

Insights into the t (6;11) (q27; q23) translocation in acute leukemia by Cytogenetic & Fluorescence in Situ Hybridization Technique

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Abstract

Acute Myeloid Leukemia (AML) is a numerous hematologic malignancy marked by means of the clonal growth of myeloid precursors within the bone marrow, resulting in disrupted hematopoiesis. Chromosomal abnormalities are pivotal inside the development of AML and serve as key indicators for prognosis and the components of healing strategies. AML is a multifaceted cancer marked through numerous genetic abnormalities. AML with t(6; 11)(q27; q23) is a super subtype of AML marked with the aid of a chromosomal translocation among chromosomes 6 and eleven, leading to the formation of the MLL-AF6 fusion gene. This fusion disrupts normal blood cell improvement and drives leukemic cellular proliferation. Patients with this translocation, more usually seen in kids, frequently face poor diagnosis and resistance to conventional remedies, with a better danger of relapse.

Key words: disease; red blood cell; genetic; cancer

Introduction

Early T-cell precursor Acute Lymphoblastic Leukemia (ETP-ALL) is a subgroup of T-cell Acute Lymphoblastic Leukemia (T-ALL) that shares similarities with both T-ALL and AML (Zhan Permikin et. Al., 2022). AML is characterized by a high degree of genetic and molecular heterogeneity, which significantly influences disease prognosis and treatment response. AML is considered as a fatal malignancy (Rong Wang, et al., 2022).

The KMT2A gene normally functions as a transcription regulator of the HOX genes and is crucial for everyday mammalian boom and hematopoiesis. The function of the KMT2A fusion genes and proteins is poorly understood, but the fusion proteins disrupt the capability of untamed-kind KMT2A to alter HOX gene expression, main to leukemogenesis (Mateusz Górecki et. Al., 2023).

KMT2A is associated with over 30 proteins, including significant components of the SWI/SNF chromatin-remodeling complex and the TFIID transcription complex. It attaches to the promoters of HOX genes by modulating histone acetylation and methylation. As a critical regulator of hematopoiesis and embryonic progress, KMT2A plays a central role in regulating the expression of HOX genes. ([http://atlasgeneticsoncology.org/haematological/1015/t\(6;11\)\(q27;q23\)](http://atlasgeneticsoncology.org/haematological/1015/t(6;11)(q27;q23))).

Afadin is a multidomain scaffold protein that be able to interact with a diverse spectrum of proteins and has been implicated in the regulation of diverse cellular phenotypes. Afadin, also known as AF6 or AFDN, is found in Adherens Junctions (AJs) that form between adjacent epithelial cells in tissues throughout the body (Jennifer H., et al., 2020). Translocations of the KMT2A gene is found in 15–20% of pediatric AML cases. The translocation forms fusion genes in which the truncated form of KMT2A and the partner gene are fused in frame, leading to a gain of function of MLL-fusion gene complexes. More than 60 fusion partners have been recognized for MLL. These fusion partners can be generally classified into cytoplasmic proteins and nuclear proteins (Kenji Mandai et al., chapter 19, 2013).

Cytogenetic investigation of bone marrow at the diagnosis of leukemia is important for optimal patient management and is increasingly being used in treatment stratification. Fluorescence in situ hybridization (FISH) is a sensitive method for identifying chromosomal breakpoints. In the case of chromosomal translocation, two methods are possible. One is a co-localization assay with probes for both translocation partners. A second method is a separation assay with probes for one locus that will split in case of a chromosomal breakpoint (Anne von Bergh et. al., 2000).

Herein we describe the case of a girl with AML with significant translocation t(6;11)(q27; q23) using Conventional Cytogenetics and Fluorescence In situ Hybridization (FISH).

Case Details:

We report a patient with AML-M5 who developed t(6;11)(q27; q23). At the time of diagnosis, a 15-year-old girl experiencing low-grade fever and weakness was admitted to the Gujarat Cancer and Research Institute in Ahmedabad, India.

