Comparative genomic hybridization arrays in complex karyotype analysis – an AML case report

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Abstract

Acute myeloid leukemia (AML) is the most common type of leukemia among the elderly. Lymphocytes of approximately 55% of AML patients show various chromosomal aberrations, including structural and numerical alterations. Such changes may not only correlate with morphological and clinical data, but also serve as prognostic factors. We describe the case of AML patient with complex karyotype examined by means of array CGH. The number of aberrations found was significantly higher as compared with GTG results, which indicates that aCGH enables more sensitive karyotype examination and may be used in the search for new prognostic and predictive factors in AML.

Key words: *acute myeloid leukemia, complex karyotype, array CGH.*

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Introduction

In Poland 6000 people are diagnosed with leukemia every year [1]. Acute leukemias constitute a group of heterogeneous lymphoproliferative disorders characterized by the presence of transformed cells clone that originate from early stages of hemopoesis. According to the schema of ontogenetic development acute leukemias may be divided into two groups: lymphoblastic and non-lymphoblastic (myeloid), and further into different subtypes depending on morphologic and immunologic features altogether with immunocytochemistry of the dominant cell clone [2].

Acute myeloid leukemia (AML) is the most common leukemia of the elderly. It covers 80-85% of acute leukemias diagnosed after 20 years of life.

Chromosomal instability detected as chromosomal aberrations is a typical feature of many neoplasms of hemopoietic system. Detailed knowledge concerning such alterations is crucial not only for accurate diagnosis but also has significant prognostic and predictive value.

That is why the choice of appropriate method of testing that results in data useful for the clinician is very important in diagnostic procedure.

In late 90. the new molecular research method was introduced, comparative genomic hybridization (CGH). It enables complex examination of chromosomal aberrations without prior knowledge concerning their presence or genomic localization.

The invention of molecular micro- and macroarrays enabling simultaneous analysis of expression of thousands of genes, and development of methods of preparing such arrays led to generation of oligonucleotide CGH arrays. Those platforms allow for very precise examination of genetic material thank to high resolution of obtained signals [3].

In this paper we present the case of AML patient with complex karyotype aberrations detected with conventional cytogenetic methods. We then assessed the genomic changes by the means of array CGH method.

Material and methods

Female patient 67 years old in a critical condition, was hospitalized in the Department of Hematooncology and Bone Marrow Transplantation, Medical University in Lublin, in July 2009. She had previous history of hospitalization in the Department of Internal Medicine due to progressive

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weakness, fever and increased night sweating. Mild anemia and low platelet count was then diagnosed. In the age of 40 the patient underwent surgery (histerectomy) followed by radiotherapy because of uterine cancer. She was also treated for type 2 diabetes mellitus and hypertension.

Blood test performed after admission to the hospital showed: Hb – 8.5 g/dl; Ht – 24.8%; E – 2.63 G/µl; L – 4.41 K/µl; Tr – 27.0 K/µl, with 58% of myeloblasts in blood smear. Bone marrow FNAB (fine needle aspiration biopsy) results showed 70% blasts that were: CD13+, CD33+, CD34+, DR+, CD117+, CD11c+, MPO+, CD2+/–, CD65+/–, CD64+/–, CD4+/–, CD15–, CD16–, TdT–. Based on these results the diagnosis of MLA-M4 was set up. Cytostatic chemotherapy was applied (Ara-C 2 × 40 mg s.c.). At the same time patient received packed red blood cells, packed platelets, plasma and antibiotics. Unfortunately, despite intensive care, patient's general condition worsened and she died after two days due to sudden circulatory arrest.

Bone marrow sample obtained from the patient was used for 24 and 72 hours unstimulated cultures on appropriate culture medium. Cultures were finished conventionally, and the metaphase spreads were stained using banding techniques [G-bands by trypsin using Giemsa (GTG), R-bands by heating using Giemsa (RHG)].

Part of fresh bone marrow sample served as a source of DNA for CGH arrays. DNA was isolated by means of QIAamp DNA Blood Kit (Qiagen), according to manufacturers protocols. Quantitative and qualitative DNA assessments were performed using spectrometric and electrophoretic methods. This procedure was aimed to get reproducible results.

Patient's genome analysis was performed by means of Constitutional Chip 4.0 arrays, PerkinElmer. This method includes several steps. At the beginning both examined and control DNA were labeled with two different fluorochromes.

Then, labeled DNA was hybridized to the arrays. Hybridization lasted 16 hours at 37°C, in the humid chamber. Next, after extensive washing in order to remove unspecific binding, the arrays were scanned in Cyclone scanner (PerkinElmer).

