Short Communication

The Assessment of Array Comparative Genomic Hybridization in Complex Karyotype Analyses

(array comparative genomic hybridization / multicolour fluorescence *in situ* hybridization / myelodysplastic syndromes / complex karyotype)

D. BYSTŘICKÁ[1], Z. ZEMANOVÁ[1], J. BŘEZINOVÁ[2], M. GANČARČÍKOVÁ[1], L. GROSOVÁ[1], I. ŠÁROVÁ[2], S. IZÁKOVÁ[2], A. BERKOVÁ[1], K. MICHALOVÁ[1,2]

[1]Charles University in Prague, First Faculty of Medicine and General University Hospital, Institute of Clinical Biochemistry and Laboratory Diagnostics, Center of Oncocytogenetics, Prague, Czech Republic
[2]Institute of Haematology and Blood Transfusion, Prague, Czech Republic

Abstract. Molecular-cytogenetic methods were used to analyse and specify complex genome rearrangements in malignant cells. Twelve samples of bone marrow cells were collected from patients with myelodysplastic syndromes (MDS). The complex karyotypes were examined by multicolour fluorescence *in situ* hybridization (mFISH), high-resolution multicolour banding (mBAND) and array comparative genomic hybridization (aCGH). For aCGH, DNA was isolated from fixed bone marrow cells in methanol and acetic acid and amplified by whole-genome amplification. Three samples were analysed by the oligonucleotide array NimbleGen on the basis of full service. BAC-based Haematochips (BlueGnome) were used for the other nine samples. Sensitivity and detection limits of both methods were compared. The results obtained by mFISH/mBAND were in most cases confirmed by the microarray technique. aCGH detected 43 unbalanced chromosomal changes that were also identified by classical cytogenetics and FISH. Moreover, aCGH discovered 14 additional changes. Cryptic amplifications and deletions were characterized with a resolution of 0.5 Mb. In one bone marrow sample with suspected monosomy 5 detected by conventional cytogenetic analysis, aCGH revealed a 22.3 Mb region of chromosome 5 inserted in another autosome within the complex karyotype. Amplified DNA was successfully used for aCGH in 11 out of 12 cases, improving resolution of unbalanced chromosomal aberrations. The combination of both approaches brought more detailed description of complex karyotypes and is highly recommended.

Introduction

The finding of clonal chromosomal aberrations in bone marrow cells is considered one of the most important independent biological and prognostic factors in malignant cells. Precise analysis of complex karyotypes has been essential to understanding tumorigenesis and progression of cancer. Conventional cytogenetics is limited by the quality of classical chromosomal preparations. Thresholds for detection vary around ten megabases. Fortunately, a variety of more sensitive molecular-cytogenetic methods are now available for genome-wide screening. In the present study, we report on multicolour fluorescence *in situ* hybridization (mFISH) and array comparative genomic hybridization (aCGH), which were used for description of complex karyotypes in leukaemic cells.

mFISH is a multicolour karyotyping technique that enables whole-genome analysis in one hybridization experiment and is suitable for identification of all chromosomes involved in a complex karyotype. The presence of metaphase spreads is necessary and each pair of chromosomes and both sex chromosomes are marked by different colour (24-colour karyotyping). The high-resolution multicolour banding technique (mBAND) allows precise determination of chromosomal rearrangements

Received January 4, 2010. Accepted January 26, 2010.

This work was supported by grants IGA MZ CR NR/9227-3, MZO VFN2005, LC 535 and MSM 0021620808.

Corresponding author: Dagmar Bystřická, Charles University in Prague, First Faculty of Medicine and General University Hospital, Institute of Clinical Biochemistry and Laboratory Diagnostics, Center of Oncocytogenetics, U nemocnice 2, 128 08 Prague 2, Czech Republic. e-mail: Dagmar.Bystricka@vfn.cz

Abbreviations: aCGH – array comparative genomic hybridization, BAC – bacterial artificial chromosome, CGH – comparative genomic hybridization, CNA – copy number alterations, Cy5 – cyanine 5, DAPI – 4,6-diamidino-2-phenylindole, DEAC – diethylaminocoumarin, FISH – fluorescence *in situ* hybridization, I-FISH – interphase FISH, ISCN – international system for human cytogenetic nomenclature, MDS – myelodysplastic syndromes, mBAND – a high-resolution multicolour banding technique, mFISH – multicolour fluorescence *in situ* hybridization, WGA – whole-genome amplification.

such as insertion, interstitial deletions and breakpoint definition. Overlapping microdissected and fluorochrome-labelled sections of chromosomes with one of five different fluorochromes are hybridized to metaphase chromosomes and unique colour combinations can be identified with the mFISH/mBAND module of the fluorescence *in situ* hybridization (FISH) imaging software with high final banding resolution (approx. 500 bands per genome) (Chudoba et al., 1999). The resolution limits for mFISH/mBAND range from 500 to 1500 kb (Sawyer et al., 1998).

