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Original Article

Homozygous inv(11)(q21q23) and *MLL* gene rearrangement in two patients with myeloid neoplasms

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Abstract: Rearrangements of the *MLL* gene located at chromosome 11q23 are common chromosomal abnormalities associated with acute leukemias. In vast majority of cases with *MLL* gene rearrangements, only one chromosome 11 or a single *MLL* allele got involved. We report two very unusual cases of myeloid neoplasms with homozygous inv(11)(q21q23) and biallelic *MLL* rearrangement. Both patients, a 12-year old boy and a 29-year old woman, presented initially with T lymphoblastic leukemia/lymphoma (T-ALL), achieved complete remission with intensive chemotherapy, then recurred as acute myeloid leukemia in one patient and therapy-related myelodysplastic syndromes in the other patient, 24 and 15 months after initial T-ALL diagnosis, respectively. In both cases, biallelic *MLL* gene rearrangements were confirmed by fluorescence in situ hybridization. *Mastermind like 2* gene was identified as *MLL* partner gene in one case. To our knowledge, homozygous inv(11)(q21q23) with two *MLL* genes rearrangement are extremely rare; it is likely a result of acquired uniparental disomy.

Keywords: Inv(11)(q21q23), *MLL-MAML2* fusion, T lymphoblastic leukemia, myeloid neoplasms, acquired uniparental disomy, homozygosity

Introduction

Chromosomal rearrangements involving *MLL* gene have been associated with lymphoid, myeloid, and mixed lineage leukemias. Over 100 different *MLL* rearrangements have been described and over 70 *MLL* partner genes have been characterized [1-4]. Among the partner genes, *MLLT2/AF4, MLLT3/AF9, MLLT1/ENL, MLLT10/AF10, MLLT4/AF6, ELL, EPS15/AF1P, MLLT6/AF17, MLLT11/AF1Q* and *SEPT6,* are the most common ones [1, 4]. These 10 partner genes account for about 94% of *MLL* rearrangements in B-lymphoblastic leukemia/lymphoma (B-ALL) and 80% of *MLL* rearrangements in acute myeloid leukemia (AML). Although all these 10 partner genes can be observed in both ALL and AML, only *AF4 and EPS15* are commonly associated with B-ALL; the other 8 are commonly associated with AML [1, 3].

MLL gene rearrangement as a result of inv(11)(q21q23) has been reported in a few cases of hematological neoplasms, including T-ALL (n = 2), AML (n = 2), and myelodysplastic syndromes (MDS, n = 1) [5-8]. *Mastermind like 2* (*MAML2)* gene, located at 11q21, has been identified as the partner of *MLL* in 4 of these cases [5, 6]. The *MAML2* gene is composed of a conserved basic domain in the N terminus that binds to the ankyrin repeat domain of *NOTCH* and may function as a transcriptional co-activator of *NOTCH* signaling [9, 10]. In the case of *MLL-MAML2*, the N-terminal portion of *MLL* may acquire oncogenic activity by transcriptional activation; however, *MLL-MAML2* did not seem to involve the activation of *NOTCH* signaling in one study [5].

MLL gene rearrangements almost always involve only one *MLL* allele. To our knowledge, only one case with rearrangement involving both *MLL* alleles has been reported [8]. The patient had a therapy-related acute myelomonocytic leukemia (AMML) showing inv(11)(q21q23)x2 by conventional cytogenetics and