Good quality images were then analyzed and genome changes were assessed with the oneClick CGH Edition software, Version 4.3.1, PerkinElmer.

Results

Cytogenetic analyses of bone marrow cells showed the complex karyotype:

41~46,XX,der(3)del(3)(p21)t(3;15)(q27;q15),-4,del(5)(q13q35),-15,-17, –18,+5mar [cp14]/44~47,sl,+21[cp6] (Fig. 1).

Examination of DNA samples by means of array CGH resulted in many new data concerning the regions in the genome that were changed (regions of loss or amplification). The sensitivity cut-off, where particular change was considered as clonal, was 30%. For selected chromosomal aberrations, changes identified using array CGH were verified by fluorescence *in situ* hybridization (FISH) technique (Fig. 2). Chromosomal ideograms showing mutated regions in patient's genome are shown below (Fig. 3-5).

Table 1 shows changes detected in the genome of our patient by means of conventional cytogenetics and array comparative genomic hybridization.

Table 2 shows the preliminary characteristics of regions of a genome that were identified using CGH arrays as rearranged/mutated. Right column includes the genes mapped to given locus, which dysfunction may be engaged in the process of development of chromosomal instabilities in case of AML with complex karyotype.

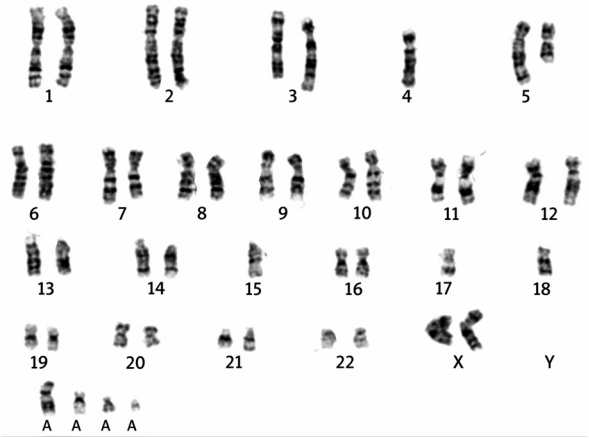


Fig. 1. Karyotype: 46,XX,der(3)del(3)(p21)t(3;15) (q27;q15), -4,del(5)(q13q35),–15,–17, –18,+4mar

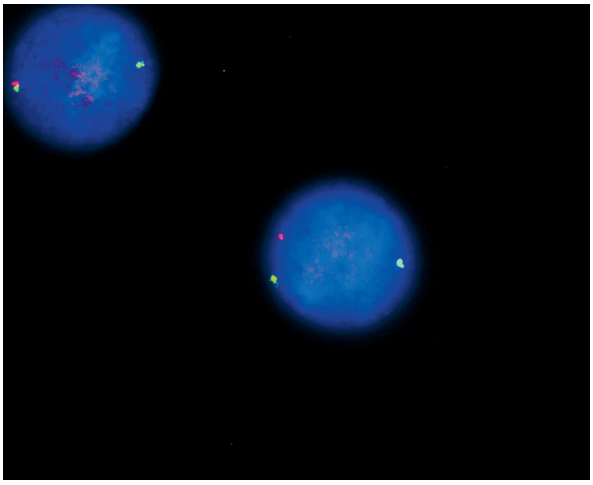


Fig. 2. Interphase FISH analysis of leukemic lymphocytes with ERG1 (5q31) Spectrum Orange/D5S23, D5S721 Spectrum Green probes (VYSIS)

