A 14.8 Mb 12p Deletion Disrupting ETV6 in a Patient with Myelodysplastic Syndrome

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Abstract

We present on a new case of myelodysplastic syndrome characterized by array Comparative Genomic Hybridization. This technique confirmed the monosomy 7, detected by conventional cytogenetics, and revealed also a deletion on the short arm of chromosome 12. This deletion extends for about 14.8 Mb and breaks ETV6 gene.

12p deletion extents in hematological malignancies may vary, but the minimally deleted region almost invariably contains ETV6, that is considered the main candidate tumor suppressor genes within the region for tumor progression. It has been shown that levels of ETV6 were significantly decreased in cases with 12p13 deletions, whereas expression of other genes in the deleted region, like BCL2L14, LRPR, DUSP16 and GPRC5D, did not show any variation, independently of their copy number status. This observation strengthens the fact that ETV6 may play a potential role in the tumorigenesis process. The role of ETV6 in our patient myelodysplastic syndrome is showed by his clinical history and his poor prognosis.

Keywords: Myelodysplastic syndrome; 12p Deletion; CGH array; ETV6

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell malignancies characterized by peripheral blood cytopenias, one or more lineage dysplasia, and ineffective hematopoiesis. Metaphase cytogenetics has become the routine test in the management of MDS as well as in hematological malignancies where the presence of specific chromosomal aberrations can be diagnostic, or highly predictive of prognosis or responsiveness to target therapeutics. Clonal cytogenetics abnormalities have been observed in about 50% of de novo MDS and in more than 80% of secondary MDS cases [1-4].

Karyotype analysis has technical limitations, mainly due to the available metaphase spreads and to the chromosome resolution. These factors result in the underestimation of the degree of chromosomal changes. In the recent years, the advent of array CGH (Comparative Genomic Hybridization) has given a great contribution to the diagnostic tools available for MDS, overcoming some of the limitations of the classic cytogenetic techniques. This method do not rely on cell division, has superb resolution for unbalanced lesion and allows for detection of copy number variation that can be related to the tumor progression [1,5,6].

Here, we present a case of MDS where we were able to find a chromosome 12 abnormality detected only by array CGH.

Material and Methods

Patient history

In October 2005, a 60-year-old male patient was admitted to our hospital for nodal marginal zone non-Hodgkin's lymphoma, stage IV (bone marrow was infiltrated), diagnosed after biopsy of a 20-mm inguinal lymph-node. At that time, conventional cytogenetics on bone marrow was normal. Patient was firstly treated with 2-chlorodeoxiadenosine for 6 cycles and subsequently, because of a poor response, according to R-CHOP (rituximab, cyclophosphamide, vincristine, prednisone) schedule. After further three months, our patient showed again enlarged lymph-nodes in abdomen and neck, thus he received R-GDP (rituximab, gemcitabine, cisplatin, and dexamethasone). In February 2008, because of a relapse in the central nervous system (CNS), he received high-dose methotrexate, intrathecal aracutin and temozolomide. This latest drug was repeated in February 2011 for a new evidence of a further disease progression in CNS, this time followed also by encephalic radiotherapy. In August 2011, for newly enlarged lymph-nodes in abdomen, he received R-bendamustine, achieving a partial remission, without significant hematological or extra-hematological toxicities. Nevertheless, in November 2012, he presented with anemia (hemoglobin = 9.7 g/dL), thrombocytopenia (platelets = 25 x 10^9/L) and leukocytosis with monocytosis (monocytes = 2.8 x 10^9/L).

The bone marrow showed an increased cellularity, with 25% of monocytic precursors, 7% of CD34+, CD11b+, CD13+ blasts, reduced erythroid and megakaryocytic lineage, with clear dysplastic features.
Patient was then diagnosed as affected by chronic myelomonocytic leukemia type 1.

Patient received epoietin and danazol, but he rapidly became transfusion-dependent. On this bases, the CPSS risk score of our patient was high [7].

For a further increase of leukocytes, patient received etoposide for three months, but he died in November 2013 because of sepsis.

Genetic analysis

Standard karyotype was performed on metaphase spreads obtained by unstimulated cultures of BM blood sample.

Array CGH was performed according to standard protocols. Genomic DNA was extracted from 200 µL of bone marrow anti-coagulated by EDTA. This DNA and a male reference DNA were labeled differentially with Cyanine 3 and Cyanine 5, using standard procedures. Microarray experiments were performed on 4x44K microarrays (Agilent Technologies, Santa Clara, CA), that have ~35 kb average probe spatial resolution. Microarrays were scanned with an Agilent scanner G256BA. Hybridization data analysis was carried out with Agilent Technologies dedicated softwares. Search for Copy Number Variation Regions (CNVRs) was performed using the Database of Genomic Variants (http://projects.tcag.ca/variation/).

WT1 gene expression and mutations of ASXL1, TP53, EZH2, and TET2 genes were evaluated according to standard procedures. DNA was extracted from the bone marrow samples by the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). About 600 ng of DNA were analysed for each patients by using a custom qBiomarker somatic mutation PCR array (Qiagen, Hilden, Germany). About 600 ng of DNA was extracted from the bone marrow samples by the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). About 600 ng of DNA were analysed for each patients by using a custom qBiomarker somatic mutation PCR array (Qiagen, Hilden, Germany). About 600 ng of DNA were analysed for each patients by using a custom qBiomarker somatic mutation PCR array (Qiagen, Hilden, Germany). About 600 ng of DNA were analysed for each patients by using a custom qBiomarker somatic mutation PCR array (Qiagen, Hilden, Germany).

The array CGH confirmed the monosomy of chromosome 7 (mean fluorescent ratio -0.66) and detected a chromosome 12p interstitial deletion, from 12p13.2 to 12p11.23 (mean fluorescent ratio -0.68), not visible at the karyotype analyzed by two expert cytogenetists. The deletion extends for about 14.8 Mb, starting from 11,897,416 to 11,897,616 bp, and contains more than 55 annotated genes, according to UCSC database (http://genome.ucsc.edu/). Interestingly, the breakpoints fall into ETV6 gene, which results partially deleted.

The mean fluorescent ratio of the deletion (-0.66) is compatible with a mosaic deletion, roughly involving about the 75% of the cells. This percentage is overlapping with the one with monosomy 7, detected both by standard karyotype and array CGH.
It can be guessed that this partial deletion results in a decreased expression of this gene [11]. These authors demonstrated that levels of *ETV6* were significantly decreased in cases with 12p13 deletions encompassing the entire gene or at least the 3'UTR, like in the present case, whereas expression of other genes in the deleted region, like *BCL2L14, LRP6, DUSP16* and *GPRC5D*, did not show any variation, independently of their copy number status. This observation highlights that expression of *ETV6* is strictly regulated in the blood cells and suggests a potential role for this gene in the tumorigenesis process. The role of *ETV6* in the clinical history of our patient is showed by the poor prognosis both of lymphoma and of MDS.

**Conclusion**

Array CGH is a new diagnostic test to be performed along with conventional karyotype in MDS, especially when chromosome quality is poor. Discovering new cases with 12p deletions, often undetectable by conventional karyotype, can be used to establish if these deletions may identify a MDS subset with a negative prognostic factor.

Future studies investigating clonal evolution should clarify whether haploinsufficiency of *ETV6* may play an early role in the process of leukemic transformation by disordering key processes of differentiation and proliferation and whether it also plays a critical role in the induction of chromosomal instability finally resulting in the development of -7 clones and/or clones with CK.

**References**