Rearrangements of the Retinoic Acid Receptor Alpha and Promyelocytic Leukemia Zinc Finger Genes Resulting from t(11;17)(q23;21) in a Patient with Acute Promyelocytic Leukemia

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Abstract

Cytogenetic study of a patient with acute promyelocytic leukemia (APL) showed an unusual karyotype 46,xy,t(11;17)(q23;21) without apparent rearrangement of chromosome 15. Molecular studies showed rearrangements of the retinoic acid receptor alpha (RARα) gene but no rearrangement of the promyelocytic leukemia gene consistent with the cytogenetic data. Similar to t(15;17) APL, all-α-trans retinoic acid treatment in this patient produced an early leukocytosis which was followed by a myeloid maturation, but the patient died too early to achieve remission. Further molecular analysis of this patient showed a rearrangement between the RARα gene and a newly discovered zinc finger gene named PLZF (promyelocytic leukemia zinc finger). The fusion PLZF-RARα gene found in this case was not found in DNA obtained from the bone marrow of normals, APL with t(15;17) and in one patient with AML-M2 with a t(11;17). Fluorescence in situ hybridization using a PLZF specific probe localized the PLZF gene to chromosomal band 11q23.1. Partial exon/intron structure of the PLZF gene flanking the breakpoint on chromosome 11 was also established and the breakpoint within the RARα gene was mapped ~ 2 kb downstream of the exon encoding the 5' untranslated region and the unique A2 domain of the RARα isoform. (J. Clin. Invest. 1993. 91:2260–2267.) Key words: novel gene rearrangements • retinoic acid receptor • leukemia

Introduction

Acute promyelocytic leukemia (APL) is characterized by the chromosomal translocation t(15;17)(q22;q21) that is present in almost all patients (1, 2). Recently, molecular studies showed that the t(15;17) results in a chimeric gene (PML-RARα) with fusion between promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARα) genes normally localized on bands 15q22 and 17q21, respectively (3, 4). RARα is a member of the nuclear receptor superfamily gene, whereas PML may be a transcription factor belonging to a new family of DNA binding proteins (5–7). The chimeric PML-RARα gene is thought to play an important (but as yet undefined) role in differentiation and/or proliferation of APL cells.

Variant translocations have been reported in APL. They are in most instances three way translocations involving chromosomes 15, 17, and a third chromosome (8). Here we present clinical and cytogenetic data on a Chinese patient with APL and a variant translocation t((11;17)(q23;21) between a newly discovered gene (9), designated as PLZF (promyelocytic leukemia zinc finger), and the RARα locus. The PLZF gene is normally situated on a band 11q23.1 and is fused to RARα as a result of the above reciprocal translocation to form two new chimeric genes PLZF-RARα and RARα-PLZF. Additionally, we show that both PLZF(A)-RARα and PLZF(B)-RARα mRNAs were expressed in the bone marrow cells isolated from this patient.

Methods

Cytological and cytogenetic studies. The diagnosis of APL (M3) was established according to the criteria of the FAB classification (10). Cytogenetic analysis was performed on 24 h in vitro cultures of bone marrow cells. GTG-banding and RGH-banding techniques were applied. Chromosomes were classified according to the international nomenclature (11).

DNA and RNA analysis. The high molecular weight DNA was extracted according to standard procedures (12). For controls, a number of DNA samples from APL with the common t(15;17) and one AML-M2 with t(11;17)(q23;21) were analyzed. DNAs were digested with EcoRI, BglII, and HindIII to completion, size fractionated on 0.7% agarose, and Southern blotted.

Molecular cloning. Using the PLZF specific cDNA probe (9), a previously described human genomic DNA library (13) was screened. A positive phage clone 20+ was obtained (Fig. 3A, JV). The restriction map was established using a series of double digestions. A 2.3-kb BamHI-BglIII fragment (probe MB) hybridizing to the PLZF probe...
and free from repetitive sequences was isolated for in situ hybridization and Southern analysis. Subsequently, a 2.1-kb BgIII-EcoRI fragment situated just 3' to the MB probe was generated to perform the chromosome walking.

In situ hybridization. Fluorescence in situ hybridization to high resolution R-banded metaphase chromosomes was performed with the biotinylated probe MB, according to (14). Chromosomes were examined with a Leitz fluorescence microscope as previously described (14).