Chromosomal comparative genomic hybridization (CGH) (Kallionemi et al., 1992) enables screening of deletions and amplifications in the entire genome in one hybridization experiment. The standard resolution is 10–20 Mb for CGH (Kallioniemi et al., 1992), 3–5 Mb for high-resolution CGH (Kirchhoff at al., 2001; Kristensen et al., 2003) and up to 100 kb for array-based CGH (Vissers et al., 2005). aCGH, also called molecular karyotyping, is a subsequent modification of chromosomal CGH (Shinawi and Cheung, 2008) and is regarded as top of cytogenetic resolution potential at the moment. The method emerged in the early 1990s at the same time as genotyping or expression microarrays. The technology of all microarray formats is set up on hybridization of known immobilized sequences (targets) and unknown tested sequences (probes). aCGH is suitable for detection of quantitative and unbalanced structural genome changes associated with copy number alterations (CNA), not for the balanced ones such as reciprocal translocations, inversions and insertions. Pathological clones smaller than 35 % cannot be identified (Evers et al., 2007). The quality and quantity of genomic DNA is critical for the successful analysis. For bacterial artificial chromosome (BAC)-based arrays, the required amount of DNA is approximately 100–400 ng of DNA, whereas oligonucleotide and cDNA platforms typically require 500 ng to 3 μg of high-quality DNA. Nevertheless, this is problematic particularly in haematological neoplasm samples as there is often just a limited amount of fixed bone marrow cell suspension available. In addition, DNA isolated from the above-mentioned archive material is commonly of poor quality. However, these problems could be resolved by whole-genome amplification.

Myelodysplastic syndromes (MDS) are clonal haematopoietic stem cell disorders clinically characterized by ineffective haematopoiesis. Chromosomal rearrangements can be found in bone marrow cells in more than 50 % of cases with primary MDS and in up to 80 % of cases with secondary or therapy-related MDS. Unbalanced chromosomal rearrangements are prevalent to balanced changes of chromosomes (Schoch et al., 2001) and are often part of complex karyotypes (classified as ≥ 3 numerical and/or structural chromosome abnormalities) (Herry et al., 2007). Deletions of the long arm of chromosomes 5 and 7, specifically del(5)(q31) and del(7)(q31), are considered to be the most frequent recurrent chromosomal changes in myeloid malignances. The exact identification of chromosomal changes facilitates diagnosis and prognosis of patients and helps select the proper therapy (Haase et al., 2007). Therefore, it is very important to provide precise molecular-cytogenetic description of aberrations present in malignant cell clones.

The aims of this pilot study were to provide detailed molecular-cytogenetic analyses of complex karyotypes in myelodysplastic disorders, to perform correlations of mFISH and aCGH findings, to determine the range of unbalanced aberrations and also to test the suitability of DNA obtained by whole-genome amplification for aCGH as an effective solution in cases of limited amounts of sample DNA. The pivotal goal was to evaluate the asset of aCGH to complex karyotype analyses.

Material and Methods

Bone marrow cells of 12 adults with newly diagnosed MDS and complex karyotype identified by conventional cytogenetics were examined by mFISH/mBAND and aCGH. Diagnoses were done according to the WHO classification and are presented in Table 1. All patients provided written informed consent approving use of their samples for research purposes.

