Two consecutive immunophenotypic switches in a child with MLL-rearranged acute lymphoblastic leukemia

An 18-month-old girl was diagnosed with pre-pre-B ALL/t(4;11) leukemia, which during the treatment and after matched bone marrow transplantation (BMT), underwent two consecutive switches from lymphoid to myeloid lineage and vice versa. The high expression of HOXA9 and FLI3 genes remaining genotypically stable in a leukemia throughout phenotypic switches, suggests that this leukemia may have originated as a common B/myeloid progenitors.

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Case report

An 18-month-old girl was admitted to the San Giovanni Rotondo Casa Sollievo della Sofferenza IRCCS Hospital presenting with a WBC of 201,000/mm³ (80% blasts), hemoglobin of 7.1 g/dL, and platelet count of 42,000/mm³. After obtaining informed consent from the patient’s parents, a bone marrow (BM) aspirate was performed. The sample was submitted to the University of Padua’s Laboratory of Pediatric Onco-Hematology laboratory, as National reference laboratory for all pediatric leukemias enrolled in the AIEOP protocols, for morphologic, cytochemical, cytogenetic, molecular, and flow cytometric evaluations. The BM aspirate contained 95% of tumor cells having the morphology of lymphoblasts (French-American-British [FAB] L1) (Figure 1A). Flow cytometric immunophenotypic analysis of blast cells showed: CD19+, CD34+, CD45+, CD133+, CD135+, NG2+, TdT+, HLA-DR (bright) (Table 1). Conventional cytogenetic analysis revealed 46, XX, t(4;11)(q21;q23) in all 20 metaphases analyzed. RT-PCR analysis showed the presence of two amplified MLL-AF4 fusion transcripts (Figure 2A). The diagnosis of pre-pre-B ALL was made.

Considering the insufficient clearance of circulating blasts (36,000/mm³) on day 8 after a steroid prophase (prednisone poor response; PPR) and despite an initial relevant cytoreduction (4,000/mm³ on day 5), the girl was assigned to the high-risk group in accordance with the AIEOP-BFM-ALL-2000 protocol. Thirteen days later her WBC count increased a second time to 96,600/mm³, the hemoglobin level was 9.8 g/dL, and platelets of 56,000/mm³. Peripheral blood and BM examination revealed, respectively, 98% and 85% blasts characteristic of monoblastic features and consistent with M5 morphology. Cytochemistry was strongly positive for myeloperoxidase (Figure 1B,C). Flow cytometric analysis of BM showed: CD15 (bright), CD33 (bright), CD45+, CD64+, CD135+, HLA-DR (dim) but CD19+, CD34+, TdT- (Table 1). The results were consistent with a typical AML (FAB M5) diagnosis. The translocation 46, XX, t(4;11)(q21;q23) was still present (20 of 20 analyzed metaphases). Consequently, her treatment was changed to the AIEOP-AML-2002 protocol. Five months later, after complete remission was achieved, she received allogeneic BMT from an HLA-matched normal donor. Two months after the BMT, the patient relapsed again. The leukemic cells displayed morphology (Figure 1D) and immunophenotype (Table 1) of the initial diagnosis of ALL. Cytogenetic analysis again revealed a 46, XX, t(4;11)(q21;q23) in all 20 metaphases analyzed. She died a few weeks later without responding to any further treatment forms.