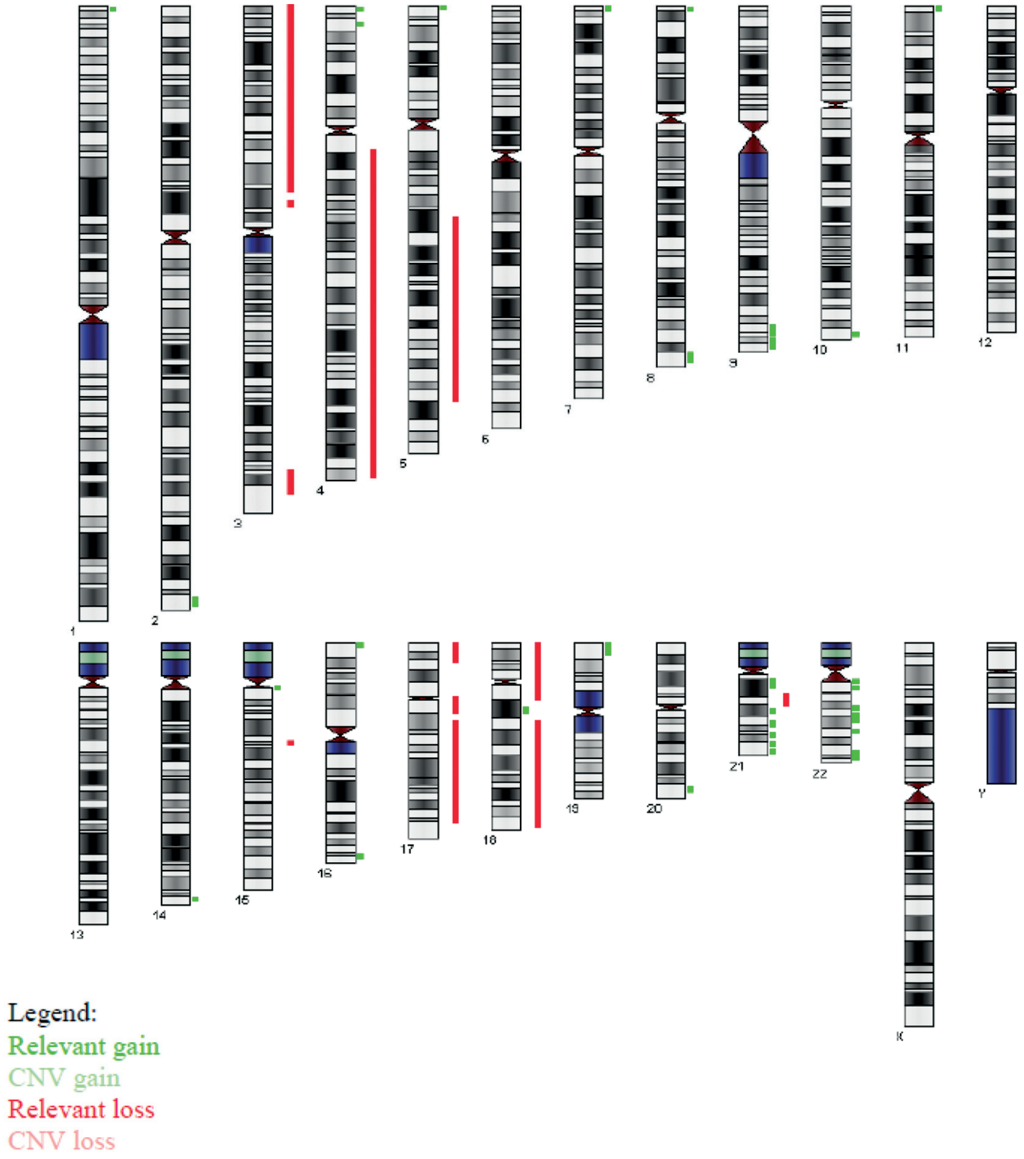


Fig. 3. Delineation of genomic losses and gains

Discussion

Non-random, clonal chromosomal aberrations are detected in 50% adult patients with AML. Such changes correlate with morphologic and clinical features of leukemia [4, 5]. Molecular characteristics of balanced translocations and inversions such as t(8;21)(q22;q22), inv(16)(p13q22) and t(15;17)(q22;q11~21), that were considered as clonal changes, have the implications for pathogenesis of different AML subtypes.

Current knowledge in the field of molecular pathogenesis of AML with complex karyotypes is rather insufficient. Gross chromosomal alterations, i.e. t(8;21), inv(16)/t(16;16), t(15;17), inv(3)(q21q26)/t(3;3)(q21;q26) and balanced translocations involving 11q23 are described elsewhere [6]. Complex karyotypes found in 10-15% adult patients with AML are associated with early myelodysplastic stage or with previous exposure to toxic factors and, almost always, with poor prognosis [7, 8].

The use of new diagnostic techniques of the array CGH type makes it possible to detect genome changes that so far could not be detected using classical cytogenetic techniques [9].