Conventional cytogenetics

For conventional cytogenetic analyses, unstimulated bone marrow cells were cultivated for 24 h in RPMI 1640 medium with 10% foetal calf serum (Sigma-Aldrich, St. Louis, MO). Chromosomal preparations were done according to standard techniques using colcemid, hypotonic treatment, fixation in methanol-acetic acid and G-banding with Wright stain. Chromosomal aberrations were described according to ISCN nomenclature (2009).

mFISH

mFISH was carried out using 24XCyte MetaSystems colour kit (MetaSystems GmbH, Altlussheim, Germany) containing combinatorially labelled painting probes specific for all autosomes and sex chromosomes of human karyotype. The probes were labelled by the following fluorochromes: diethylaminocoumarin (DEAC, NEN Life Science Products, PerkinElmer, Boston, MA), Spectrum Orange (Vysis, Abbott Laboratories, Abbott Park, IL), Texas Red (Molecular Probes, Invitrogen Corporation, Carlsbad, CA), Alexa 488 (Molecular Probes, Invitrogen Corporation), Cy5 (Amersham Life Sciences, Arlington, IL), and 4,6-diamidino-2-phenylindole (DAPI) (Abbott Laboratories) was used as counterstaining. Hybridization and post-hybridization washes followed standard procedures recommended by the manufacturer's protocol. Image capturing and acquisition was processed with an Axioscope Zeiss and the Isis imaging system (MetaSystems).

mBAND

High-resolution multicolour banding was performed with region-specific partial chromosome 5 paints generated by micro-dissection and labelled with different

fluorochromes and/or their combinations, as it is described in detail by Chudoba et al. (1999). Partial probes are labelled by the same five fluorochromes as quoted for the 24XCyte mFISH kit (MetaSystems GmbH).

aCGH

For aCGH analysis, bone marrow cells fixed in methanol and acetic acid (ratio 3 : 1) were used, stored for different times in -20 °C. After washing in PBS, DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The amount of 50–250 ng of obtained DNA was amplified with GenomePlex® Complete Whole Genome Amplification (WGA) Kit (Sigma-Aldrich, St. Louis, MO). Both methods were done according to the manufacturer's protocol. DNA concentrations, before and after amplification, were measured in NanoDrop ND-1000 (Thermo Scientific, Wilmington, MA) and are presented in Table 1.

Haematochip: Fluorescent Labelling System (BlueGnome, Cambridge, UK) and BlueGnome Focus Haematology Array were treated according to the recommended protocol. The hybridized slides were scanned with GenePix 4200A scanner (Axon Instrument, Union City, CA) and for image analysis, BlueFuse Multi array CGH analysis software (BlueGnome) was used. In the three cases, we used full service offered by NimbleGen (Roche, Basel, Switzerland) and we sent sample DNA to their laboratory. After analysis, we obtained a CD with aCGH results and the required software.

Results and Discussion

Our study offered comparison of mFISH and aCGH results. Most of aCGH findings corresponded with mFISH and cytogenetic conclusions, and non-balanced chromosome changes associated with translocations were verified and specified. The aCHG and mFISH analysis results are summarized in Tables 1 and 2. The extent of the clone with deletion of the long arm of chromosome 5 was specified by means of interphase FISH (I-FISH) with VYSIS LSI probe for the 5p15.2 and 5q31 regions (Table 3).

In sample No. 6 with suspect monosomy 5 detected by conventional cytogenetics and/or FISH, further detailed analysis by aCGH revealed that part of chromosome 5 material originating from the region p11-p14 (22.3 Mb) was retained as insertion within the complex karyotype. The aCGH finding was successfully confirmed by means of I-FISH with appropriate BAC probes (data not shown). The result corresponds to the study of Herry at al. (2007), which redefined monosomy 5 in 23 subjects with MDS/AML.

Table 1. Diagnoses of 12 analysed samples, quantification of DNA isolated from fixed bone marrow cells before and after amplification, type of used aCGH approach and discrepancies between aCGH and mFISH analyses

Sample No.	Diagnosis	DNA concentration ng/µl	Purity of DNA 260/280	Input amount of DNA (ng)	Amplified DNA concentration ng/µl	Purity of amplified DNA 260/280	Type of aCGH	New aberration detected by aCGH compared to mFISH and their extent
1.	MDS RAEB I.	19.7	1.63	50	258.5	1.88	BAC	add(21)(q22.3q22.3) [0.74 Mb]
2.	MDS RAEB I.	11.3	1.58	50	117.8	1.91	BAC	del(20)(q11.23q12) [3,9 Mb] del(20)(q12q13.12) [2,5 Mb]
3.	MDS RAEB II.	9.7	1.76	50	266.1	1.93	BAC	
4.	MDS RAEB II.	39.1	2.01	250	269.8	1.93	BAC	
5.	MDS RARS	39.5	1.80	50	204.3	1.94	BAC	
6.	RAEB-T	47.9	1.93	250	282.5	1.92	oligo	del(5)(p14p15.3)(q11.1q35.3); [22.3 Mb] fragment was detected
7.	RCMD	280.9	1.94	250	247.1	1.93	BAC	del(3)(p12.3p26.3) [77,98 Mb] del(7)(p12.1p22.3) [53,71 Mb] del(12)(p12.2p13.2) [7,87 Mb] add(12)(p13.32p13.33) [7,3 Mb]
8.	RCMD	78.1	1.95	250	344.2	1.93	oligo	Chromosome 5 specific aCGH
9.	sMDS	119.7	1.94	250	345.7	1.93	oligo	del(11)(q22q23) [22,12 Mb] -16
10.	sMDS	56.6	1.53	200	352.3	1.88	BAC	add(21)(q22.13qter); [9,99 Mb]
11.	MDS CMML	86.2	1.72	200	337.7	1.90	BAC	
12.	MDS RAEB II.	30.2	1.53	200	332.6	1.93	BAC	add(1)(p36.31p36.32) [3,01 Mb] del(7)(q22.1q22.1) [1,91 Mb] del(8)(q24.21q24.21) [0,42 Mb]

