Conventional and fluorescence in situ hybridization analysis of three-way complex BCR-ABL rearrangement in a chronic myeloid leukemia patient

ABSTRACT
Chromosomal analysis was carried out in bone marrow sample of an 11-year-old girl suspected of myeloproliferative disorder. Conventional G-banding study detected a complex three-way translocation involving 7, 9 and 22, which has resulted in the formation of a variant Philadelphia chromosome causing rearrangement of abl and bcr genes in 87% cells. Fluorescence in situ hybridization (FISH) confirmed the fusion of bcr-abl oncogene. Thus the bone marrow karyotype was observed as 46,XX (13%) / 46,XX,t(7;9;22)(q11;q34;q11) (87%). Hyperdiploidy was present in two cells. In this study, both conventional cytogenetic and FISH diagnosis proved to be significant to identify the variant nature of the Philadelphia chromosome and hyperdiploid condition for introduction of a suitable treatment regimen and estimation of life expectancy of the young girl.

KEY WORDS: BCR-ABL fusion, chronic myeloid leukemia, fluorescence in situ hybridization, t(7;9;22), variant Philadelphia chromosome

INTRODUCTION
Chronic myeloid leukaemia (CML), a clonal myeloproliferative disease, occurs following an acquired somatic mutation in a pluripotent bone marrow stem cell. In more than 90% of classic CML patients, a simple two-way exchange between breakpoint cluster region gene (bcr (22q11)) and Ableson murine sarcoma virus gene (abl (9q34)) results in the cytogenetically obvious chromosomal translocation, t(9;22)(q34;q11), the product of which is a small derivative chromosome 22,[1] which is well known as the Philadelphia chromosome (Ph).[2] The translocation causes fusion or rearrangement of the two genes and forms a chimeric bcr-abl oncogene[3] that contains the protein coding sequences.

During bcr-abl translocations, the breakpoint on 9 involves a large (~200kb) region within the abl alternative exons 1 and 2, while chromosome 22 breakpoints are clustered within the major breakpoint cluster region (M-bcr, a 5.8 kb region spanning exons 12-16).[4] The juxtaposition of abl gene with bcr gene results in transcription of a chimeric mRNA of 8.5 kb instead of normal abl transcript of 6-7 kb and consequently translated to an activated bcr-abl gene product of 210 kd, instead of the normal abl gene product of 145 kd.[5] Abl protein is normally a nuclear protein whereas the bcr/abl protein is located in the cytoplasmic surface of the cell membrane and acquires a novel function in transmitting growth regulatory signals from cell surface receptors to the nucleus via the signal transduction pathway of the RAS protein.[6] This displays an upregulated tyrosine kinase activity that leads to the recruitment of downstream effectors of cell proliferation and cell survival and consequently cell transformation.

In other CML patients, approximately 5-8%, bcr-abl gene association arises from complex rearrangements which may clearly involve one or more chromosomes in addition to 9 and 22 or which may be masked by an apparently normal karyotype.[7] The hybrid bcr-abl product is known as variant Philadelphia chromosome and disease is known as variant CML. In variant CML, consistent bcr-abl mutation is obviously seen beside involvement of other chromosome(s). Investigation of the mutation in such complex translocation is sometime not possible by conventional cytogenetic technique; however, molecular techniques are efficient to discern the complex bcr-abl translocation. The clinical symptoms of CML are generally present, however, the prognosis and life expectancy could differ due to presence of other gene rearrangement. The distinction between classical and variant CML has become increasingly important in view of
We report a case of myeloproliferative disorder carrying a complex translocation involving abl and bcr genes and chromosome 7 and confirmed as a variant CML.

CASE REPORT

The present case, an eleven-year-old girl, was referred for cytogenetic study by cell culture. The trephine biopsy diagnosed her as a case of myeloproliferative disorder.