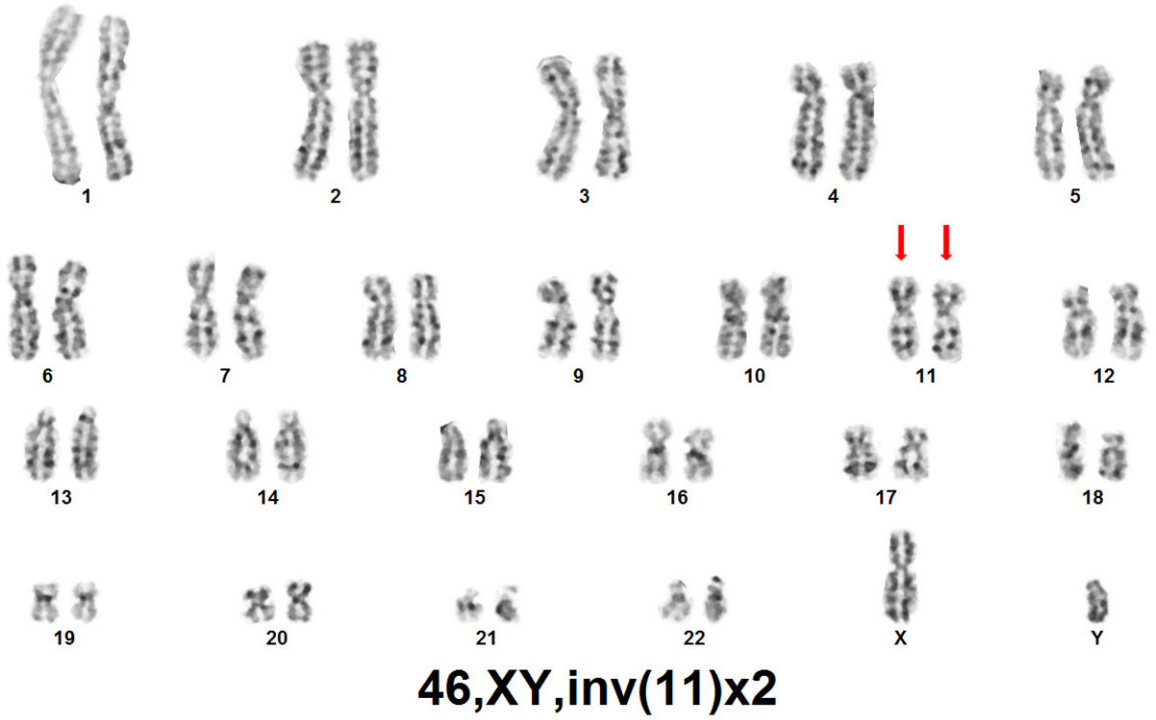

Figure 1. Conventional cytogenetics of bone marrow from case 1 during the recurred acute myeloid leukemia. Inv(11)(q21q23) was noted on both chromosomes 11.

MLL gene rearrangement by Southern blot analysis. Here we present two highly unique cases. Both cases initially presented with T-ALL and went into complete remission, but later recurred as AML in one patient and therapy-related MDS in the other patient. Both cases showed homozygous inv(11)(q21q23) and biallelic *MLL* gene rearrangement in the myeloid neoplasms.

Case report

Case 1: A 12-year-old boy presented with a 2-month history of fatigue, decreased appetite, and 20-pound weight loss. He presented to emergency room (ER) after 2 days of intermittent but worsening chest pain and fever. A complete blood count (CBC) showed pancytopenia with white blood cell count (WBC) 1.3 x 10^9/L (normal range, 5.1 ~ 15.5 x 10^9/L), hemoglobin 6.7 g/dL (normal range, 9.5 ~ 14.8 g/dL), platelet count 49 x 10^9/L (159 ~ 353 x10^9/L), and 15% of circulating blasts. Bone marrow (BM) biopsy revealed 80% blasts. Flow cytometric immunophenotyping (FCI) of the BM showed a pre-T immunophenotype: positive for cytoplasmic CD3 (cCD3), CD5, CD7, CD56 (partial), and CD117 (partial); and negative for CD1a, CD2, surface CD3 (sCD3), CD4, CD8, CD10, CD13, CD14, CD15, CD20, CD22, CD33, CD34, CD41, CD61, CD64, HLA-DR, TdT and myeloperoxidase (MPO). While conventional cytogenetics was insufficient for analysis, fluorescence in situ hybridization (FISH) analysis revealed one *MLL* gene rearrangement in 35% of interphases. The patient was diagnosed with T-ALL and treated with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high dose methotrexate and cytarabine) chemotherapy. By the end of induction, the patient achieved complete remission, no minimal residual disease detected by morphology, flow cytometry, and FISH analysis. However, during the 6th cycle of consolidation therapy (about 24 months after the initial presentation of T-ALL), the patient presented at ER with rhinorrhea, cough and fever. Peripheral blood (PB) showed 4% of blasts. Bone marrow biopsy showed 22% of blasts. FCI analysis showed that the blasts gained the expressions of myeloid antigens (CD13, CD33) and CD34, lost the expression of T-cell antigens (cCD3 and CD5), increased the expression of CD117 and decreased CD7 expression, MPO and TdT remained negative. Chromosomal analysis showed 46,XY, inv(11)