MDS – myelodysplastic syndromes; RAEB – refractory anaemia with excess blasts; RARS – refractory anaemia with ring sideroblasts; RAEB-T – refractory anaemia with excess blasts in transformation; RCMD – refractory cytopoenia with multilineage dysplasia; sMDS – secondary MDS; BAC – Haematochip (Bluegnome, Cambridge); oligo – NimbleGen array format (Roche); aCGH – array comparative genomic hybridization; FISH – fluorescence *in situ* hybridization; del – deletion; add – amplification; Mb – megabase

Table 2. Karyotype designations according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009 as identified by mFISH and summary of the extent of revealed deletions (del) and amplifications (add) by aCGH. Aberration extent is in megabases [Mb], chromosomal changes revealed by aCGH only are assigned in bold.

Sample No.	Karyotype (conventional cytogenetics, mFISH, mBAND)	aCGH
1	47,XX,del(5)(q13q33)[1]/idem,-21,+22,+der(22)ins(22;21)(q?;q?)[14]	del(5)(q14.3q34) [78 Mb] del(21)(q11.2q22.3) [27,5 Mb] **add(21)(q22.3q22.3) [0,74Mb]** +22
2	42~45,XY,der(1)t(1;19)(p13;?),+der(1)t(1;20)(?;?),der(5)t(5;12)(q11;q?),del(7)(q21),der(12)t(5;12)(q?;q12),der(14)t(14;20)(q31;?),-16,der(17)t(16;17)(?;p11),der(19)t(19;20)(q11;?),-19[cp8]	del(5)(q13.2q35.3) [119,33 Mb] del(7)(q22.1qter) [59,61 Mb] del(12)(p11.1p13.33) [33,13 Mb] del(16)(q11.2q24.3)43,52 Mb] del(17)(p13.1p13.3) [9,8 Mb] **del(20)(q11.23q12) [3,9 Mb]** **del(20)(q12q13.12) [2,5 Mb]**
3	44,XY,-3,del(5)(q),-7,del(12)(p12.1p13.2) [5]/46,XY,idem,+20,+22[3]/39~70,XY,idem[cp4]	-3 del(5)(q11.2q34) [105,63 Mb] -7 del(12)(p12.1p13.2) [13,88 Mb]
4	45,XX,der(2)t(2;3)(q31;q12)ins(3;3)(q13.2;p24.2p25),-3,del(5)(q13.3q33.3)[3]/49,XX,idem,+1,+11,+21,i(22)(q10),+del(22)(q11)[cp3]	del(2)(q37.1q37.3) [12,82 Mb] del(3)(p11.2p24.2) [60,85 Mb] del(3)(p26.1p26.3) [6,63 Mb] del(5)(q14.3q34) [84,45 Mb] add(22)(q11.22q12.3) [9,25 Mb]
5	42~47,XY,der(9)t(1;9)(?;p22),+19[cp8]/46,XY[2]	add(1)(q21.1q44) [104,13 Mb] add(19)(q13.11q13.43) [31,05 Mb]
6	37~54,XY,der(3)t(3;4)(?;?),-4,- 5,+8,der(10)ins(10;4)(q21;?),der(16)t(16;17)(q12;?),-17,+21[cp16]	del(3)(p11.1p26.3) [90,3 Mb] del(4)(q35.1q35.1) [1,39 Mb] del(5)(p14p15.3) [23,87 Mb] del(5)(q11.1q35.3) [131,22 Mb] -7 +8 del(16)(q12q13) [7,14 Mb] del(16)(q21q22.1) [10,29 Mb] del(16)(q23.2q24.3) [10,35 Mb] del(17)(p11.1p13.3) [28,56 Mb] +21