Reverse transcription PCR (RT-PCR) analysis. RNA was extracted with the guanidium thiocyanate CsCl gradient method. The general conditions of RT-PCR were according to the previously described procedure (15). PLZF specific primers were designed according to the PLZF cDNA sequence (9) whereas primers homologous to PML and RARα were according to the previously published sequences (6) (see Fig. 6A) for the positions of each oligos. In both PML-RARα and PLZF-RARα analyses, the same set of RARα derived oligonucleotides were used as retrotranscription primer (oligo g: 5' GTTCGTA- GTATTTGCCCAGCTGGCAGAG 3') and the 3' PCR primer (oligo f: 5' GCCTTGTAGTGCTTTCCG 3'). The 5' primer used in PML-RARα RT-PCR was derived from PML exon 3 (5' ATGCTGTAGCAGGGGTAAG 3') allowing the detection of both long (L) and short (S) PML-RARα isoforms (15). For analyzing the PLZF-RARα isoforms, different PLZF specific 5' primers were used: oligo a (situated upstream of the alternatively spliced exon): 5' GACAAT- GACACGGAGGCCAC 3'; oligo c (situated within the alternatively spliced exon): 5' AACCACAAGGGTAC-GCTGT 3'. Different oligonucleotide probes were also used including: oligo e (for RARα): 5' GTGGGACATCTTCTCA 3'; oligo c (for the alternatively spliced PLZF exon): 5' ATGAGCATGCGCTTTCCG 3'; oligo b (upstream to the alternatively spliced PLZF exon): 5' AGGACCGCAGGCTCGGTAC 3' and an oligo probe specific for PML exon 3: 5' AGCTTTGCATACCGAGG 3'.

Sequence analysis. DNA sequence was established according to the dideoxynucleotide method using the Sequenase Version II Kit (U.S. Biochemical Corp., Cleveland, OH).

Results

Clinical data. The patient was a 67-yr-old man complaining about weakness and anorexia for 1 mo, as well as coughing and gingival bleeding for a few days. Physical examination noted pallor, signs of bronchitis, purpura of the tongue without other obvious bleeding signs, cervical lymphadenopathy, and absence of hepatosplenomegaly. The renal and hepatic function tests were normal. Disseminated intravascular coagulation was not diagnosed, but occult blood in the stool was detected. The peripheral white blood cell count was 4.1 × 10⁹/liter. Bone marrow was hypercellular with 69% APL-like promyelocytes, but without Auer rods. The myeloperoxidase reaction was strongly positive.

The patient was classified as APL and was given all-trans retinoic acid (ATRA) at a dose of 60 mg/d. On day 8, the white blood cell count increased to 61 × 10⁹/liter with 72% promy-
elocytes and the bone marrow contained 77% promyelocytes. The white blood count increased to $131 \times 10^9$/liter on day 11 (promyelocytes 75%) and hepatosplenomegaly was noted for the first time. On day 16, the white blood cells decreased to $71 \times 10^9$/liter with 63% promyelocytes present in bone marrow smears, although an increase in more mature myeloid cells began to appear (Fig. 1). The ATRA treatment was continued until day 19. However, the patient developed pneumonia and respiratory failure, and he died on day 20.

**Cytogenetics.** Out of 20 metaphases examined, two were normal, 46,XY, and 16 were abnormal: 46,XY,t(11;17) (q23;21) (Fig. 2). The two remaining metaphases showed the t(11;17) and chromosome random losses.

**Molecular evidence demonstrating the fusions between the RARα and PLZF genes.** Southern analysis of DNA obtained from the patient with t(11;17) APL revealed no rearrangement of the PML gene (data not shown) and using a PLZF genomic DNA probe (MB) (see Fig. 3 A, IV) revealed rearranged bands following EcoRI and HindIII digestion (Fig. 3 B). No rearrangements or PLZF were detected in bone marrow DNAs in three normals, 10 APL patients with t(15;17), and one AML-M2 with a t(11;17) (data not shown).

The RARα gene was also rearranged in this case and the rearranged bands in EcoRI and HindIII revealed by both probes had the same size. According to our previously established RARα restriction map (Fig. 3 A, I) (13), this rearrangement is located in the intron between exon 3 encoding the 5' untranslatable region and the unique A2 domain of the RARα isoform and exon 4 encoding the B region of RARα.

The PLZF gene is localized on chromosomal band 11q23.1 by in situ hybridization. In situ hybridization of the 2.3-kb MB probe to normal metaphases showed hybridization signal as twin spots on the chromatids of one chromosome 11, subband 11q23.1 in 18 metaphases. Only one metaphase had double spots on both chromosome 11. Two metaphases showed aberrant double spots on bands 1p34 and 15q21 respectively, in addition to the localization on 11q23. It was concluded that the 2.3-kb MB probe representative of the PLZF gene was localized on 11q23.1 (Fig. 4).

**Molecular characterization of partial genomic PLZF gene region harboring the breakpoint on chromosome 11.** The genomic studies allowed us to clone a partial PLZF region of 70 kb containing the 3' part exonic sequence in the open reading frame (Fig. 3 A, IV). We identified the exon just upstream of
the breakpoint and two exons downstream (Fig. 5 for sequence). The chromosome 11 breakpoint was within an intron which separate exons encoding the second and the third PLZF zinc fingers, respectively. There is an uncloned region in this intron as shown in Fig. 3A, predicting PLZF to be a very large gene.

