Cancer Genetics 205 (2012) 255–260

Cancer Genetics

BRIEF COMMUNICATION

Acute panmyelosis with myelofibrosis with *EVI1* amplification

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EVI1 is located on chromosome 3q26 and is up-regulated mostly through an inv(3)(q21q26) or t(3;3)(q21;q26). Chromosomal aberrations involving 3q26 comprise 1–2% of all acute myeloid leukemia (AML). These changes result in overexpression of the *EVI1* oncogene. *EVI1* transcriptional activation has been reported in up to 10% of AML patients, even in the absence of 3q26 changes, and is an independent indicator of adverse prognosis. Rearrangements of the *EVI1* locus are often associated with monosomy 7. We present a case of acute panmyelosis with myelofibrosis with a unique *EVI1* amplification within a derivative 8 chromosome, characterized by karyotyping and fluorescence in situ hybridization, conventional high resolution comparative genomic hybridization, as well as by gene expression studies. We conclude that *EVI1* overexpression as a consequence of *EVI1* gene amplification causes similar biological effects to the changes caused by the typical 3q26 aberrations such as an inv(3)(q21q26) or t(3;3)(q21;q26) with *EVI1* gene rearrangements.

Keywords *EVI1*, acute myeloid leukemia, amplification, panmyelosis, myelofibrosis

Human ecotropic virus integration site 1 (*EVI1*) is a proto-oncogene located on human chromosome 3q26 which is activated in up to 10% of cases of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and the blast phase of chronic myeloid leukemia (CML) carrying 3q26 rearrangements (1–3). The *EVI1* gene encodes a zinc finger protein localized in the nucleus and is not normally expressed in hematopoetic cells (4). Previous experiments on human and mouse cell lines have shown that *EVI1* prevents the terminal differentiation of bone marrow (BM) progenitor cells to granulocytes and erythroid cells; however, it favors the differentiation of hematopoietic cells to megakaryocytes (5). Elevated expression of *EVI1* predicts an adverse outcome in AML (6). The improper activation of *EVI1* usually occurs through chromosome 3 translocations or inversions that induce the constitutive expression of the gene, which presumably leads to the development or progression of leukemia (2). In addition, *EVI1* may be expressed aberrantly in patients without a 3q26 translocation as a result of yet-unknown reasons (6–8). All these anomalies lead either to the formation of *MDS1-EVI1*-fusion transcripts or to elevated expression of the unaltered *EVI1* messenger RNA (mRNA).

The most common recurrent chromosomal changes involving the *EVI1* gene locus are t(3;3)(q21;q26) and inv(3)(q21q26), which cause abnormal thrombocytosis and rapid disease progression known as the *3q21q26 syndrome* (9). Translocations in *EVI1* with other partners such as t(3;11)(q26;p13), t(3;5)(q26;q34), t(3;9)(q26;q34), and t(3;8)(q26;q24) have also been described (10,11). Reported investigations suggest that a subgroup of AML with overexpression of *EVI1* is associated in most cases with a complex karyotype that commonly includes chromosome 7 abnormalities and, clinically, is characterized by poor chemoresponsiveness and a very poor prognosis (12,13). It has been hypothesized that *EVI1* acts as a survival factor in tumor cell lines, preventing therapeutically induced apoptosis and rendering the tumor cells more resistant to current treatments (14).

Here we present the clinicopathologic, cytogenetic, and molecular findings from a case of acute panmyelosis with

Received December 13, 2011; received in revised form February 1, 2012; accepted February 9, 2012.
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doi:10.1016/j.cancergen.2012.02.003

myelofibrosis (APMF) with unique *EVI1* amplification within a derivative 8 chromosome.

Materials and methods

Cell culture and karyotyping

A fresh BM sample obtained after trepanobiopsy was processed in accordance with standard protocols. Cells were harvested according to standard procedures, treated with colcemid, exposed to hypotonic solution and fixed in Carnoy's solution. Chromosomes were G-banded and Wright stained. Twenty metaphases were analyzed using a microscope Axioskop2 (Carl Zeiss, Jena, Germany) and documented with an IKAROS (Metasysytems, Altlussheim, Germany) Imaging System. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature (2009) (15).

Fluorescence in situ hybridization (FISH)

FISH was performed on bone marrow cells from overnight culture. The following commercially available probes were used: EVI1 Break Apart (Cytocell, Cambridge, UK), MYC Break Apart, CEP7 (Vysis, Downers Grove, IL) and whole chromosome painting probes WCP-3,WCP-5,WCP-7,WCP-8,WCP-17 and WCP-22 (DAKO, Glostrup, Denmark). The procedures for commercial probes were applied according to the manufacturer's protocol. Slides were analyzed using an epifluorescence microscope Axioskop2 (Carl Zeiss, Jena, Germany) and documented using an ISIS (Metasysytems, Altlussheim, Germany) Imaging System.