7	85~89,XX,del(5)(q14.3q33.3),del(11)(q?),-12,-16,-16,-X,+ 2mar, inc[cp18]/46,XX[4]	**del(3)(p12.3p26.3) [77,98 Mb]** **del(5)(q14.3q34) [74,97 Mb]** **del(7)(p12.1p22.3) [53,71 Mb]** **add(12)(p13.32p13.33) [7,3 Mb]** **del(12)(p12.2p13.2) [7,87 Mb]**
8	45,XX,dic(3;7)(p11?;p11?),del(5)(q13.1q33.3)[3]/44,XX,idem,der(12)t(12;15)(p11.1;q11.1),-15[9]	del(5)(q12.3q31.1) [87 Mb] Chromosome 5 Tiling array
9	45,XY,der(3)t(3;6)(p11.3;?),del(5)(q13q33),-6 [3]	del(5)(q12q34) [96,18 Mb] del(11)(q22q23) [22,12 Mb] -16
10	44~45,XX,der(5)t(5;13)(q21;q21),-7,-13,der(22)t(7;22)(p15.3;q13)[cp7]/44~45,XX,der(5)t(5;13)(q21;q21),der(7)t(7;22)(q11.21;q13),-13,-22,+mar[cp3]	del(5)(q14.3qter) [94,42 Mb] del(7)(p15.3qter) [134,88 Mb] del(13)(q14.11qter) [70,94 Mb] **add(21)(q22.13qter) [9,99 Mb]** del(22)(q12.1qter) [22.13 Mb]
11	41~45,XY,der(3)del(3)(p14)del(3)(q12),der(5)del(5)(q14.3qter)t(3;5)(?;q14.3),-7,del(12)(p?13), del(13)(q?32)[cp9]/46,XY[1]	del(3)(p11.2pter) [86,62 Mb] del(5)(q14.3qter) [89,26 Mb] del(7)(p11.2pter) [55,73 Mb] del(7)(q11.21q21.13) [10,93 Mb] del(7)(q21.3qter) [61,15 Mb] del(12)(p12.1p13.2) [12,06 Mb]
12	46,XX,der(8)t(1;8)(?;q24)[10]/46,XX,idem,t(13;19)(q13;?)[7]/46,XX[2]	**add(1)(p36.32pter) [3,01 Mb]** add(1)(q21.1qter) [244,92 Mb] **del(7)(q22.1q22.1) [1,91 Mb]** **del(8)(q24.21q24.21) [0,42 Mb]**

Table 3. Identification of deletion del(5)(q) by I-FISH, mFISH/mBAND and aCHG

Sample No.	I-FISH* chromosome 5 deletion Size of clone	mFISH/mBAND extent of deletion	aCGH extent of deletion	Deleted region [Mb]
1.	90.0%	del(5)(q13q33)	del(5)(q14.3q34)	78.00
2.	52.5%	der(5)t(5;12)(q11;q?)	del(5)(q12.1qter)	119.33
3.	73.0%	del(5)(q13q33)	del(5)(q11.2q34)	105.63
4.	40.5%	del(5)(q13.3q33.3)	del(5)(q14.3q34)	84.45
6.	82.5%	-5	del(5)(p14p15.3)(q11.1qter)	155.09 (23.87 p + 131.22 q)
7.	77.5%	nd	del(5)(q14.3q33.3)	74.97
8.	68.5%	del(5)(q13.1q33.3)	del(5)(q12.3q31.1)	87.00
9.	91.5%	del(5)(q13q33)	del(5)(q12q34)	96.18
10.	91.0%	del(5)(q21qter)	del(5)(q14.3qter)	94.42
11.	96.4%	del(5)(q14.3qter)	del(5)(q14.3qter)	89.26

I-FISH – interphase fluorescence *in situ* hybridization, mFISH – multicolour fluorescence *in situ* hybridization, mBAND – high-resolution multicolour banding, aCGH – array comparative genomic hybridization, nd – not done, del – deletion, Mb – megabase

Note: In samples No. 5 and No. 12, chromosome 5 was not aberrant.

*I-FISH was performed with Dual Color Probe LSI EGR1 (5q31)/D5S721,D5S23 (Vysis).

