CASE REPORT

Identification of homogeneously staining regions in leukemia patients

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Homogeneously staining regions (HSR) or double minute chromosomes (dmin) are autonomously replicating extra-chromosomal elements that are frequently associated with gene amplification in a variety of cancers. The diagnosis of leukemia patients was based on characterization of the leukemic cells obtained from bone marrow cytogenetics. This study report two cases, one with Acute Myeloblastic Leukemia without maturation (AML-M1), aged 23-year-old female, and the other with chronic myelogenous leukemia (CML)-blast crisis, a 28-year-old female associated with double minute chromosomes. Most cases of acute myeloid leukemia with dmin in the literature (including our cases) have been diagnosed as having acute myeloid leukemia.

Key words: AML, cancer, chronic myelogenous leukemia, homogeneously staining regions dmin, HSRs, Iran, incidence, leukemia, new case

INTRODUCTION

Double minute chromosomes (dmin) or Homogeneously Staining Regions (HSRs) was first described in a direct preparation of cells from patient with untreated bronchogenic carcinoma.[1] Sait *et al*., (2002)[2] reported for the first time that dmin are from chromosome 19. Homogeneously staining of regions or Dmin are the cytogenetic hallmarks of genomic amplification in cancers.[3] Furthermore, dmin/HSRs are from the breakpoint region of translocation event.[4] Although found in a variety of human tumor cells, their presence in hematologic malignancies is rare.[5] Also, their role in leukemogenesis is not clear but they have been reported to be associated with rapid progression and short survival time.[5] Dmin are found in tumor cell proliferations, characteristically varying in number from cell to cell. They are thought to be involved in tumor genesis and in drug resistance.[6] Dmin are small chromatin particles that represent a form of extra chromosomal gene amplification.[7] Gene amplification causes an increase in the gene copy number and, subsequently, elevates the expression of the amplified genes, which modify normal growth control and survival pathway.[8-10] In this connection, *C-myc* was the most frequently amplified gene, but cases with mixed lineage leukemia (MLL) gene amplification have also been reported in the current literature.[2,11] The semi conservative replication of Deoxyribonucleic acid (DNA) in dmin has been demonstrated to occur in both human and mouse cell line.[12] Here, we present two cases with leukemia associated with dmin. The latest data regarding dmin was reported by Mitelman database (http://cgapanci.nih.gov/chromosomes/Mitelman)[9] and own peer reviewed publication.[13]

MATERIALS AND METHODS

We receive bone marrow (BM) and peripheral blood (PB) specimen from AML and CML diagnosed adult patients at initial presentation. The diagnosis of leukemia patients was based on characterization of the leukemic cells, obtained from bone marrow and molecular cytogenetic, when appropriate. In each patient, 0.5 - 1.0 ml BM/PB was obtained and studied using; (a) a 24 h unstimulated culture technique and (b) Methotrexate cell synchronization method,[14] with some modification. For culture, 3-5 × 10^6 cells were cultured in 4 ml medium (RPMI 1640, Gibco-BRL Grand Island, NY, USA) supplemented with 15% heat inactivated fetal bovine serum (Gibco-BRL Grand Island, NY, USA) at 37°C in an atmosphere containing

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5% CO_2. For methotrexate (MTX) synchronization, BM/PB cells were synchronized with 10^{-7} M MTX after 1.0-5.0 h of culture. The S-phase block of synchronized cells was released after 17 h by the adding of 10^{-5} M Thymidin for 3.0-6.0 h. Standard MTX cell synchronization procedures for cultures, collection of samples, and slide preparation were modified and performed in our laboratory.[14] Briefly, the cultured cells were then treated with Colcemide (Gibco-BRL Grand Island, NY, USA) final concentration, 10 μg/ml and incubated at 37°C for an additional 3 min. The contents of the tube were then centrifuged for 10 min at 1,000 rpm and re-suspend in 10 ml of 75 mM KCl (0.56%) pre-warmed to 37°C for 20 min. At this stage, 1 ml of Carnoys Fixative (3:1 methanol: Acetic acid) was added in to the tube, and this fixation step was repeated four times. Ten slides were prepared for each culture and stained for 3 min with Giemsa G banding.[15] Slides were examined with Yisis/ Applied imagine system. Eighty well-spread metaphases were analyzed for each subject. Karyotypes were described according to International System for Chromosome Nomenclature (ISCN).[16]

CASE HISTORY

Two patients with CML and AML were seen between 1994 and 2010, at Shahid Beheshti University of Medical Sciences and various private clinics, in Tehran, Iran.

