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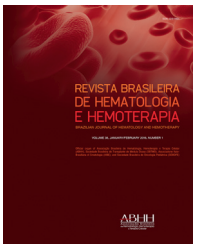

Case Report

DNA microarray expression profiling of a new t(8;13) AML case allows identification of possible leukemogenic transformation markers

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Introduction

Acute myeloid leukemia (AML) is a disease characterized by clonal proliferation and accumulation of myeloid progenitor cells in the bone marrow (BM), inhibition of cell differentiation, increased proliferative index and defective apoptosis.[1] AML secondary to myelodysplastic syndrome (MDS–sAML) is characterized by diverse cytogenetic and molecular changes including del(5q), as well as changes in the RNA splicing pathway, *TET2*, *EZH2*, *FLT3*, *NRAS*, *NPM1*, *RUNX1*, *DNMT3a*, *IDH1*, *IDH2*, *TET2*, *TP53* genes, etc.[2] Established prognostic factors in AML include age, the cytogenetic and molecular profile and history of hematologic disorders, such as MDS.[1] About 40% of elderly AML patients were previously diagnosed with MDS, which is usually refractory to chemotherapy.[1] We present a case of a sAML, which showed a single and previously not described abnormality, a chromosomal translocation between chromosomes 8 and 13 t(8;13) identified by conventional cytogenetics, and several altered genes detected by DNA microarray assay, suggesting that the t(8;13) rearranged region results in altered gene expression patterns.

Case report

The patient was a 72-year-old female admitted to the Instituto Nacional de Cancer (INCA), Rio de Janeiro, Brazil. She presented with a history of increasing fatigue and dyspnea. Past medical history included arterial hypertension, coronary artery disease, myalgia and pneumonia. The laboratory results at diagnosis revealed: white blood cell count 36.9×10^9/L with 41% monocytoid blasts, hematocrit 23%, hemoglobin 7.6 g/dL, platelets 131.0×10^9/L and lactate dehydrogenase 806 IU/L. A

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bone marrow aspirate showed 34% myeloid and monocytoid blasts (FAB M4) with dysplastic features in erythroid and myeloid cells. Flow cytometry immunophenotyping identified $CD33^+$, $CD14^+$, $CD11b^+$, $CD13^+$, $CD38^-$, HLA-DR^-, $CD15^+$, MPO^-, $CD117^+$, TDT^-, $cCD79a^-$, $CD2^-$, and $CD7^-$ cells. Reverse transcription-polymerase chain reaction (RT-PCR) for RUNX1/RUNX1T1, BCR/ABL1 and MYH11/CBFB, showed no fusion transcripts. G-banding revealed an abnormal karyotype and the gene expression profile was investigated for altered genes in the rearranged t(8;13) region.

This study was approved by the institutional review board of INCA (number 110/06). The study was conducted in accordance with the Helsinki Declaration as revised in 2008.

Methods

Cytogenetic analysis was performed on unstimulated BM cells for 24 h according to standard protocols with the karyotype being described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013).[3] The gene expression profile was investigated using an Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix Inc., Santa Clara, CA, USA) following the manufacturer's instructions to identify differentially expressed genes in the rearranged t(8;13) region. Array data were extracted and processed with the open software packages from the Bioconductor Project (www.bioconductor.org). In brief, the data was normalized with Robust Multi-Array Average expression measure. Subsequently, a non-specific filter was applied with the genefilter package[4] in order to remove Affymetrix probes and genes that exhibited low variance across samples; differentially expressed genes were selected using the linear models for microarray data package and summarized at $\log_2 > 2$. RNA extracted from sAML peripheral blood was compared to normal peripheral blood using a pool of six healthy donors. Patient's relative gene expression levels were compared to the control sample level and lists of 'up-regulated' and 'down-regulated' genes were generated by the program.

Results

G-banding revealed an abnormal karyotype with a translocation involving chromosome 8 and 13, resulting in the previously undescribed karyotype 46,XY,t(8;13)(q22;q11)[4]/46,XX[28] (Figure 1) according to the catalogs of cancer cytogenetics.[5] The microarray analysis labeled 874 as differentially expressed in over 28,000 genes. The gene expression levels (fold change), shown in Table 1, demonstrated down-regulated genes involved in myeloid development or apoptosis, including *CEBPB*, *RARA*, *GATA3*, *TRIB1*, *TRIB2* and *TNFRSF10C* (*TRAIL-R3*). In addition, up-regulated genes related to proliferation, differentiation and drug resistance, such as *KIT*, *IDH1*, *ERG*, *BIRC3* and *ABCC4* were observed by microarray analysis. The gene *RUNX1* (*AML1*) required for the generation of definitive hematopoietic stem cells during embryogenesis was up-regulated. The *brain and acute leukemia cytoplasmic* (*BAALC*) gene, located in the 8q22 region, and the *FLT3* and *RB1* genes, located in the 13q12 and 13q14 regions, respectively, appear to be intimately linked to the t(8;13). These genes were up-regulated suggesting that they could be altered due to the rearrangement of this region. Another gene that may have been inactivated due to this rearrangement is the *TRIB1* gene, located in the 8q24.13 region, which is a potent negative regulator of MAPK signaling.

Discussion

sAML develops in approximately 40% of patients with MDS and the clinical discrimination between AML and MDS is based on cytomorphological analysis, since patients with MDS have dysplastic hematopoiesis and a myeloblast count of less than 20%, whereas those with a myeloblast count of 20% or more have AML.[6] sAML has clinical and biological heterogeneity linked to chromosome aberrations or molecular changes with the association between them suggesting that those mechanisms are significantly involved in leukemogenesis.[1] This case

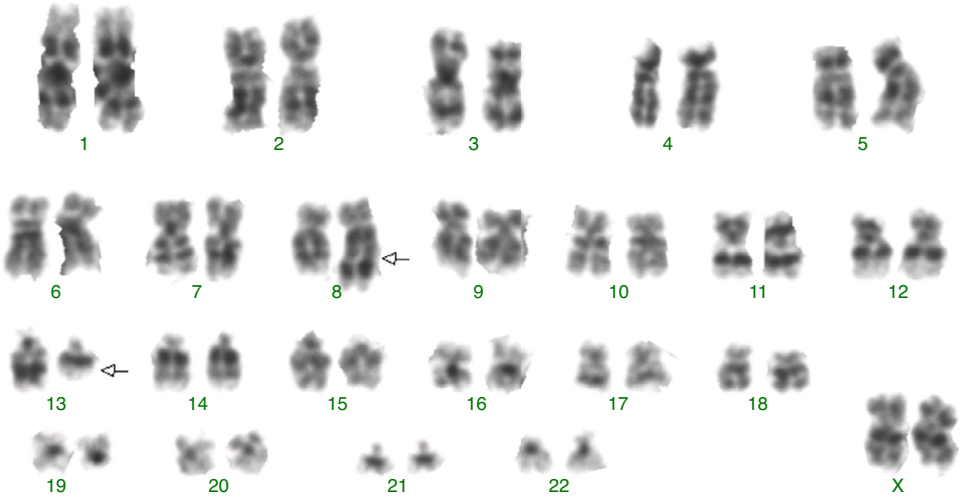

Figure 1 – Abnormal karyotype identified by G-banding with a translocation involving chromosome 8 and 13: 46,XY,t(8;13)(q22;q11)[4]/46,XX[28].

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