Complex karyotype analyses of cases No. 1 and 2 proved by mFISH and aCGH are shown in Fig. 1. In both cases, aCGH results mostly corresponded with mFISH findings. In case No. 1, imbalances of chromosomes 5, 21 and 22 were found by both methods and moreover, aCGH detected additional 0.74 Mb amplification on chromosome 21: add(21)(q22.3q22.3). In case No. 2, unbalanced deletions of chromosomes 5, 7, 12, 16, 17 and 20 were found by mFISH as well as by aCGH.

Bone marrow cells of sample No. 7 were evaluated by classical cytogenetics as a high-grade polyploidy, mFISH was not done, and aCGH discovered a malignant cell clone with deletions of chromosomes 3, 5, 7 and 12 and amplification of chromosome 12 (see Table 2). The revealed chromosomal changes are recurrent and we found them as the main aberrations of the malignant cell clone.

We obtained discrepant results by aCGH and by mFISH in sample No. 9 only. Using aCGH, we found 22 Mb deletion del(11)(q22q23) and loss of chromosome 16, while findings of mFISH, except for deletion of chromosome 5, were not confirmed. We suppose that the quality of DNA for oligonucleotide aCGH before whole-genome amplification was poor and initial errors were amplified by whole-genome amplification.

In twelve analyses of complex karyotypes, we described 46 deleted and 11 amplified chromosomal regions by aCGH (see Fig. 2). The most frequently rearranged were chromosomes 5 (10×), 7 (7×), 3 and 12 (5×), 21 (4×), 16 and 22 (3×). Using aCGH, 14 cryptic imbalances were found (10 deletions and 4 gains). On the other hand, aCGH did not detect 10 chromosomal rearrangements identified by mFISH. Most probably, these aberrations were part of balanced chromosomal rearrangements (see Table 2).

In 10 of 12 samples, deletions of long arm of chromosome 5 were studied. Conventional cytogenetic analyses showed seven interstitial deletions and in three cases, deleted chromosome 5 was involved in structural rearrangements. In two cases, chromosome 5 was not affected. The size of clone with del(5)(q) detected by I-FISH ranged from 40.5 % to 96.4 % and the extent of deletion varied from 74.97 to 119.33 Mb as determined by aCGH. In this study, the minimal deleted region lost in all 10 cases was q14.3q31.1. An example of chromosome 5 deletion proved by mFISH and aCGH is shown in Fig. 3.

One sample (No. 8) was analysed for chromosome 5 only by oligonucleotide tiling-path aCGH (NimbleGen) and aCGH analysis established del(5)(q12.3q31.1), subsequently ascertained by mFISH in extent of 87 Mb.

Standard requirements for the DNA amount vary among different aCGH protocols from hundreds of nanograms to micrograms for one hybridization experiment. In addition to the quantity, DNA quality is fundamental for microarray analyses. Our samples were fixed in acetic acid and methanol and stored in -20 °C. Whole-genome amplification solved the deficiency and insufficient quality of nucleic acid and on principle, the input amount of DNA to the amplification reaction did not affect the results. For our analyses, we selected the GenomePlex Whole Genome Amplification (WGA) system, which is tolerant to poor quality of highly degraded DNA. The method is based upon random fragmentation of the genome into a series of overlapping short templates, which are efficiently primed and replicated using linear, isothermal amplification. The linear amplification is in the initial stages followed by a limited round of geometric amplifications and generates DNA fragments of variable size (200–2000 pb). The accuracy of the WGA system for aCGH was studied by Little et al. (2006), and the data obtained and the reproducibility of experiments with amplified DNA were more precise in comparison to unamplified DNA or DOP-PCR-amplified DNA.

The advantage of DNA amplification is not the increase of genomic DNA concentration only, but also a fundamental improvement of DNA purity. The oligonucleotide array format is more sensitive to DNA integrity.

