

Variant complex chromosome translocation with chronic and acute myeloid leukemia

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ABSTRACT

A large number of simple or complex translocation involving the CML and AML chromosomal abnormalities has been described. This study was aimed to investigate the complex chromosome aberrations in the series of myeloid malignancies including CML and AML. The present report deals analyzed 187 consecutive with CML and AML patients, using Methotrexate cell synchronization and un-stimulated cultures of cells to determine the incidence of chromosomal aberrations and association of complex variant chromosome anomalies according to French American British morphological subtypes. The results revealed an abnormal karyotype with a novel complex translocation involving chromosomes 1,2,4,9,11,22. Complex variant translocations were found in two cases of AML and two cases of CML. The present reports provides sufficient grounds for assuming that a chromosomes involving of complex abnormalities plays an important role in the development of malignancy.

Keywords: CML; AML; Complex variant translocation; Malignancy; Iran

INTRODUCTION

Chromosomal abnormalities in neoplasia appear to be nonrandom and tend to cluster to certain chromosomes. All human chromosomes except the Y chromosome have been involved in complex chromosome translocation [1]. Acute Myeloid Leukemia (AML) is a malignant neoplasm of hematopoietic stem cells characterized by an abnormal proliferation of myeloid precursors, a reduced rate of apoptosis, and an arrest in cellular differentiation[2]. Variant t(8;21) translocations have been reported in 6–10% of cases with the RUNX1/CBFA2T1 fusion gene [3]. The majority of these patients shows three way complex translocations involving another chromosome in addition to 8q22 and 21q22 [4], whereas few reports have shown the occurrence of four-way rearrangements [5].

Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia chromosome (Ph) and the formation of the BCR-ABL1 fusion. More

than 85% of cases of CML are characterized by the presence of Ph chromosome in their bone marrow, the remaining of the cases a translocation between chromosome 22 and other than 9, or complex three or four way translocation are identified [6]. Variants of the Philadelphia translocation and complex translocations involving BCR have been reported in myeloproliferative disorders [6]. A rare translocation, t(9;22)(p24;q11.2), resulting in a novel BCR-JAK2 fusion has been reported in a handful of cases of chronic and acute myelogenous leukemia [7].

MATERIALS AND METHODS

The total of 187 of de novo AML and CML patients were selected on the basis of complexity of the chromosome abnormalities. Complexity was defined as the presence of three or more different chromosome abnormalities in the malignant clone and/or variant i.e., changes from cell to cell despite the presence of a clonal origin.

Cytogenetic analysis

As part of thesis research and project study, we used these data to investigate the chromosome 1,2,4,9,11,22 abnormalities in complex chromosome aberrations in myeloid malignancies. During the last twelve years, chromosome banding studies were performed on 187 unselected consecutive either adults sex patients with de novo CML and AML admitted to the major referral hospitals affiliated of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conventional cytogenetic studies of a 24- to 48-h culture were carried out on bone marrow cells by standard methods and evaluated by giemsa-trypsin-giemsa banding according to the ISCN [8]. A minimum of 20 metaphases were analyzed. Approximately 0.5 ml of bone marrow / peripheral blood was obtained from each participant. The blood sampling and cell culture procedures were essentially the same for all the participants. Briefly, heparanized blood was immediately mixed with 4 ml RPMI – 1640 (Gibco BRL, USA) cell cultured medium supplement with 15-20% heat inactivated fetal bovine serum (Gibco BRL, USA), 100mg/ml Phytohemagglutinin (PHA) in a Vacutainer tube (Becton Dickinson Co, Ltd USA). This tube cultured for 70 hours at 37°C under the aeration of 5% Co₂ in the incubator. After an incubation period, the cultured cell harvested by 75 ml colcemide 10µg/ml (Gibco BRL USA) and incubated at 37°C for 30 minutes. The contents of the tube were then centrifuged for 10 min at 1000 rpm and resuspended in 10 ml of 75 mM KCl 0.56% (Sigma, Co) prewarmed to 37°C for 20 min. At this stage 1ml of 20°C Carnoy's Fixative 3:1 methanol: acetic acid (Fisher Scientific) was added in to the tube to stop further cell swelling. This fixation repeated four times. Then cells dropped on to clean slides, and cultured for 3 days at 60°C on the slide warmer. Slides then banded for 10 second with 0.2 X trypsin (Difco, Co USA) and stained for 3 min Giemsa (Harleco, Co) [9]. Slides were examined with an Olympus model BH-2 light microscope. Eighty well spread G-banded metaphases were analyzed.