Changes detected in our patient's genome by means of array CGH technique involve both additions and deletions and clearly indicate poor prognosis. Large chromosomal

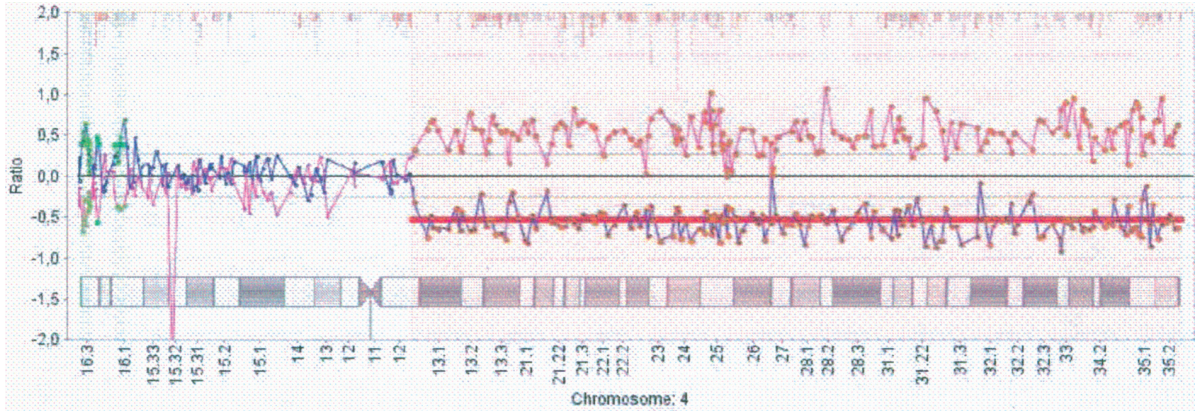


Fig. 4. Ideogram of chromosome 4

areas that were found are losses in chromosomes: 3, 4, 5, 17 and 18. Additions were found in chromosomes: 9, 21 and 22. Smaller areas including additions also appeared. Among the regions that were lost we identified the part of chromosome 4 (4q12-q35.2), known to be a locus of *NF-κB* and *KIT* genes (Table 2).

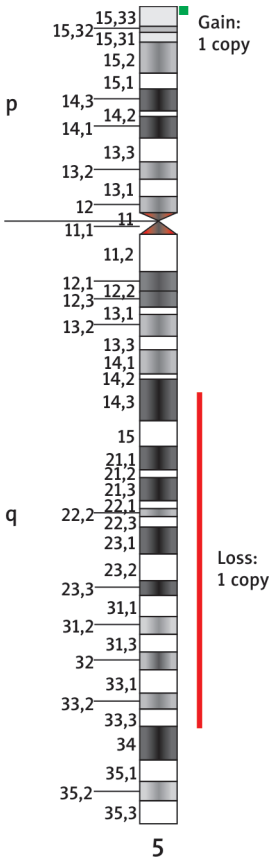
Nuclear factor κB (NF-κB) represents a family of transcription factors that affect the level of expression of BCL2 (B-cell leukemia/lymphoma) family genes and other genes of antiapoptotic function, such as *IAP2* and *FLIP* [10]. NF-κB was found to increase the resistance of the cells to apoptosis. Increased NF-κB activity leads to increased expression of genes involved in the control of the intrinsic BCL2 gene family, extrinsic DED family (death effector domains family) and common final IAP family (inhibitors of apoptosis family) pathways of caspase activation. In addition, NF-κB inhibits P53 function via MDM2 phosphorylation [11, 12].

Nuclear factor κB exists in the cytoplasm of a cell in connection with IκB family proteins. IκB binds NF-κB forming complexes to prevent their entrance to the nucleus. Kinase activation and IκB degradation leads to NF-κB release and translocation to the nucleus.

Overexpression or increased activity of NF-κB was described in many cancers. In the case of lymphoproliferative disorders such phenomenon was found in 50% of cases derived from B lymphocytes: NHL and B-CLL (non-Hodgkin lymphoma and B-cell chronic lymphocytic leukemia) [13].

In our patient also *TP53* locus was found missing (Table 2). *TP53* is well-known tumor-suppressor gene. Its protein product serves as transcription factor enabling proliferation of only those cells with intact genome. *P53* protein stands for very important factor in the process of apoptosis induced by oncogenes, such as *C-MYC*, *E1A* and *RAS*, as well as in the case of loss of pRb [14, 15].

TP53 gene inactivation as a result of mutation in 17p13.1 locus is found in more than 50% of human cancers [16]. P53 may be also inactivated due to *MDM2* oncogene overexpression.



