematopoietic transcription factor such as c-myb40 and PU.114 by homologous recombination in ES cells have shown that they are required for proper differentiation of several but not all hematopoietic lineages and, therefore, their roles (if any) in self-renewing multipotential progenitor and/or stem cells remain unclear. The recent knockout of the murine GATA-2 gene, which results in severe impairment of development of all hematopoietic lineages and early embryonic lethality, suggests that this protein may play a critical role in the maintenance of self-renewing and undifferentiated phenotypes of such cells.42 However, it is likely that regulation of gene expression in a multipotent hematopoietic progenitor and/or stem cell involves the concerted action of a network of transcription factors, rather than one master regulator. A number of indirect observations reported here suggest that the PLZF protein may be a component of such a regulatory gene network. By analogy to GATA-2,43 the mPLZF gene is expressed at higher levels in IL-3–dependent progenitor cell lines than in more mature hematopoietic cells. Consistent with the above data, we find the hPLZF protein(s) in CD34+ cells isolated from normal human BM and expression of the mPLZF gene in mesonephros, which is part of the intraembryonic region (aorta, gonad, mesonephros [AGM] region) possessing hematopoietic stem cell activity.44,45 The striking similarities in patterns of expression between the PLZF and GATA-2 genes, which are also seen during erythroid and myeloid differentiation of FDCPmixA4 cells (see above and Cross et al46) and which are not restricted to hematopoietic cells, suggest that there may be a cross-regulatory interaction between them and/or that their products may be required for regulation of developmental expression of the same target genes.

It is interesting that lower levels of PLZF expression in more mature hematopoietic cells are restricted to the myeloid lineage (eg, 416B promyelocytic cells) and, therefore, persistent expression of the PLZF gene may be required for proper myeloid maturation. It is possible that, like the krüppel protein, which at high and low concentration can function as repressor and activator, respectively,37 at varying concentrations the PLZF gene product could exert different functions in more mature myeloid cells than in their multipotential progenitors. The potential role of the PLZF protein in maintenance of the undifferentiated phenotype of hematopoietic cells could be a factor in the mechanism underlying the pathogenesis of APL with t(11;17) (see also Chen et al47 and Licht et al48). Both the function of the wild-type PLZF gene product in hematopoiesis and the degree to which it may be affected in APL remain to be tested directly.

It is important to note that the POZ-domain is present in the PLZF-RARα chimeric protein expressed in APL cells with t(11;17)49; hence, this potentially oncogenic protein may cause transformation by interfering with functions of the wild-type PLZF and/or related POZ-domain containing proteins, as well as with the retinoid signalling pathway(s). It is interesting that, in addition to having similar subnuclear localization patterns, both PLZF and PML proteins contain structural motifs that can mediate protein-protein interaction, nucleic acid binding (Zn-fingers), and transcriptional regulation (proline-rich regions). Furthermore, these functional regions are present in the RARα chimeric proteins, suggesting that both PML- and PLZF-RARα chimeras may possess similarly altered RARα activities. This speculation is corrob-
EXPRESSION OF THE PLZF GENE DURING HEMATOPOIESIS

orated by recent results showing that both chimeric proteins similarly interfere with the function of the wild-type retinoid receptors in transfected cells and bind to the RA-response elements in vitro (data not shown). Because of their specific subnuclear localization, it is likely that both PML and PLZF proteins as well as their fusions with RARα possess sequences that target them to specific sites of action in the nucleus. In this respect it is worth noting that, as the result of t(5;17), the RARα is fused to a protein that normally possesses nucleolar localization. Although it is not yet known whether, as in the case of PML-RARe, expression of PLZF-RARe causes delocalization of the wild-type PML, retinoid X receptor, and some other nuclear proteins, given a large number of parallels that begin to emerge between the wild-type PLZF and PML, it is tempting to speculate that, at least in part, similar mechanisms may be responsible for APLs associated with the two different RARα gene translocations. An important question that remains to be answered is whether functions of the wild-type PML and PLZF can both be affected by the presence of either translocation.

ACKNOWLEDGMENT

We thank L. Wiedemann, T. Enver, J. Kabarowski, and other members of the institute for helpful comments and discussions. We are grateful to P. Chambon and M.-P. Gaub for the anti-RARα antibody and to F. Bedford, I. Titely, L. Healy, and M. Wiles for providing the B6SUTA RNA sample, purified CD34+ cells, the FDCPmixA4 cell culture, and RNAs from ES cells, respectively. We are also thankful to H. Paterson and C. Price for help with the confocal microscope.

REFERENCES

28. Kan O, Whitton AD, Heyworth CM: Development of haemo-

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.


Leukemia translocation gene, PLZF, is expressed with a speckled nuclear pattern in early hematopoietic progenitors

A Reid, A Gould, N Brand, M Cook, P Strutt, J Li, J Licht, S Waxman, R Krumlauf and A Zelent