In patient 1, a 23-year-old female, in previous good health, presented with weight loss, tiredness. Her peripheral blood hemoglobin was 6.2 g/dl with a platelet count of 100×10^9/l (differential: 77% blast, 1% neutrophils, 22% lymphocytes). The marrow aspirate was cellular with reduction in all normal marrow elements and marked increased in Sudan Black-positive myelobalst. G-banding analysis in patient one, AML-M1 showed that all of 20 metaphases cells revealed both numerical and structural abnormalities, including 46, XX, t (9;22)(q34.1;q11) + dmin; 45, XX, t (9;22)(q34.1;q11), -15. Sixteen of the 20 cells (80%) also had 3 – 19 dmin in each cell. Her past medical history noticed without history of prior toxic exposure.

In patient 2, with CML-blast crisis, a 28-year-old female, was the subject of dms. On an admission, splenomegaly was found, count was $15.1 \times 10^4/mm^3$, hemoglobin was 7.2 g/dl, RBC $224 \times 10^4/mm^3$ and bone marrow examination revealed hypercellularity with promyelocyte 14.2% and myeloblast 49.7%. G-banding analysis revealed that 14 of the 80 metaphases cells (70%) [Figure 1], had dmin, including [t (9;22)(q34.1;q11)] + dmin. Her past medical history was notable only for occupation for cultivation as Farm Yard Manure. There were no results detected of HSR in chromosomes for both the patients.

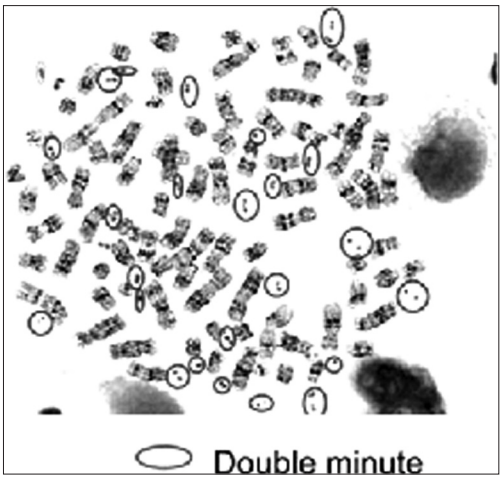

Figure 1: Giemsa staining of bone marrow cell containing dmin in the metaphase of leukemia patients

DISCUSSION

The presence of dmin with two leukemia patients, which were identified in our laboratory, was also observed in other parts of the world. These two new cases of chromosomes will be discussed in the following sections utilizing the largest chromosome aberrations database in cancers (http://cgapanci.nih.gov/chromosomes/Mitelman).[9] Also, Thomas *et al*., (2004)[5] documented 33 cases with dmin in acute myeloid leukemia.

The relationship of dmin/HSRs and malignancies seems well established and, indeed, dmin/HSRs have not, so far, been observed in non malignant cell.[3] Gene amplification cause an increase in the gene copy number and, subsequently, elevate the expression of the amplified genes, which modify normal growth control and survival pathway.[7-10] Dmin represent a mechanism for upregulated oncogene expression and are generally associated with a poor prognosis. This type of gene amplification has been found in various solid tumors, such as colon, pancreatic, breast carcinoma, brain tumors, and neuroblastoma (http://cgapanci.nih.gov/chromosomes/Mitelman).[9] However, it appears to be less common in AML.[5] Generally, *C-myc* amplification results in an over expression of the myc protein, which is known to be a critical nuclear transcription factor.[10,11] Some results suggested that dmin and HSR are alternative manifestations of the same biological phenomenon.[17]

Amplification of the *ETS1, FL11, SRPR, NFRKB*, and *KCNJ4* genes located at 11q23-24 distal to MLL was demonstrated in a patient with AML.[12,18] Many oncogenes have been identified on dmin, for example, *MYCN, C-myc, EIFA2*, and *MDM2*.[5,9,19]

Several early reports concluded that dmin, whose number and size vary from cell to cell, were not viral or bacterial

contamination. Also, past medical history of both cases presented here, recorded without history of any micro organism contaminations and history of previous malignancies. In case 2, past medical history was notable only for occupation for cultivation as farm yard manure. It is coincident with previous reports indicating that dmin do occur in leukemic patients without previous history of malignancy.[20] On the contrary, a relation between dmin and previous mutagenic exposure has been suggested by other scientists.[21] It is reasonable to suggest that the demonstration of dmin in patients with AML in the present study might be an indication that these patients have a previous history of malignant disease treated with irradiation or chemotherapy or both.

The identification of two new cases of dmin presented here are secondary, apparently non-random associated with leukemia. However, further studies and accumulation of new cases are needed in the hope of defining it as specific abnormalities in the field of leukemia.

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