Conventional comparative genomic hybridization (CGH)

Comparative genomic hybridization (CGH) was performed according to standard procedures. In brief, test and control DNA were labeled by nick translation kit (VYSIS, Downers Grove, IL) with SpectrumGreen-dUTP and SpectrumOrange-dUTP (VYSIS, Downers Grove, IL) respectively. Two hundred ng of labeled test DNA, 200 ng of labeled reference DNA and 12.5 μg Cot-1 DNA were co-precipitated, denatured and hybridized to normal denatured metaphase spreads. After incubation at 37°C for three nights, standard post hybridization washes were performed. Metaphase images were evaluated using an epifluorescence microscope Axioskop2 (Carl Zeiss, Jena, Germany) fitted with a CCD camera RrogRes (Jenoptik, Jena, Germany) and appropriate single band pass filter sets. Image analysis and karyotyping was performed using the ISIS analysis system (MetaSystems, Altlussheim Germany). The CGH quality was analyzed using a 99.5% confidence interval.

Preparation of mRNA and reverse transcription polymerase chain reaction (RT-PCR)

Real-time quantitative PCR was run in a Fast 7500 (Applied Biosystems, Foster City, CA) with primers, probes and PCR parameters described elsewhere (12). The expression level of transcripts was determined using the $\Delta\Delta C_t$ relative quantification method using a porphobilinogen deaminase (*PBGD*) control gene as described previously (12). To define high *EVI1* and *MDS1-EVI1* expression, a cut off value of 50 (in reference to control gene expression) was chosen.

Results

Case history

A 66-year-old man was admitted to the Department of Haematology of the Institute of Haematology and Transfusion Medicine (Warsaw, Poland) with suspicion of an acute myeloid disorder. In July 2009, complete peripheral blood (PB) count showed leukocytosis (white blood cell count (WBC) 31 $\times$ 10^9 cells/L with 85% blasts), mild anemia (hemoglobin (Hgb) 11.7 g/dL) and normal platelet count (PLT 334 $\times$ 10^9 cells/L). Laboratory studies revealed an elevated level of lactate dehydrogenase (LDH 390 U/L; normal range 135–225 U/L). An abdominal ultrasound showed neither hepatomegaly nor splenomegaly. A BM aspirate confirmed the presence of 73% blasts. A BM immunophenotype detected 82% myeloblasts with aberrant immunophenotypes: CD117+, CD34+, or CD11c+. Results from a trephine biopsy and cytogenetic and molecular analyses were not yet available at that time. A preliminary diagnosis of AML was made. The patient received daunorubicin and cytosine arabinoside in a 2 + 5 induction regimen. A BM aspirate test revealed 40% blasts on day 16 after chemotherapy. A complete PB count showed a normal WBC (4.7 $\times$ 10^9 cells/L), severe anemia (Hgb 8.4 g/dL), and a normal PLT (159 $\times$ 10^9 cells/L). The patient required blood transfusions for a prolonged time. In August 2009, the trephine biopsy result was available and a diagnosis of acute myeloid leukemia not otherwise specified (AML-NOS): acute panmyelosis with myelofibrosis (APMF) was made in accordance with the World Health Organization (WHO) classification 2008 (16). The trephine biopsy revealed that the BM was hypercellular with an increased number of immature granulocytes. The presence of 40% CD34+ cells with prominent dysplastic megakaryocytes (CD61+) was described. Severe reticulin fibrosis was detected (grade 3, European Myelofibrosis Network (EUMNET) consensus). A chromosomal analysis (conventional G-banding) performed on BM cells showed a complex karyotype. A BM molecular analysis excluded the *JAK2 V617F* mutation. Due to the poor response to standard chemotherapy, the patient's comorbidities, and the high risk nature of AML-NOS (APMF), palliative care was implemented. Hydroxyurea was administered and blood transfusions continued. In October 2009, rapid disease progression occurred. A PB examination showed leukocytosis (WBC 206 $\times$ 10^9cells/L with 90% blasts), severe anemia (Hgb 7.2 g/L), and mild thrombocytopenia (PLT 93 $\times$ 10^9 cells/L). Hepatomegaly (2 cm below the costal margin) and splenomegaly (14 cm) occurred. The disease was stabilized by treatment with leukapheresis and cytosine arabinoside. Unfortunately, infectious complications with severe pneumonia appeared. The patient died because of respiratory failure during the course of pneumonia. The overall survival from the time of diagnosis was 2.6 months.