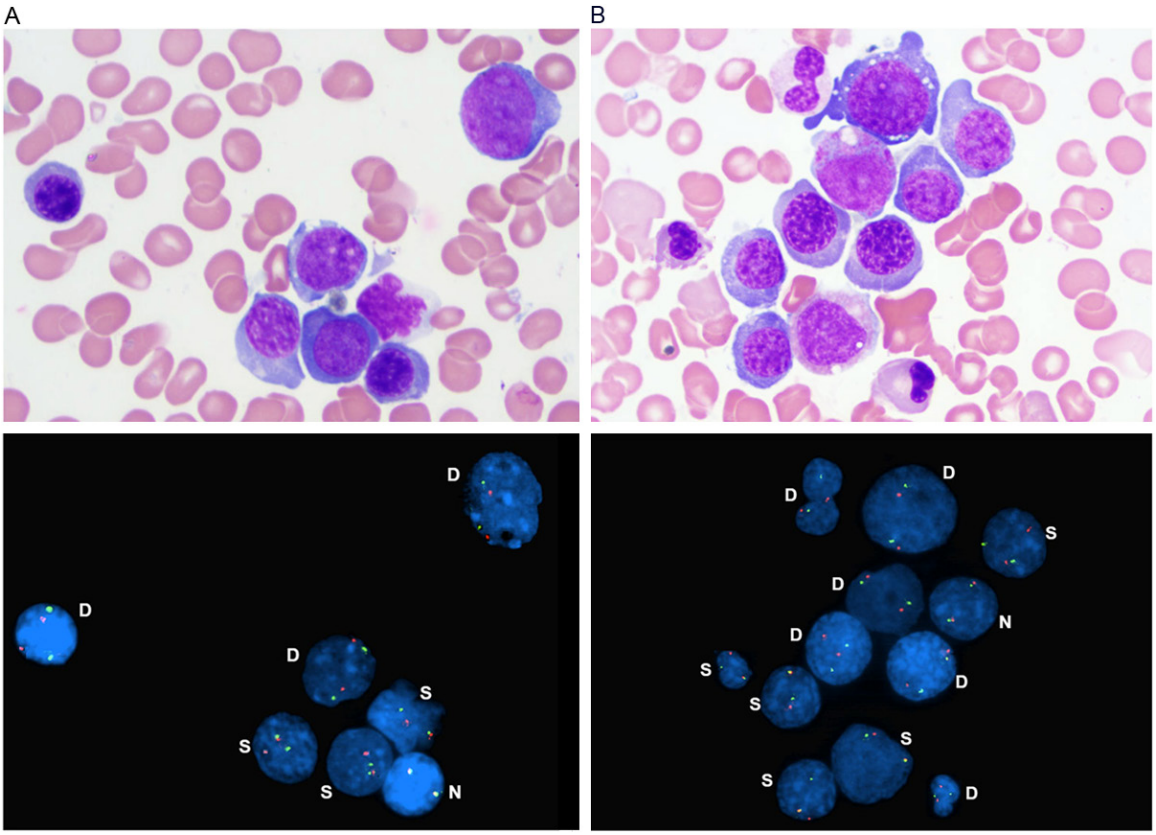

Figure 2. Morphology combined with Fluorescence in situ hybridization analysis using *MLL* dual-color break-apart probe on bone marrow. A: Acute myeloid leukemia sample in case 1. B: Myelodysplastic syndrome sample in case 2. *MLL* gene rearrangements occurred in myeloid lineages. D: Dual copies of *MLL* gene rearrangement. N: Normal pattern, negative for *MLL* gene rearrangement; S: Single copy of *MLL* gene rearrangement.

(q21q23)x2 [19]/46,XY [1] (**Figure 1**). FISH-morphology analysis revealed 41% of cells (myeloid lineages) with two and 7% with one *MLL* gene rearrangement (**Figure 2A**). The patient was diagnosed as AML and started chemotherapy under St. Jude protocol AML-02. The patient responded to the AML protocol and went into partial remission. Five months after the diagnosis of AML, patient received haploidentical HSCT. Up to now, the patient has been followed-up for 10 months post HSCT and has been in a complete remission.