Clinical symptoms

Chromosome study following conventional G-banding in her bone marrow cells detected a complex three-way translocation in 87% cells involving 7, 9 and 22 with breakpoints at 7(q11), 9(q34) and 22(q11) respectively where chromosome 22 appeared as a Philadelphia chromosome [Figure 1]. Since derivative 9 was different from its form in classical t(9;22), FISH test on interphase nuclei with DNA probes (Vysis, USA) confirmed fusion of abl and bcr genes [Figure 2]. The chromosomal pattern in bone marrow was confirmed as a complex three-way translocation with 46,XX,t(7;9;22)(q11;q34;q11) (87%)/46,XX (13%) constitution. The diagnosis was confirmed as chronic phase of variant chronic myeloid leukemia (CP-CML).

Chromosomal breakpoints and genomic recombination products of the complex rearrangements, including abl and bcr relocations, are presented in Figure 1. Normal chromosomes are shown on the left of each homologue pair and derivative chromosomes to the right.

DISCUSSION

In all complex bcr-abl rearrangements, the 5' part of bcr recombines with abl to form the 5' bcr-abl fusion gene. However, the 3' part of bcr, which unites with the 5' abl remnant in the standard t(9;22)(q34;q11), usually recombines with one of the additional chromosomes in the complex translocations or with part of chromosome 9 outside the abl gene.[9] Thus, in the present case, the 3' part of bcr has joined with chromosome 7 within the disrupted band q11.23. The similar mechanism could have worked in our previous reported cases (Ganguly et al., 2006, Ganguly and Agarwal, 2005). Tefferi et al.[10] described that the 3' part of M-bcr recombined within or immediately adjacent to Alu elements at the additional sites in complex bcr-abl rearrangements and suggested that Alu sequences have an affinity for the bcr-abl recombination process in complex rearrangements and provides additional evidence for the association of these elements with somatic rearrangements, which cause human leukemia. The DNA sequence surrounding the 3' M-bcr junction sites showed limited clusters of 2-4 bp homology, when M-bcr sequences were aligned directly against the recombination sites on chromosome 7q11.23. Alu sequences are also over represented in Zhang et al.[11] study at sites of recombination in the abl gene compared with their overall incidence throughout the genome. From a number of studies it is interpreted that Alu sequences may have a role in bcr-abl recombination, which initiates CML.

Alu elements may hold chromosome regions near each other so that recombination is more likely to occur in their vicinity. Two-dimensional FISH studies of interphase lymphocytes revealed that the bcr and abl genes map closer together more often than expected by chance, providing the first evidence that physical juxtaposition of chromosomal parts may influence the frequency of t(9;22) recombination.[12] However, the mechanisms that determine chromosome geography in the interphase nucleus remain controversial.[13]

The Philadelphia chromosome, though is the genetic etiology of CML, gives good prognostic outcome to patients observed in many patients. The evolution of the disease in presence
of a third rearrangement in Philadelphia positive patients has not been fully understood due mainly to limited number of variant CML patients. The altered genes at 7q11 could possibly have some passive effects due to dominance of \( bcr-abl \) mutation. However, gene-gene interaction could lead to progress or diminish the disease. It has been observed that CML patients with deletions and variant Ph Chromosome had poorer survival. A long-term clinical and cytogenetic follow-up could lead to better understanding of the clinical impact of the complex three-way rearrangements.

The present unbalanced three-way translocation directed \( bcr-abl \) fusion on 22 from chromosome 9, rearrangements between 22 and 7 and 7 and 9. In CML, the impact of the rearrangement in derivative 9 has not gained much importance because of the oncogenic potentility of that in 22. Chromosome 7q11 harbors genes for William syndrome, Zellweger syndrome, NADPH-dependent cytochrome P-450 reductase and \( NCF1 \) in autosomal inherited chronic granulomatous disease and others. Thus the rearrangement in derivative 7 will have some obvious effect on the prognosis or evolution of such variant CML.

The present case was important to understand the molecular mechanism of hybrid \( bcr-abl \) formation and its relevance to disease progression in variant CML. Diagnostically, investigation of variant translocation was important. However, more such cases need to be discussed to get the insight of the disease mechanism and its future.

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