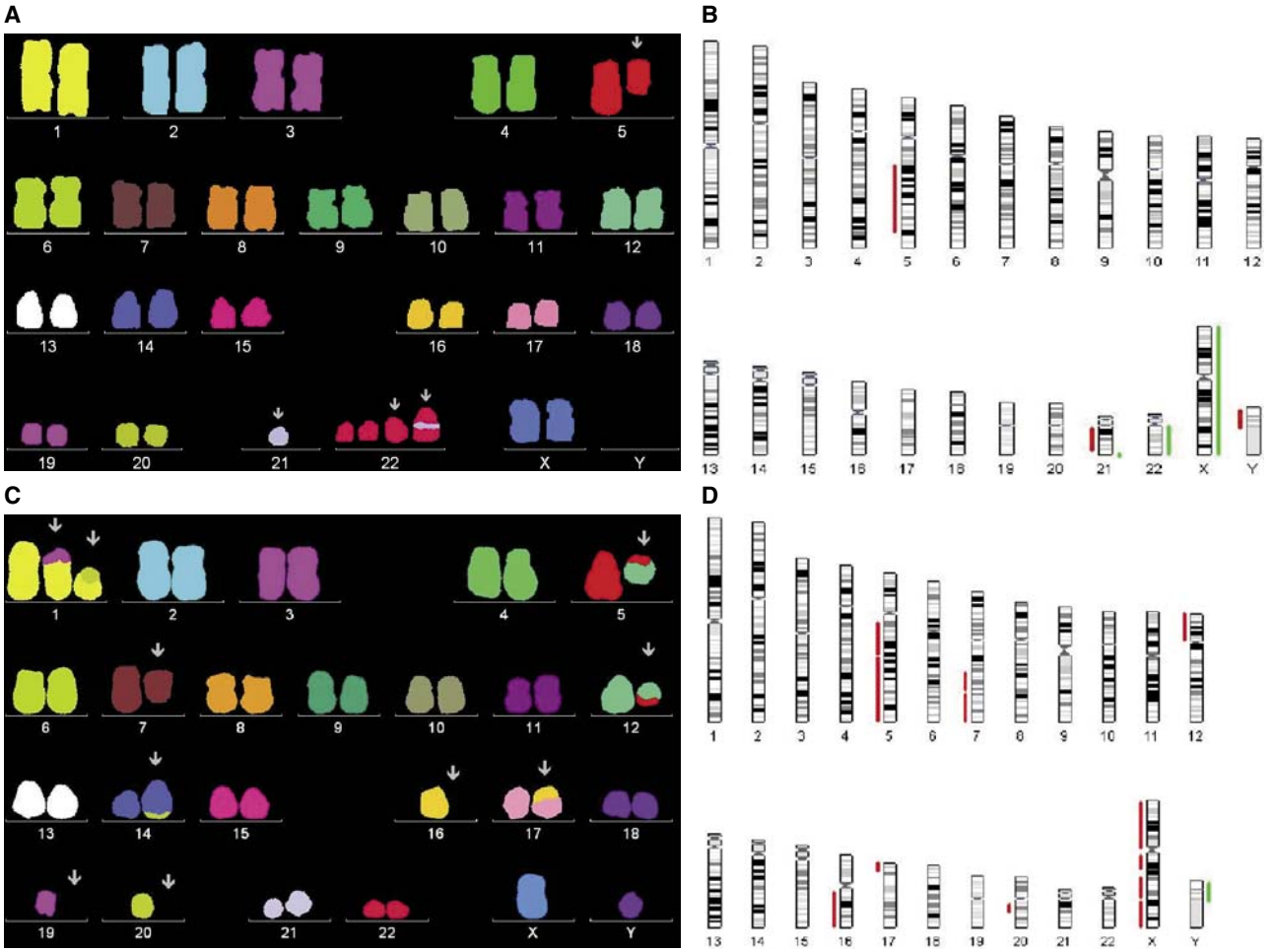

Fig. 1. Complex karyotype analyses by mFISH (A) and aCGH (B) in case No. 1 and by mFISH (C) and aCGH (D) in case No. 2. In mFISH figures, white arrows indicate derivative chromosomes. In aCGH figures, gains are on the right – the green bars – whereas losses are on the left – the red bars. In both cases, aCGH results mostly corresponded with mFISH findings. In case No. 1, imbalances of chromosomes 5, 21 and 22 were found by both methods and moreover, aCGH detected additional 0.74 Mb amplification on chromosome 21: add(21)(q22.3q22.3). In case No. 2, unbalanced deletions of chromosomes 5, 7, 12, 16, 17 and 20 were found by mFISH as well as by aCGH. Detailed description of complex karyotypes obtained by mFISH and aCGH are shown in Table 2.

