

A novel cytogenetic abnormality r(7)(::p11.2->q36.3::) in a Philadelphia-positive chronic myeloid leukemia case

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ABSTRACT

The so-called Philadelphia (Ph) chromosome is present in more than 90% of chronic myeloid leukemia (CML) cases. It results in juxtaposition of the 5' part of the *BCR* gene on chromosome 22 and the 3' part of the *ABL1* gene on chromosome 9. An additional acquired monosomy 7 or deletion of 7q is associated with poor prognosis in a variety of myeloid disorders. Here we report a novel Ph chromosome positive CML case with a ring chromosome 7 [r(7)]. Immunophenotyping was compatible with CML, although 4.5% of total leucocytes appeared like acute myelogenous leukemia (AML) subtype M2. The r(7) was characterized in detail by array-proven multicolor banding (aMCB), the latter being of enormous significance to characterize breakpoint regions in detail. Underlying mechanisms and prognostic are discussed, as ring chromosomes are rare cytogenetic abnormalities in hematopoietic malignancies.

Keywords: Chronic Myeloid Leukemia (CML); Ring Chromosome 7; Del(7p); Fluorescence *in Situ* Hybridization (FISH); Reverse Transcription Polymerase Chain Reaction (RT-PCR); Array-Proven Multicolor Banding (aMCB)

1. INTRODUCTION

The so-called Philadelphia (Ph) chromosome is typical

for by far over 95% of patients suffering from chronic myeloid leukemia (CML), a clonal malignant disorder of a pluripotent hematopoietic stem cell. A reciprocal translocation t(9;22)(q34;q11) leads to the formation of the Philadelphia (Ph) chromosome and a derivative of chromosome 9. The 3' portion of the *ABL1* oncogene is translocated from 9q34 to the 5' portion of the *BCR* gene on 22q11.2. This leads to the formation of a chimeric *BCR/ABL* gene on the derivative chromosome 22 [1]. It is known since years that the expression of the chimeric *BCR/ABL* protein has an increased tyrosine kinase activity and plays an essential role in the pathogenesis of CML [2].

Ring chromosomes are rare cytogenetic abnormalities that occur when the two ends of a chromosome fuse together and form a ring shape. Breaks in the chromosome arms and fusion of the proximal broken ends can lead to ring formation with loss of distal chromosomal material. Alternatively, rings can be formed by telomere dysfunction [3].

Complete or partial loss of chromosome 7, predominantly monosomy 7 or deletion of 7q, is associated with a variety of myeloid disorders, including de novo pre-leukemic myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) in children and adults, as well as therapy-related ones in the latter [4,5]. Also it has been reported in adult ALL, in which they frequently occur as secondary aberrations associated with a Ph chromosome and it is an adverse factor in both childhood and adult Ph+ ALL [6]. Additionally, deletions of 7p confer an inferior outcome in children with ALL, regardless of the presence of other poor prognostic features,

whereas deletions of 7q are not associated with a worse outcome [6].

Here we reported a novel case of a Ph chromosome positive CML with r(7) and immunophenotyping consistent with CML, although 4.5% of total leucocytes showed AML M2 subtype.

2. MATERIALS AND METHODS

2.1. Case Report

A 55-year-old male was diagnosed as suffering from CML in chronic phase (CP). In June 2010 the white blood cell count (WBC) was $93 \times 10^9/l$ with 49.3% neutrophils, 19.6% lymphocytes, 14.1% monocytes, 3.4% eosinophils, and 13.6% basophils. The platelets count was $181 \times 10^9/l$ and the hemoglobin level was 10.6 g/dl. A previous physical examination revealed splenomegaly. Serum lactate dehydrogenase (LDH) was 2057 U/l (normal up to 480 U/l), serum alanine aminotransferase (ALT) was 46 U/l (normal up to 41 U/l) and serum aspartate aminotransferase (AST) was 42 U/l (normal up to 40 U/l). Afterwards he was lost during follow-up.

2.2. Chromosome Analysis

Chromosome analysis using GTG-banding was performed according to standard procedures [7] before chemotherapeutic treatment. A total of 20 metaphase cells derived from unstimulated bone marrow culture were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [8].

2.3. Molecular Cytogenetics

Fluorescence *in situ* hybridization (FISH) using LSI BCR/ABL dual color dual fusion translocation probe (Abbott Molecular/Vysis, USA) was applied according to manufacturer's instructions [9]. FISH using a chromosome-7-specific aMCB probe set based on microdissection derived region-specific libraries was done as previously reported [10]. A total of 20 metaphase spreads were analyzed, each using a fluorescence microscope (AxioImager.Z1 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes plus the counterstain DAPI (4',6-diamino-2-phenylindole). Image capturing and processing were carried out using an ISIS imaging system (MetaSystems, Altlussheim, Germany).

2.4. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for BCR/ABL Fusion Transcripts

RT-PCR was carried out as previously described [11].

2.5. Immunophenotyping

Immunophenotyping of leukemic blasts was performed as previously described [12].

3. RESULTS

Prior to chemotherapy treatment banding cytogenetics revealed a karyotype 46, XY, t(9;22) [7]/46, XY, idem, r(7) [11]/47, XY, idem + 8 [2] (**Figure 1(a)**). Dual-color-FISH using a probe specific for BCR and ABL revealed that a typical Ph chromosome with BCR/ABL-translocation was present (**Figure 1(b)**). RT-PCR analysis of the fusion transcript showed a band corresponding to the b2a2 transcript, most often found in CML (data not shown). Together with aMCB-result (**Figure 1(c)**) the final karyotype was determined as: 46, XY, t(9;22)(q34;q11)[7]/46, XY, r(7)(::p11.2->q36.3::), t(9;22)(q34;q11)[11]/47, XY,+8,t(9;22)(q34;q11)[2]/.