Both PLZF (A)-RARα and PLZF (B)-RARα chimeric transcripts are expressed in patient with t(11;17) positive APL. Recently, we have described the existence of two PLZF isoforms (A) and (B), differing by a proline-rich 369-bp exon that probably resulted from an alternative splicing mechanism (9). As the proline-rich domain has been implicated in transcription regulation, it was important to see if both PLZF isoforms could be transcribed in the PLZF-RARα fusion gene mRNA. Therefore, we performed RT-PCR using primers homologous to this exon and to the RARα B region. A specific band was obtained in the t(11;17) sample but not in two t(15;17) samples (Fig. 6B). Furthermore, when the 5' primers were replaced by those just upstream of this exon, two amplified bands were observed (Fig. 6B). These results were confirmed by sequence analysis and demonstrated that two PLZF-RARα isoforms existed in the t(11;17) patient, which differed from each other by the presence or absence of one PLZF exon. As shown in previous work, two isoforms of RARα-PLZF transcripts were also present in this case by the use of RARα1 or RARα2 promoters. This is consistent with the genomic structure of the reciprocal fusion gene RARα-PLZF (Fig. 3 A, III) (9).

Discussion

Cytogenetic study of a patient with APL showed an unusual karyotype 46,XY,t(11;17)(q23;21) without apparent rearrangement of chromosome 15. Molecular studies showed rearrangement of the RARα gene but no rearrangement of PML consistent with the cytogenetic data. The leukemic cells resembled the abnormal promyelocytes seen in the usual APL, with
Figure 4. Fluorescence in situ hybridization of the MB probe to control R-banded metaphase cell. A hybridization signal is visible on the two chromatids of a chromosome 11, on band 11q23. Counterstaining by propidium iodide.

GGATCCAGGGATGTTTCCATGGGTGCCCCTCCTGGTTCCCTCCGCTGTACCTGCCACGAG 60
AGGGATGCTGGCAAGCCCCAGGTAGGGGATGGCCCTGCCTCTTGTCCAGCCAGAGCAG 120

Zf1
K L S G M K T Y G
CAGCAACCTCTCTTCTCTCCCTACAGGAAGCTGCACAGTGGGATGAAGACGTACGG

GTGCGAGCTCTGCCGAGAAGCGGTTCCCTAGTTTGGGCCGTAAGGTGACATTACTGCC

Figure 5. Nucleic acid sequence of the 525-bp PstI genomic DNA fragment containing the PLZF exon. The splicing acceptor (AG) and donor (GT) signals are underlined. The amino acid sequence encoded by this exon is shown in one-letter codes above the DNA sequence. One histidine at the end of the first PLZF zinc finger and two cysteins and two histidines constituting the second C2H2 zinc finger are circled. This exon is situated upstream of the PLZF breakpoint and is fused to the RARα B region in PLZF-RARα chimeric transcripts.

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the exception that Auer rods were not present. Similar to t(15;17) APL, ATRA treatment exacerbated a leukocytosis that was followed by morphologic evidence of myeloid maturation, but the patient died before treatment could be fully evaluated. These data are consistent with the diagnosis of APL, with a variant translocation.

Variant translocations have been reported in APL. These may involve three or more chromosomes including 15 and 17. In other cases, only two chromosomes were apparently involved and the translocations were between 17 and another chromosome in most instances. In these cases, the two 15 chromosomes were morphologically normal (16). This is reminiscent of the variant Ph translocations in chronic myelogenous leukemia, in which a BCR-ABL rearrangement was consistently found even when chromosomes appeared normal under the microscope (17). There is, however, no data presently available describing molecular rearrangements associated with variant translocations of APL. The RARα gene was not rearranged in one patient without apparent involvement of chromosome 17 (5). Rearrangement of the RARα and PML genes was pres-