On admission, the laboratory investigations of manual differential count (DC) revealed a hemoglobin level of 7.2 g/dL, hematocrit (HCT) of 23%, and red blood cell (RBC) count of 2.56×10^6 cells/ μ L. The white blood cell (WBC) count was significantly elevated at 154.18×10^3 cells/ μ L, with a platelet count of 14×10^3 cells/ μ L. The differential count showed polymorphs at 3%, lymphocytes at 4%, and monocytes at 2%, accompanied by a notable observation of 91% blasts and promonocytes. The complete blood count (CBC) corroborated these findings and provided additional metrics, including a mean platelet volume (MPV) of 10.1 fl, platelet distribution width (PDW) of 16, mean corpuscular volume (MCV) of 89.8 fl, mean corpuscular hemoglobin (MCH) of 28.3 pg, mean corpuscular hemoglobin concentration (MCHC) of 31.3 g/dL, and red cell distribution width (RDW) of 18.1%. Nucleated red blood cells (NRBCs) were observed at 1.19%. Diagnosis: AML (AML-M5). X-Ray Reports of the chest were normal. The patient was treated with CYTARABINE 100 mg, METHOTREXATE 15 MG-MTX, FOSAPREPITANT 150 MG, DAUNORUBICIN 20 MG, HYDROXYUREA 500 MG. Patient expired within 23 days (about 3 and a half weeks) of treatment which indicates poor prognosis. The institutional review board approved the present study and patients' general consent was taken.

Immunophenotype:

The Bone Marrow Immunophenotyping (IPT) Report revealed that 83% of blasts in the bone marrow predominantly expressed myeloid markers, including MPO, CD13, CD33, CD14, CD64, CD117, along with HLADR and CD34. The primary markers showed MPO at 48% (moderate positive) and CD34 at 33% (moderate positive), while secondary markers included HLADR at 97%, CD13 at 91%, CD33 at 94%, and CD64 at 74% (all moderate positive). Flow cytometry, performed using a Canto Ten-color flow cytometer, confirmed the diagnosis of Acute Monocytic Leukemia (AML-M5)

Next Generation Sequencing

Blood sample was analyzed for Next Generation Sequencing using Ion Torrent Genexus Integrated Sequencer. Results revealed positive results for fusion of KMT2A and AFDN gene.

Materials and Method:

Conventional Cytogenetics

A bone marrow sample was aseptically collected in a Sodium Heparinized vacutainer. For the conventional cytogenetic study, a short-term culture was conducted following standard protocols, and the slides were banded using the Giemsa Trypsin G-banding technique. High-grade metaphases were captured using a Zeiss automated karyotyping system and analyzed with IKAROS software. The karyotype was then described according to ISCN 2020 guidelines.

Fluorescence in situ hybridization (FISH) Using an Epi-fluorescence microscope (AXIO Imager.Z2, Zeiss, USA) equipped with appropriate filter sets, images were captured and processed with the ISIS FISH imaging system (Metasystems, Germany). Direct harvest bone marrow sample was used for FISH. The FISH probe for 11q23 is labelled with: Orange signal for the telomeric region of KMT2A gene on chromosome 11 & Green signal for the centromeric region of KMT2A gene on chromosome 11. The FISH probe 9_22 is labelled with: Orange signal for the ABL gene on chromosome 9q34 & Green signal for the BCR gene on chromosome 22q11.2. Whole Chromosome Painting (WCP) Probe

The WCP FISH was done to define the translocation's nature. WCP probe for chromosome 6 and 11 (Abbott Molecular, USA) used in metaphases. WCP 11 spectrum green and WCP 6 spectrum orange probe was used.

Results:

Conventional Cytogenetic:

Conventional chromosome examines at diagnosis of GTG banded metaphase were carried out. 20 metaphases were karyotyped. All metaphases showed 46,XX,t(6;11)(q27;q23) [20] described in figure 1 .

FISH:

BCR-ABL DC DF probe results revealed metaphases with 2O2G signals. The sample showed no fusion for the BCR.ABL gene. The FISH result interprets that the sample is negative for gene fusion. FISH report was nuc ish(ABL1, BCR) x2[200]. 11q/ KMT2A probes results revealed a metaphase Split signal for the gene. The sample shows split signals for the KMT2A gene. The FISH result interprets that the sample is positive for KMT2A gene rearrangement. FISH report was nuc ish(MLLx2) (5'MLL sep 3'MLLx1) [200] shown in figure 3.

To verify this translocation in AML, a gene specific probe for this specific translocation was not utilized. The WCP FISH was carried out to define the nature of the translocation. The WCP FISH probes for chromosome 11 Spectrum Green (SG) and chromosome 6 with Spectrum Orange (SO) were applied on metaphase cells as per manufacturer's instructions. Results of WCP FISH revealed that there was uneven translocation between chromosome 6 and 11. Green chromosomal material was observed in q arm of chromosome 6, which is labeled with spectrum orange indicating unbalanced translocation of chromosome 6 & 11 as presented in figure 2.