Case 2: a 29-year-old female presented with chest pain and persistent cough for few weeks. Chest X-ray revealed a mediastinal mass and left pleural effusion. Biopsy of the mediastinal mass showed a blastic tumor with extensive necrosis. The tumor cells were positive for CD3, CD5, and TdT by immunohistochemistry. FCI analysis on a pleural fluid sample revealed a cortical T immunophenotype: positive for CD1a, cCD3, CD5, CD7, CD4/CD8 (co-expression), TCR alpha/beta and TdT; and negative for B cell and myeloid antigens. BM biopsy showed 75% of blasts with an immunophenotype similar to the cells in pleural fluid. Conventional cytogenetics of BM showed a normal female karyotype and FISH analysis was negative for *MLL* gene rearrangement. Patient was diagnosed with T-ALL and treated with hyper-CVAD chemotherapy and radiation to the mediastinal mass followed by POMP maintenance (6-mercaptopurine, methotrexate, vincristine, and prednisone). Patient went into remission. However, BM biopsy at 15th month after initiation of the chemotherapy showed marked dyserythropoiesis and 4% of blasts. Chromosomal analysis revealed 46,XY,inv(11)(q21q23)x2 in 11 out of 20 metaphase. FISH revealed 42% of cells with two and 19% of cells with one *MLL* gene rearrangements. At 20th month, bone marrow showed trilineage dyspoiesis and 7% blasts. The blasts had a myeloid immunophenotype (positive for CD13, CD33, CD117, and MPO; negative for CD2, cCD3, CD5, CD7, TdT and all B

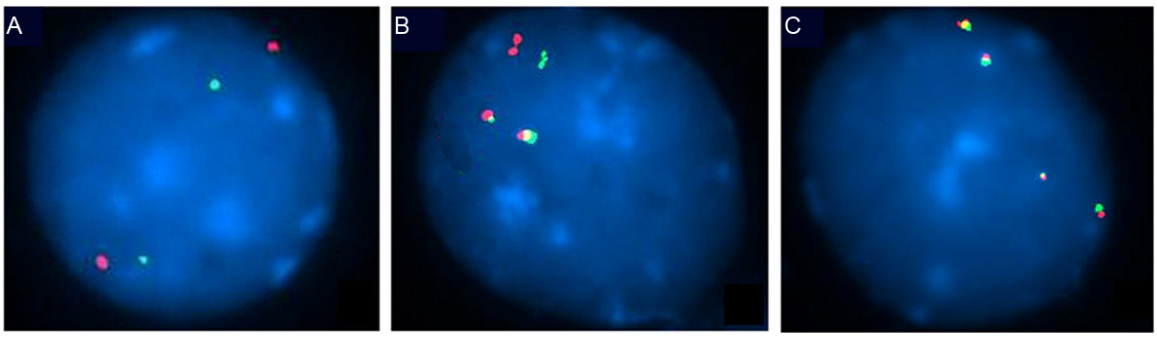

Figure 3. Fluorescence in situ hybridization using *MLL* (green) and *MAML2* (red) probes on bone marrow from case 1 during the recurred acute myeloid leukemia. A: Normal pattern; B: Inv(11)(q21q23)x1 resulting in two *MAML2/MLL* gene fusion signals; C: Inv(11)(q21q23)x2 resulting in four *MAML2/MLL* gene fusion signals.

cell antigens). All 20 metaphases analyzed showed 46,XY,inv(11)(q21q23)x2, and FISH-morphology analysis showed 72% of cells (myeloid lineages) with two and 17% of cells with one *MLL* gene rearrangements (**Figure 2B**). Patient was diagnosed with MDS and received haploidentical hematopoietic stem cell transplant (HSCT) at 22nd month. Up to now, patient has been followed-up for 8 years post HSCT and has been in a complete remission.

