

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

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Received Date: Oct 27, 2016; Accepted Date: Mar 16, 2017; Published Date: Mar 21, 2017

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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*, *LRP6*, *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-chloro-deoxyadenosine 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 aracytin 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 = $25 \times 10^9/L$) and leukocytosis with monocytosis (monocytes = $2.8 \times 10^9/L$).

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 μ L 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) for *ASXL1*, *EZH2*, *TET2*, and TP53 gene. This method can detect as low as 1% somatic mutations in the background of wild-type genomic DNA. Allele specific amplification is achieved by Amplification Refractory Mutation System (ARMS) technology, which is based on the discrimination by Taq polymerase between a match and a mismatch at the 3' end of the PCR primer. The analysis of data was performed using a pool of five healthy donors as control and calculating the relative abundance of mutant DNA templates by the formula $\Delta Ct \text{ sample} = (Ct \text{ mut} - Ct \text{ ref}) < \Delta Ct \text{ controls}$ where the reference assay is designed on a non-variable region of the same gene which carries the mutation.

Results

Standard cytogenetic analysis detected a monosomy of chromosome 7 in about 70% of the metaphases. WT1 gene expression was in the normal range, and we did not find any mutations of *ASXL1*, *TP53*, *EZH2*, and *TET2* genes.

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 26,698,489 bp, and contains more than 55 annotated genes, according to UCSC database (<http://genome.ucsc.edu/>). Interestingly, the 12p13.2 breakpoint falls into ETV6 gene, which results partially deleted.

The karyotype was given as arr[GRCh37] 7p22.3q36.3 (92532_158909679) \times 1 [0.7], 12p13.2p11.23 (11897416_26698489) \times 1 [0.7] according to ISCN 2016 nomenclature.

Discussion

12p deletions are detected in a broad spectrum of hematological malignancies in acute myeloid leukemia (AML) they are usually associated with complex karyotypes, whereas in myelodysplastic syndromes these anomalies are often seen along with monosomy 7 [8-11]. Rucker et al. showed that about half of the AML with complex karyotype (CK-AML) presented 12p13 deletions, when analyzed by high-resolution arrays [12]. Moreover, in a series of 125 patients with secondary MDS, unbalanced translocations involving chromosome 12p were found in 8% of cases [4].

Deletion extents 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 [13,14]. Other genes within the minimal deleted region like *CDKN1B*, *BCL2L14*, *LRP6*, *DUSP16* and *GPRC5D* may play an additional role in tumorigenesis and leukemogenesis [11]. All these genes are deleted in the case here presented; his deletion extends to band p11.23 where the oncogene *KRAS2* is harbored.

ETV6 (ets translocation variant gene 6) is a member of the ETS transcription factor family, and putative tumor suppressor gene since it induces G1 arrest and blocks Ras-induced transformation, promotes apoptosis, and activates TP53 dependent pathways [13].

Alterations of ETV6, as a consequence of translocations, deletions, or somatic mutations, has been detected in myeloid malignancies and MDS with highly variable frequency; this variability mainly depends on the characteristics of the study population and on the technique used. Anyway, it can be assumed that at least 5% of MDS/AML have *ETV6* deletion [15,16]. This frequency has been shown to be much higher in association with monosomy 7 [12,17,18]. Wall et al. tested *ETV6* deletion in a series of 38 patients with diagnosis of MDS/MPN (myeloproliferative neoplasms) or AML with monosomy 7 [19]. 6 out of 38 patients (16%) showed an *ETV6* deletion, that, in 5/6 was undetectable by conventional cytogenetic methods, as observed in our case. The breakpoints were heterogeneous: deletions could be complete, involving the entire *ETV6* gene, or only partial, with the 5' of the gene retained. As it can be seen in Figure 1, in our patient *ETV6* deletion is partial: the probes till to 11,847,532 bp resulted not deleted, whereas the probes starting from 11,897,616 bp indicates a monoallelic deletion (Figure 1).



Figure 1: Array CGH profile. Array CGH profile showing all the chromosomes, with chromosome 7 partial monosomy and chromosome 12p deletion highlighted (top left, red circles). Profile of chromosome 12 is enlarged, showing the 12p deletion in detail (top right). *ETV6* gene is partially deleted, as shown by the fluorescent ratios of the probes covering this gene (bottom).

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 useful 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.

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