Discussion:

AML is a prototypical hematologic malignancy with multiple genetic tests available for diagnosis and prognosis. Cytogenetic testing plays an important role in the diagnosis of AML and helps classify AML into beneficial, intermediate, and aggressive genetic risk groups (Rong He, MD et. al.,2015). Research suggests that translocation is associated with poor prognosis due to the association between rapid disease progression and chemotherapy resistance. Cytogenetic methods can but do not always detect subtle chromosomal changes, which are important that is confirmed using FISH ((Rong He, MD et. al.,2015). FISH, a molecular cytogenetic approach, uses fluorescently classified probes to target particular DNA sequences, imparting an extra sensitive method for detecting the t [6;11] translocation. The KMT2A rearrangement, such as t [6;11], can be identified using FISH probes concentrated on each KMT2A on 11q23 and AF6 on 6q27 (Nikolai Lomov, et. Al.,2020). FISH is specifically effective in detecting cryptic or complicated rearrangements that might be ignored by traditional cytogenetic

evaluation. It allows the identification of submicroscopic adjustments, helping in the accurate prognosis of AML subtypes (Debra F. Saxe, PhD. Et. Al., 2012). FISH additionally allows early detection of MLL/AF6 fusions, which is crucial in guiding the treatment approach. Given the terrible response of KMT2A rearranged AML to conventional treatment plans, the usage of FISH can support the decision to pursue opportunity treatments (J.-H. LIM et. Al., 2014). The maximum recurrent cytogenetic abnormalities in pediatric AML are balanced chromosomal rearrangements, leading to the formation of chimeric fusion genes, just a few of which can be additionally found in adults (Ursula Creutzig, MD et al.; 2016) Among them, center binding aspect (CBF) leukemia, represented by means of $t(8;21)(q22;q22)/RUNX1-RUNX1T1$ and $inv(16)(p13q22)/CBFB-MYH11$, are associated with an excellent analysis, whereas $11q23/KMT2A$ (MLL) rearrangements are Genes accompanying with an intermediate or adverse prognosis, depending on the KMT2A partner gene concerned (Hiromi Yamazaki et. Al., 2014). In uncommon cases, inclusive of $inv[3][q21q26]$, the balanced rearrangement results in a positional have an effect on with the relocation of enhancer sequences to the area of a proto-oncogenes, whose expression develops upregulated (Stefan Grochel et. Al., 2014). Uneven abnormalities, consisting of monosomies of chromosomes 5 and 7, are much less not unusual in kids, however they are associated with a terrible outcome, as experiential in adults (Ursula Creutzig, MD et al.; 2016). According to the Mitelman Database and associated assets, there are about 171 documented cases of this unique translocation in the context of acute leukemia (Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 2024). It represents a pretty erratic however clinically considerable chromosomal abnormality linked to MLL (KMT2A) gene rearrangements, generally observed in AML and sometimes in ALL. The $t(6;11)(q27; q23)$ translocation is a rare chromosomal abnormality found in acute leukemia, specifically in AML, with full-size diagnostic and prognostic implications (William Blum et. Al., 2004). This translocation involves the fusion of the KMT2A (mixed lineage leukemia) gene located on chromosome 11q23 and the AF6 (MLLT4) gene on chromosome 6q27. Detection and characterization of this translocation through cytogenetic and FISH strategies offer treasured insights into its role in leukemia development and control (David Grimwade et. Al., 2010). The KMT2A gene on 11q23 is often worried in various translocations discovered in AML and is associated with an aggressive scientific route. Patients with $t(6;11)$ translocations regularly exhibit an AML phenotype with monocytic or myelomonocytic differentiation (Josette Derre et. Al., 1990). The presence of the $t(6;11)(q27; q23)$ translocation is a key prognostic indicator in AML Patients with this translocation often showcase more aggressive disorder characterised by way of excessive white blood cell counts and further medullary involvement. Studies have proven that $t(6;eleven)$ is related to an multiplied chance of relapse and resistance to standard chemotherapy, underscoring the need for more competitive healing techniques (Alberto Hernández-Sánchez wt. Al., 2024). Early detection of the MLL/AF6 fusion, the use of cytogenetic and FISH techniques, is critical for figuring out patients who may additionally gain from allogeneic hematopoietic stem cell transplantation or targeted treatments. Recent trends in focused treatment options that inhibit KMT2A fusion proteins provide promising avenues for remedy (Shandong Tao et. Al., 2021).

Conclusion:

The $t(6;11)(q27;q23)$ translocation represents an essential chromosomal abnormality in AML with huge diagnostic and prognostic relevance. Cytogenetic analysis, accompanied via FISH, offers a robust technique for

detecting subtle translocation. Given the challenges usually related to $t(6;11)$ translocations and KMT2A rearrangements, early detection is vital for steering therapeutic decisions efficaciously. Further research into MLL-centered treatments holds the capacity to enhance effects for sufferers with this tough leukemia subtype.

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