Molecular analysis of acute promyelocytic leukemia and its variant fusion transcripts: A Hospital based study

Abstract:

Acute promyelocytic leukemia (APL) is distinguish by the augmentation of promyelocytes in bone marrow. Nearly 95% of patients with this disease belong to typical APL, which express PML-RARA and and they exhibit magnificent clinical outcome. Compared to typical APL, variant APL showed quite different characteristics, and at present it is still challenged how to recognize, diagnose, and treat variant APL. Herein, we shared our clinical experiences about variant APL . By contrast, three regions of the PML locus are involved in the translocation breakpoints: intron 6 (bcr1; 55% of cases), exon 6 (bcr2; 5%), and intron 3 (bcr3; 40%). Through this article, we hoped to provide genetic perspective of variant APL.

Introduction

The t(15;17) is associated with acute promyelocytic leukemia (APL), a distinct AML subset with M3 cytomorphology.148 The chromosomal breakpoints regions have been variously mapped to 15q22-q24 and 17q11-q21. The chromosomal break sites were isolated by four groups using distinct experimental approaches.149–152 The two genes involved in t(15;17) are PML, coding for a putative novel transcription factor, on chromosome 15149–152 and the retinoic acid receptor-a (RARA) gene on chromosome 17.153,154 The chromosome 17 breakpoints are localized within a 15 kb DNA fragment of the RARA intron 2 (Figure 14a). By contrast, three regions of the PML locus are involved in the translocation breakpoints: intron 6 (bcr1; 55% of cases), exon 6 (bcr2; 5%), and intron 3 (bcr3; 40%). Chimeric PML-RARA and RARA-PML transcripts are formed as a consequence of the reciprocal translocation between the PML and RARA loci. The existence of different breakpoint regions in the PML locus and the presence of alternative splicing of PML transcripts are responsible for the great heterogeneity of PML-RARA junctions observed among APL patients (reviewed in Ref. 155). Moreover, the alternative usage of two RARA polyadenylation sites generates extra PML-RARA transcripts of different size.155 The observation that RARA-PML transcripts are present in most but not all APL cases, has favored the use of PML-RARA transcripts as PCR target for detection of APL cells at diagnosis and during monitoring. The vast majority of analyzed series compared the two major PML-RARA isoforms, referred to as long (L) transcripts (including PML bcr1 and bcr2) and short (S) transcripts (PML bcr3). Because bcr2 (also referred to as 'variant' or V form) and bcr1 are located in PML exon 6 and intron 6, respectively, sequencing of all L transcript cases would be needed to clearly distinguish these two isoforms. Such distinction is usually not reported in clinical studies with a large number of patients.157

Material and method

Patients samples

A group of 14 patients with acute promyelocytic leukemia (APL) were studied at diagnosis in the department of Pathology, Mahavir Cancer Sansthan Patna. The diagnosis of 14 cases was based on standard morphological criteria. Samples chosen for molecular analysis of APL phenotyping were obtained from Acute promyelocytic leukemia: patient characteristics at diagnosis (PB): all specimens contained at least 80% and in most cases more than 90% leukemic cells.

Primer design for Reverse Transcriptase-PCR analysis

Primers for PML RARA was designed BIOMED 1 Concerted action paper. (Ref)

PCR protocol

RNA extracted by Trizol method, cDNA was prepared by using random hexamers. Nested PCR was performed by using specific primers of PML-RARA) followed by agarose gel electrophoresis or polyacrylamide gel electrophoresis for detection of PCR products of a particular size.

Statistical analysis

Proportional differences between patient groups were analyzed by $\chi 2$ or Fisher exact tests. Depending on the distribution of variables, correlation analyses were performed by computing contingency tables, Pearson, or Spearman correlation coefficients. Comparison of gene expression between groups was performed using the Mann–Whitney U test.14 A P value less than 0.05 was considered significant.

Primers for RT-PCR analysis of PML-RARA (M3)

Primer code	Sequence(5-3)
PML-A1	CAGTGTACGCCTTCTCCATCA
PML-A2	CTGCTGGAGGCTGTGGAC
RARA-B	GCTTGTAGATGCGGGGTAGA
PML-C1	TCAAGATGGAGTCTGAGGAGG
PML-C2	AGCGCGACTACGAGGAGAT
RARA-D	CTGCTGCTCTGGGTCTCAAT

Table . Acute promyelocytic leukemia: patient characteristics at diagnosis

No of patients	14
Males/Females	
Median age (range) years	
Hypergranular (M3)	
Hemorrhagic syndrome	
Hb median (range) g/dl	9.9 (4-13.2)
WBC median (range)/mmc	3.4 (0.6-57.5)
Platelets median (range)/rnmc	36 (9-83)
Fibrinogen median (range)ldl	100 (35-375)
FDP median (range) pg/ml	80 (10-160)

Result

The main clinical characteristics of the 14 APL patients are depicted in Table 1. The M/F ratio is …. , the median age was …….. yr. Low fibrinogen levels associated to high FDP levels were frequently observed (see Table 1). Microscopic analysis performed in all cases revealed that the typical PML-RARA. By molecular analysis in 14 cases we found that 8 cases are bcr1 positive, 6 cases are bcr3 positive and we did not find bcr2 positive cases.

Variant of PML-RARA	No of cases positive
bcr1	8
bcr2	0
bcr3	6

In order to cover all three breakpoints in the PML gene, two extra forward primers were designed. Primer sets A1 ↔ B and C1 ↔ D cover the bcr1 and bcr2 breakpoints, whereas primer sets A2 ↔ B and C2 ↔ D can detect PML-RARA transcripts derived from PML breakpoints in bcr3. The precise sequence information and relative position of the six primers is given in Table . No extra bands are usually found in PCR products run in 1.5% agarose gels and visualized with ethidium-bromide staining. However, multiple bands can appear in PCR products from bcr1+ patients, if primers are used for bcr3 breakpoint identification. These extra bands are caused by alternative splicing of PML exons.

Conclusion (Genetic and phenotypic paper)

This article demonstrated the amount of possible APL variants and translocations. We have reported 14 variants of APL (Fig.); However, these variants are uncommon and have been only reported as case reports or case series, thus limiting the possibility of providing conclusions about those variants. Primary observations include that these variants are different from the classical APL in many ways that include their pathological and cytochemical features. Future considerations should include the improvement of our detection abilities for these variants and investigation of more therapies that might provide a better outcome, including hematopoietic cell transplantation. The role of transplantation in classical APL management is usually reserved for patients with relapsed APL, with autologous being favored over allogeneic hematopoietic cell transplantation [134,135]. In APL variants, transplantation was rarely used as a modality. As inferred by the current literature on APL variants, managing these patients might be challenging due to late diagnosis and rapid deterioration. The future research agenda will be limited by the rarity of these cases, necessitating more robust in vitro studies and registry based or multi-institutional studies, to further characterize these variants. As described above, APL variants are very rare; thus, no defined characterization has been achieved of their clinical features. Clinical, laboratory, and cytochemical characteristics might not be reliable to suspect APL translocation variants. Therefore, genetic studies with confirmed translocation will confirm the diagnosis. APL variants pose a management challenge as there are no defined management regimens.

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