Figure 6. (A) Schematic representation of the protein-coding region of the two PLZF-RARα isoforms that differ by the presence (∆PLZF(B)-RARα) or absence (∆PLZF(A)-RARα) of PLZF B isoform exon (here temporarily referred to as exon 2). The exon which was fused to RARα B-region is defined by genomic DNA analysis (exon 4, see Fig. 5). The sequence between exons 2 and 4 could be one PLZF exon (exon 3). The exon composition in the region 1 remains to be established. The first and last amino acids, as well as those at the ends of exons 1 to 4, are represented by three-letter codes and are numbered according to Chen et al. (9). The black triangles indicate the junction between PLZF and RARα coding sequences. The positions of oligonucleotides used in PCR analysis of PLZF-RARα isoforms are indicated either by arrows (primers) or by bars (probes). The nucleic acid sequences of these oligonucleotides are shown in Materials and Methods. (B) RT-PCR experiments showing the specific presence of PLZF(A)-RARα and PLZF(B)-RARα isoforms in leukemic cells with the t(11;17). RNA derived from the t(11;17) cells (lanes 1, 4, and 7), the t(15;17) cells expressing the long (L, lanes 2, 3, and 8) or short (S, lanes 6, and 9) PML-RARα fusion transcripts were analyzed. The retrotranscription was carried out in all cases using oligonucleotide g corresponding to the B region of RARα. For detecting the PML-RARα fusion transcripts (lanes 1–3), 5’ primer homologous to the PLZF exon 4 and 3’ primer situated in RARα B region (oligonucleotide f) were used. For analyzing PLZF-RARα isoforms, two different 5’ primers were used, which allow to amplify either both isoforms (oligonucleotide a, lanes 4–6) or only the PLZF(B)-RARα isoform (oligonucleotide c, lanes 7–9) when the same 3’ primer (oligonucleotide f) were used. PCR products on the same blot were hybridized sequentially to different probes specific for RARα B region (oligonucleotide e, I); PML exon 3 (II); PLZF region 1 (oligonucleotide b, III); and PLZF B form exon (oligonucleotide d, IV). Molecular standards are shown in basespairs on the left. Note that the PML-RARα PCR products were present only in the t(15;17) samples, while those of the PLZF-RARα were detected only in t(11;17) cells. In addition, in different sets of PCR, two bands corresponding to the PLZF(A)-RARα and PLZF(B)-RARα (lane 4, I and III) or one band specific for the PLZF(B)-RARα (lane 7, I and IV) were obtained when appropriate primer pairs were used.
ent in another patient with an unusual abnormal karyotype without apparent chromosome 15 rearrangement (Baranger et al., manuscript submitted for publication).

A variant t(11;17) positive APL was previously reported (18) but not studied for molecular rearrangements. In the present case, the t(11;17) was associated with rearrangement of the RARα gene while the PML gene was structurally intact. The chromosome 11 gene (PLZF) involved in rearrangement with RARα in this unique case of APL has recently been cloned (9). This gene has some homology with the zinc finger gene MZF-1 (19) and is also retinoic acid responsive and preferentially expressed in myeloid cells (19). Because of t(11;17), the PLZF gene is disrupted in its zinc finger containing region, with fusion of two zinc fingers to the RARα B region in the PLZF-RARα fusion gene, while seven zinc fingers joined the RARα region in the reciprocal RARα-PLZF chimeric gene. It is noteworthy that the RARα gene was disrupted in its third intron as commonly found in the standard t(15;17). The association of both PML-RARα and PLZF-RARα fusions with the APL phenotype argues for a key role for RARα in the hybrid genes. In support of this is the observation that two cases of myelodysplastic syndrome transformed to APL with trisomy 11 without RARα rearrangements, failed to respond to ATRA (20). However, since the role of PML and PLZF in leukemic cell proliferation and differentiation remains largely unknown, it is also possible that PML and PLZF have an equivalent function when rearranged with the RARα gene.

Chromosomal band 11q23 is rearranged in several varieties of hematopoietic malignancies (17). Breakpoints of chromosome 11 in acute leukemias with t(4;11), t(6;11), t(9;11) and t(11;19) have been localized within 300 kb downstream of the CD3D gene (21–24). The breakpoint was found more distal in a leukemia with t(11;19) than in leukemias with one of the other three translocations (25), suggesting that there is a heterogeneity in the localization of the breakpoints within the band 11q23, as already shown for the t(11;14)(q23;p32) translocation of lymphoid malignancies (26, 27). The localization of the PLZF gene on 11q23.1, which is centrometric to the other breakpoint localizations of most of the recurrent translocations of hematopoietic malignancies, also argues in favor of the breakpoint heterogeneity of malignancies with 11q23 rearrangements. Interestingly, the molecular study of another translocation t(11;17) detected in a patient with acute myeloblastic leukemia M2, and thus clinically and cytologically different from APL, did not show rearrangements of the RARα nor PLZF genes. In our most recent studies, we have identified a second case of t(11;17) APL (in collaboration with Wilson Miller) which expressed the PLZF-RARα fusion gene. This is concordant with the specificity of the gene rearrangements in APL. The PLZF gene is a putative transcription factor that appears to be associated with myeloid differentiation (9) and may be deranged in other myeloid malignancies. The identification of PLZF justifies that molecular studies of other APL variant translocations should be systematically performed to determine if genes other than PML (or RARα) may be involved in these APLs. 

Acknowledgments

This work was supported by the National Chinese Foundation for the Natural Sciences, the Leukaemia Research Fund of Great Britain, the Samuel Waxman Cancer Research Foundation, and the Ligue Nationale Contre le Cancer.

References