Partner gene identification

Since the *MAML2* gene has been the only *MLL* partner gene identified on the band of 11q21 so far, we performed additional FISH studies targeting *MLL* gene and *MAML2* gene to identify *MLL* fusion partner gene. We used Bacterial Artificial Chromosome (BAC) clones: RP11-91A14 (11q23.3, 118245309-119399215, encoding *MLL* gene, labeled with spectrum green) and RP11-111I14 (11q21, 95811640-95976114, encoding *MAML2* gene, labeled with spectrum red), and performed FISH analysis on the AML BM sample from case 1 following the standard procedure described previously [11]. *MLL-MAML2* fusion signals are expected to be in yellow. There were 2 fusion signals corresponding to inv(11q)x1 or one *MLL* rearrangement (9% of nuclei), and 4 fusion signals corresponding to inv(11q)x2 or 2 *MLL* rearrangement (31% of nuclei), were detected (**Figure 3**). Due to the lack of appropriate material, FISH for *MLL-MAML2* rearrangement was unable to perform on case 2.

Discussion

Here we report two highly unique cases: both cases initially presented with T-ALL and received intensive chemotherapy, case 1 recurred with AML and case 2 developed secondary MDS. Both cases showed a unique cytogenetics abnormality of inv(11)(q21q23)x2 with two *MLL* gene rearrangements in the myeloid neoplasms

In case 1, it is debatable if the AML with inv(11)(q21q23)x2 represents a therapy-related AML (t-AML) or a relapse leukemia with immunophenotype switch. At the time of T-ALL diagnosis, although no sufficient metaphases were obtained for chromosomal analysis, FISH revealed a rearrangement involving one copy of *MLL* gene in the T-lymphoblasts. Additionally, the AML and original T-ALL shared some common antigens such as CD7 and CD117 expression. Therefore, we believe that this AML more likely represents a clonal evolution of T-ALL, rather than a t-AML. In case 2, on the other hand, the MDS was likely to be therapy-related. The conventional chromosomal analysis recovered 20 normal diploid metaphases and FISH analysis was negative for MLL gene rearrangement in the T-ALL sample. In the MDS sample, the myeloblasts showed no immunophenotypic resemblance to the original T-ALL. Putting all together, we believe that inv(11)(q21q23) in the case 2 was likely an emerged clone secondary to cytotoxic therapy administered for T-ALL.

Therapy-related or relapsed myeloid neoplasms, in both cases, homozygous inv(11)(q21q23) is likely a result of acquired uniparental disomy (aUPD) or loss of heterozygosity (LOH). aUPD is a common event in AML, usually detected by single nucleotide polymorphism (SNP), occurring in about 20% of primary AML and 35-40% of relapsed AML or therapy-related AML [12-15]. Majority (87%) of aUPD is due

to mitotic recombination (segmental homozygosity with one or two breakpoints). Only a small subset (13%) is due to nondisjunction (whole chromosome homozygosity with no breakpoint) which is almost exclusively seen in aUPD involving chromosome 13 [12, 13]. Chromosome 11 is one of the most common chromosomes susceptible to segmental aUPD [12]. aUPD frequently occurs in chromosomes harboring mutated genes involving leukemogenesis, such as *FLT3* gene on 13q, *WT* gene on 11p, *CEBPA* on 19p, and *RUNX1* on 21q [12, 13, 15]. aUPD can generate homozygosity for a mutated gene, which provides survival advantage for tumor cells by promoting cell proliferation and/or developing chemoresistance. Although we were unable to perform SNP microarray analysis on neither of our cases, the breakpoint likely resided at or above the band of 11q21. This apparent mitotic recombination resulted in homozygosity of inv(11)(q21q23) with two copies of *MLL* rearrangement. Interestingly, these two cases as well as the case reported by Obama [8] were all myeloid neoplasms (AML and MDS). This indicates that aUPD of inv(11)(q21q23) may steer the stem cells towards myeloid differentiation, whereas, one copy of MLL rearrangement can be seen in myeloid as well as lymphoid leukemia.

Study has shown that cases with aUPD exhibited a worse overall survival and event-free survival in primary AML with a normal karyotype as well as in primary or secondary AML with an abnormal karyotype [14]. The patient reported by Obama [8] died 3 months after the diagnosis of t-AML. However, both of our patients received HSCT after the diagnosis of myeloid neoplasms, which made it difficult to evaluate the clinical significance in term of prognosis.

Disclosure of conflict of interest

None.

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