RESULTS

Cytogenetic analysis of bone marrow cells revealed an abnormal karyotype with a novel complex translocation involving chromosomes 1,2,4,9,11,22. Complex variant translocations were found in two cases of AML and two cases of CML. This study demonstrates unique cases of two complex variant translocations designated as, the three-way translocation was confirmed with spectral karyotyping t(2;4;21) and other with t(2;4) and monosomy 21 seen independently in the same patient M2-AML. Another patient with a rare or not yet described chromosomal aberration, exhibited t(9;22), der(22) (9;22), ring chromosome 15 and two marker chromosomes, seen in 5 cells in M2-AML.



Figure. 1 Karyotype of bone marrow cell showing 46,XX,t(2;5;9;22).

In CML patient, in whom complex translocation was noticed, showed 46,XY, t(1,9,11,22) in 5 cells. In the second patient, a 24 years old female, cytogenetic study indicates clonal abnormality involving complex Ph translocation viz., 46,XX, t(2;5;9;22) in 8 cells (Figure.1). In all cases had complex chromosomal abnormalities without any history of previous malignant diseases or

occupational or therapeutic exposure. The percentage of all abnormal cytogenetic cells was recorded to be between 25 to 100% . The

distribution of total chromosomal changes revealed as Figure2 [10-11].

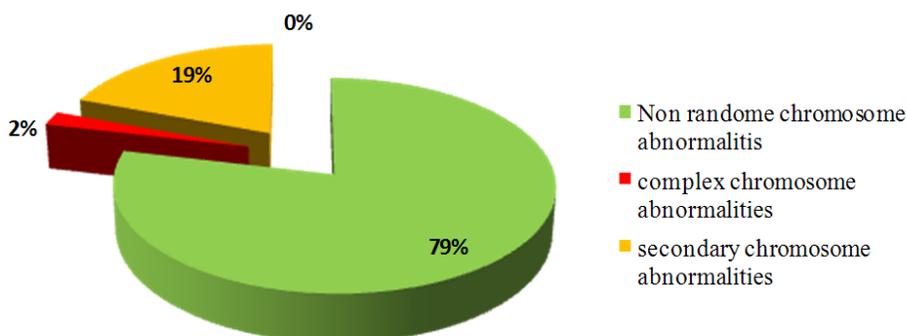


Figure 2. Distribution of chromosomal damages in myeloid leukemia

DISCUSSION

A large number of simple or complex translocation involving the CML and AML chromosomal abnormalities have been described in the current literature [12-18]. The present report describes cases of CML and AML-M2 with a unusual translocation involving chromosomes 1,2,4,9,11,22 with the various break points. Complex translocations of t(8;21), involving chromosome 1, 5, 6, 11, 12, 15, 17, or 19 have been reported elsewhere[19-20], in patients with AML (M2) with similar clinical features as those of t(8;21), including good response to therapy. These cases with complex translocations, including the importance of AML1-MTG8(ETO) transcript in the leukemogenesis of M2/t(8;21) as a result of AML-M2 previously demonstrated [21]. In the majority of t(8;21) AML cases with complex translocations a third chromosome is generally involved in the rearrangement [19-21]. Few cases bearing a four-way variant t(8;21) have been described and a detailed molecular cytogenetic characterization of the breakpoints has never been attempted[15]. Using appropriate BAC/PAC clones we have defined the chromosomal breakpoints of a complex t(8;11;16;21) in an AML case, accompanied by large chromosomal deletions which did not affect the chromosome 8 sequences. Indeed, the 5_RUNX1/3_CBFA2T1 fusion gene was

observed, as expected, on der(8). The precise breakpoints characterization the presence of submicroscopic deletions of chromosome 11 and 16 sequences reported, by Albano and co-workers, in the year 2005[15]. The general results of this study are in good agreement with previously reported findings in the series of complex chromosomal abnormalities, with CML and AML patients.

CONCLUSION

In conclusion, these findings confirmed previous data showing that in hematologic malignancies several reciprocal complex chromosomal translocations appearing as malignancy.

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