Chromosome 5
Loss of 1.00 copy(ies), Ratio = 0.65, Region = 85.94-161.08
Samples: Array 0
Gain of 1.00 copy(les), Ratio = 1.41, Region = 0.11-2.00
Samples: Array 0
arr cgh 5q14.3q34(RP11-72L22→RP11-62G13)×1, 5p15.33(RP11-811/15→CTD-2194D22)×3

Fig. 5. Ideogram of chromosome 5

Table 1. Results of GTG and array CGH analyses

GTG	Array CGH
41~46,XX,der(3)del(3)(p21)t(3;15)(q27;q15),-4,del(5)(q13q35),-15,-17,-18,+5mar [cp14]/44~47,sl,+21 [cp6]	del(3)(p26.3p12.3),del(3)(p12.3p12.2),del(3)(q27.22q29),del(4)(q12q35.2),add(4)(p16.3),add(4)(p16.1),del(5)(q14.3q34),add(5)(p15.33),add(7)(p22.3p22.2),add(8)(p23.3),add(8)(q24.3),add(9)(q34.11q34.13),add(9)(34.13.34.3),add(10)(q26.3),add(11)(p15.5p15.4), add (14)(q32.33), add(15)(q11.1q11.2),del(15(q15.1q15.3), add(16)(p13.3),add(16)(q24.2q24.3),del(17)(q11.1q12)(del(17)(q12q25.3),del(17)(p13.3p13.1),add(19)(p13.3),add(20)(q13.33),add(21)(q11.2q21.1), add(21)(q21.3q22.1),add(21q22.11q22.12),add(21)(q22.13q22.2),add(21)(q22.3),del(21)(q21.1q21.3),add(22)(q11.21),add(22)(q21),add(22)(q12.1q12.2),add(22)(q12.2q12.3),add(22)(q13.1q13.31),add(22) (q13.32q33)

Table 2. Genomic losses and gains

Loss or Gain	Chromosome	Candidate Genes
Gain	1p36.33p36.32	*CDK11A*
Gain	2q37.3	*CXCR7*
Loss	3p26.3-3p12.3	*MLH1, EVI1*
Loss	3q27.2q29	*BCL6, IGF2BP2*
Gain	4p16.3-4p16.1	*FGFR3*
Loss	4q12q35.2	*KIT, NFkB1*
Gain	5p15.33	
Loss	5q14.3q34	*PDGFRB*
Gain	7p22.3	*GPER*
Gain	8p23.3	
Gain	8q24.2	*MYC, MAFA*
Gain	9q34.11q34.13	*ADAM12*
Gain	10q26.3	
Gain	11p15.5p15.4	*HRAS, EIF3SS*
Gain	14q32.33	*DBM, IGH*
Gain	15q11.1q11.2	*BCL8*
Loss	15q15.1q15.3	*SPINT1, THBS1*
Gain	16p13.3	*CREB BP*
Gain	16q24.2q24.3	*FBXO31*
Loss	17p13.3p13.1	*TP53*
Loss	17q11.1q25.3	*RARA*
Loss	18p11.32q23	
Gain	18q12.1	*DSG2*
Gain	19p13.3	*ICAM1*
Gain	20q13.33	*EEF1AL*
Gain	21q11.1q22.3	*ERG, ETS2*
Loss	21q21.1q21.3	*ADAMTS1*
Gain	22q11.1q13.33	*CHEK2, NF2, MKL1*

TP53 gene mutations are observed in aggressive tumors that are resistant to the therapy. The presence of such mutation correlates with short survival [17].

Lymphoproliferative disorders constitute 14% of described cases of *TP53* mutation, most commonly they are found in patients with secondary AML and MDS (myelodysplastic syndromes). Clinical course in such cases is unfavorable with small percentage of complete remissions and short survival.

We also noted the change of the long arm of chromosome 21 in our patient – it was amplification of 21q11.1-q22.3 (Table 2). Genes *ERG* and *ETS2* have been mapped to this region. Both genes belong to the family of transcription factors. Santoro and colleagues described *ETS2* amplification in AML patient characterized by hypodiploid karyotype with complex translocation t(6;18;21), whereas *ERG* gene was found to be involved in t(16;21)(p11;q22), rearrangement leading to establishment of a fusion gene *FUS/ERG* [18, 19].

Further research performed by Rücker *et al.* on bigger group of AML patients also showed *ETS* amplification in some of them, what points to important role of this phenomenon in pathogenesis of AML with complex karyotype [20].

Still little is known about the role of these genes in leukemogenesis. One of the hypotheses suggests that transcription factor ETS2 overexpression may lead to deregulation of processes that are critical for leukemogenesis [21].

Presented case proves that array CGH technique enables precise examination of karyotype changes, what is of great importance in case of AML patients, where identification of complex chromosomal aberrations involving critical regions of a genome has significant diagnostic and prognostic value.

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