DNA and RNA from BM samples were collected throughout the disease course (diagnosis, myeloid phase and relapse) of this patient. Samples were studied for Ig/TCR clonality evaluation by PCR heteroduplex analysis and for gene expression profiling. A heteroduplex and homoduplex bands corresponding respectively to V\textsubscript{H}4-J\textsubscript{H} and V\textsubscript{H}4-J\textsubscript{H} gene rearrangements were identified from the diagnosis, myeloid phase and relapse samples (Figure 2B). The sequence analysis revealed an identical V\textsubscript{H}4-J\textsubscript{H}6, V\textsubscript{H}4-J\textsubscript{H}4 and V\textsubscript{H}11-J\textsubscript{y}1.3/2.3 junctions region between the specimens analyzed.1 Additional RT-PCR analyses showed...
Figure 2. (A) RT-PCR analysis of the MLL-AF4 fusion gene showing expression of two different products in diagnosis, 13-day and relapse RNA leukemic cells. Total RNA was extracted from BM cells of diagnosis, 13-day and relapse using TRIzol® (Life Technologies, NY, USA). Integrity of the RNA was assessed by capillary electrophoresis (Agilent Technologies, Palo Alto, CA, USA). (B) Heteroduplex PCR analysis of IGH and TCRG gene rearrangements. Blast cells at diagnosis as well as 13-day and relapse contained a biallelic heteroduplex (he) VH6-JH and a monoclonal homoduplex (ho) V4V1/2.3 gene rearrangement. PCR products were detected by silver staining on a 12% non-denaturing polyacrylamide minigel. Mononuclear cells were separated by Ficoll-Paque centrifugation and DNA was extracted and purified using Gentra kit (Gentra System, Minneapolis, MN, USA). Mw marker, molecular weight marker; Mock, negative control; normal MNC, normal mononuclear cells. (C) Hierarchical clustering based on HG-U95Av2 expression data of diagnosis ALL (ALL-1), 13-day (AML) and relapse (ALL-2). The genes used in this analysis are the top 100 genes chosen by t-test statistic that are the most differentiated among the tumor samples. The normalized expression value for each gene is indicated by color, with red representing high expression and blue representing low expression. cRNA was prepared according to the standard Affymetrix protocol (BioArray™ High Yield RNA Transcipt Labeling Kit; Enzo Diagnostics, Farmingdale, NY, USA). Expression values were determined using Affymetrix MAS 5.0 software. (D) Scatter plots indicating statistical correlations among the samples on the basis of their gene expression values. Tighter the dot cloud around the diagonal stronger the correlation among samples. The correlation between ALL-1 and ALL-2 is stronger than between ALL and AML samples.
the presence of two MLL-AF4 amplified products also during the myeloid phase and relapse (Figure 2A). The sequencing of these PCR products showed two alternatively spliced MLL-AF4 transcripts joining MLL exon 10 to either AF4 exon 4 or exon 5. Gene expression analysis confirmed the MLL signature for each disease phase analyzed (ALL and AML). Furthermore, a closer examination of these genes showed a significant over-expression of HOXA9 and FLT3 gene targets during all phases of the acute leukemia (data not shown).

Several hypotheses have been suggested to explain lineage conversion in acute leukemia, but its precise mechanism remains unclear. We have described a case of an acute leukemia in which the blast cells rapidly changed lineage from pre-pre-B ALL to AML after 13 days of high-risk AIEOP-BFM chemotherapy. The patient achieved a complete remission by conventional AML-type treatment which included BMT. Eight months later she relapsed again and the blast cells showed a clear return to lymphoid B-cell phenotype. Analyzing their characteristics, as shown in Table 1, the Ig/TCR gene rearrangements and the cytogenetic t(4;11) abnormalities demonstrated that the leukemic cells switched throughout each disease phase while maintaining the same clonal relationship. Re-examining the blast cells (85%) at day 13 showed that <1×10^5 were CD34+/CD19+, indicating that it was unlikely that these cells contaminated the myeloid phase. Therefore, the CD34+/CD19+ lymphoid-restricted cells at diagnosis were not fully B-lymphoid committed but also able to differentiate into monocyted and blasts retaining the Ig/TCR and MLL-AF4 leukemic-specific rearrangements. In addition, the expression of the progenitor/stem cell-related markers, such as CD133 and CD34, only during the lymphoid phase could indicate that an immature lymphoid progenitor develops the potential to address different lineages. Gene expression studies of MLL-rearranged ALL demonstrate that these leukemias represent a unique disease when compared to other ALLs. Moreover, the differences in gene expression support the hypothesis that the cell of origin of MLL is an early hematopoietic progenitor with both myeloid and B-lymphoid potential. In our patient this is further supported by the high level expression of HOXA9 and FLT3 genes in all phases of the acute leukemia. HOXA9 and FLT3 are expressed in early hematopoietic progenitors and both are necessary for the appropriate expansion of the hematopoietic stem cell. Considering this hypothesis together with our finding of related Ig/TCR and t(4;11)/MLL-AF4 gene rearrangements during the lineage switches, it indicates that the leukemia population in our patient could have originated from a common B/myeloid progenitor with the capacity to differentiate into committed cells of either lymphoid or myeloid lineage. In contrast, the translocation t(4;11) that leads to a MLL-AF4 fusion gene has been preferentially associated with B-cell phenotype lineage10, but in this case the leukemic clone retains the possibility to induce both myeloid or lymphoid gene expression, suggesting that an immature progenitor/stem cell may be the target of the chromosomal translocation. Therefore, an interpretation of this case is that the MLL-rearranged leukemic clone is able to differentiate as lymphoid and myeloid under therapeutic effects by amplifying or suppressing the normal differentiation programs for their survival/expansion.

In conclusion, this report confirms that some forms of acute leukemia may arise from very immature cells belonging to a common myeloid/lymphoid progenitor. Moreover, it provides further information into the mechanism of leukemic lineage switches and underlines that it could be useful to test new therapeutic protocols fit to these particular severe leukemias.

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References