The specimen submitted has a high WBC count $>100,000$ cells/mm³. Neutrophils (86% of all leucocytes), showed abnormal intensity staining patterns for CD16 (49.4%), CD32 (62.7%), CD10 (31%), CD33 (49.4%), CD15 (57.1%), CD13 (43.3%), CD11b (27.2%), and CD11c (41.5%). These cells expressed CD123 (67.5%). Overall, this result was indicative for chronic

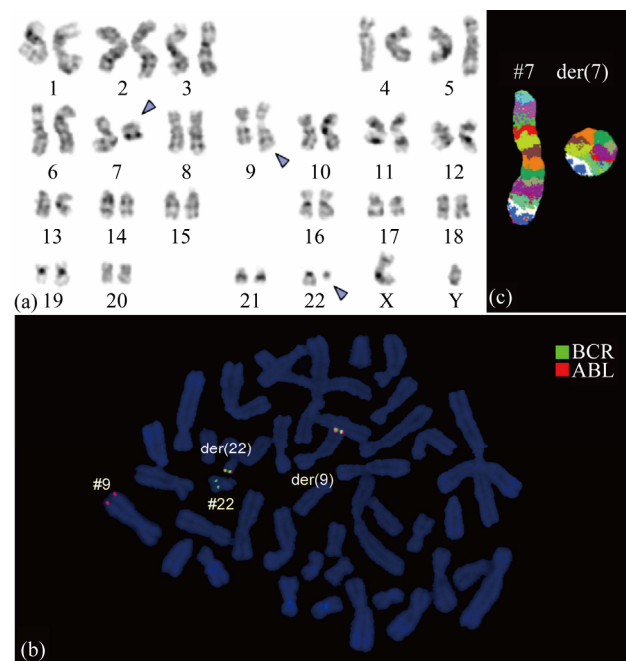


Figure 1. (a) GTG-banding revealed a r(7)(::p11.2->q36.3::) besides derivative chromosomes 9 and 22. All derivative chromosomes are highlighted by arrow heads; (b) Metaphase FISH using probes for BCR (green) and ABL (red) confirmed Ph chromosome presence; (c) The application of aMCB 7 characterized the r(7)(::p11.2->q36.3::) comprehensively. Abbreviations: # = chromosome; der = derivative chromosome; Ph = Philadelphia-chromosome.

myeloproliferative disorder, most likely a CML, subsequent to an MDS. However, there was as well another cell population, which represented ~4.5% of all leucocytes, showing an AML-M2 phenotype. These cells have high forward scatter, low side scatter pattern and were CD45⁺ dim (4.5%), CD34⁺ (4.9%), CD33⁺ (4.4%), CD38⁺ (4.8%), CD32⁺ (4.9%), CD123⁺ (4.9%). These cells expressed CD13 (2.6%), CD15 (2.5%), CD11c (1.9%), HLADr (2.8%), CD117 (2.3%) heterogeneously, and were CD16⁻, CD64⁻, CD11b⁻, CD10⁻, CD41a⁻, CD235a⁻, CD3⁻, CD19⁻.

4. DISCUSSION

According to the literature, a r(7) involved the short arm is a rare but recurrent cytogenetic abnormality observable in AML {r(7)(p15q35) and r(7)(p22q31)} [13], hepatosplenic T-cell lymphoma [r(7)(p?:q?) [14]] and acute megakaryoblastic leukemia [15]. To the best of our knowledge, the present case is the only one ever seen case of a Ph chromosome-positive CML-CP with de novo a r(7)(:p11.2->q36.3::); notably there was another Ph-positive clone with trisomy 8 as secondary abnormality [14].

The progression of CML from CP to blast crisis (BC) is frequently associated with nonrandom secondary chromosomal aberrations such as +8, i(17q), +19 and an extra Ph chromosome [16].

Ring chromosomes are rare cytogenetic abnormalities that occur in less than 10% of hematopoietic malignancies but have been reported in up to 70% of mesenchymal tumors [3].

Monosomy 7 or deletion of 7q in these disorders is associated with poor prognosis [17]. It has been hypothesized that there is a tumor suppressor gene (TSG) on chromosome arm 7q that contributes to the pathogenesis of these diseases [18]. However, monosomy 7 or structural abnormalities resulting in the deletion of 7p were infrequent in childhood ALL to confer increased risk of treatment failure in Ph-positive cases, and it has been hypothesized that a TSG on 7p may contribute to the poor outcome of these patients [6].

Both monosomy 7 and del (7p) are of the additional chromosomal abnormalities and they associated with adverse prognostic factors in CML patients treated with IM as a frontline therapy [19,20].

Recent studies have reported that major route abnormalities such as trisomy 8 at diagnosis were related to a worse outcome t(9,22) [20,21].

In conclusion, we reported here a novel case of a Ph chromosome positive CML in chronic phase with a new cytogenetic abnormality r(7) resulting in the deletion of 7p and immunophenotyping was consistent with CML although 4.5% of total leucocytes showed acute myelogenous leukemia (AML) subtype M2. The r(7) and

del 7p might be a marker for adverse prognosis in CML.

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