In case No. 9, very poor quality of isolated and amplified DNA probably led to the misleading results of aCGH. In the case of BAC arrays, DNA amplification can cause some discrepancies in detection of chromosome losses. This could be due to imprecise hybridization of too short fragments of labelled DNA to BAC clones. DNA obtained by amplification is fragmented and it could influence efficiency of the dye incorporation. The probability of yielding an adequately labelled short DNA fragment is much lower when compared to longer DNA sequences as they can be lost through the purification processes. Whole-genome amplification provided the shortest 200 pb fragments; the size of BAC clones is 1 kb. Repeatedly, another reason can be assigned to the extent and number of represented malignant clones. For recently used aCGH techniques, a lower amount of input DNA (cca 50 ng) is required and amplification steps are already included in their protocols.

The NimbleGen whole-genome Tiling Set array design utilizes short oligomere targets, which provide comprehensive coverage of genic and intergenic regions. Chromosome 5 Tiling array is more detailed. The density of probe placement enables ultra-high resolution mapping of chromosomal aberrations and breakpoints linked with unbalanced rearrangements. The results can be validated by PCR amplification and sequencing. In this way, we can create "custom" designed array. BlueGnome BAC-based array covers the whole genome and is focused on regions associated with known haematological aberrations. Spotted clones may be ordered as labelled FISH probes to directly validate the obtained results. The exploited aCGH approaches provided whole-genome screening with high resolution efficiency (less than 0.5 Mb). Both microarray techniques specified the 5q deletion and established its extent (Table 3). The resolution of the oligonucleotide-array CGH is more exact in comparison with the BAC array format

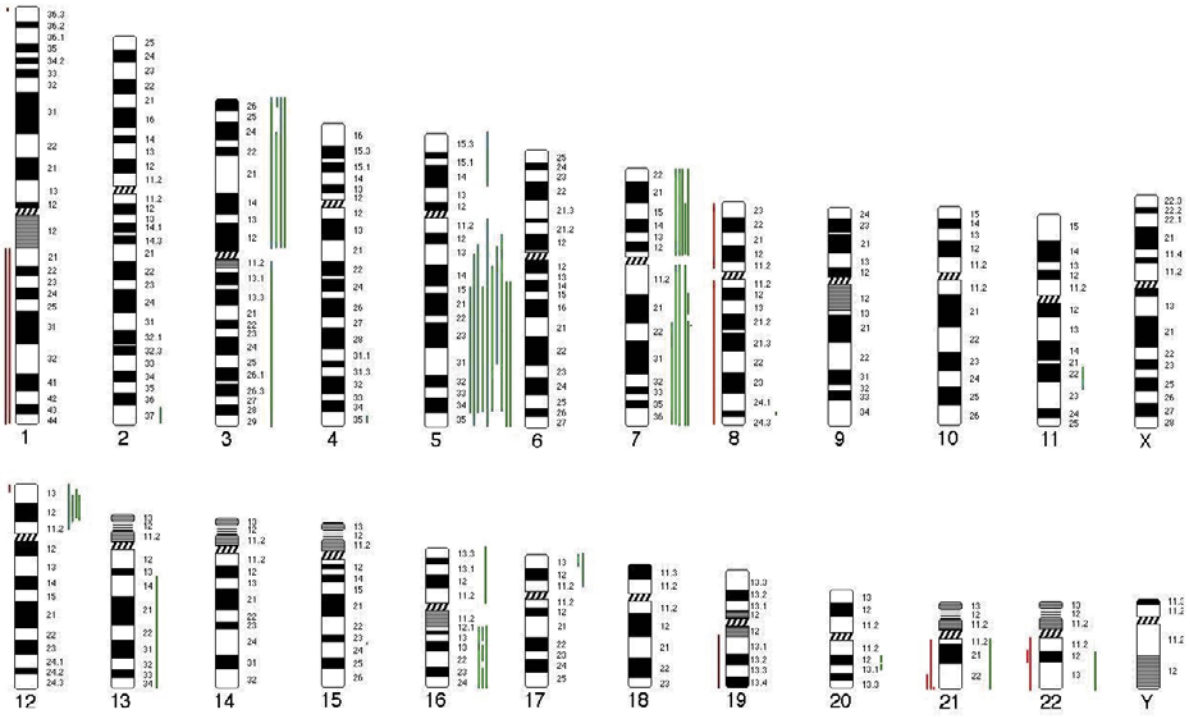

Fig. 2.The results of 12 aCGH analyses of MDS cases with complex karyotype: gains (left side, red bars) and losses (right side, green bars). Chromosome ideograms were adapted from http://www.biologia.uniba.it/rmc/.

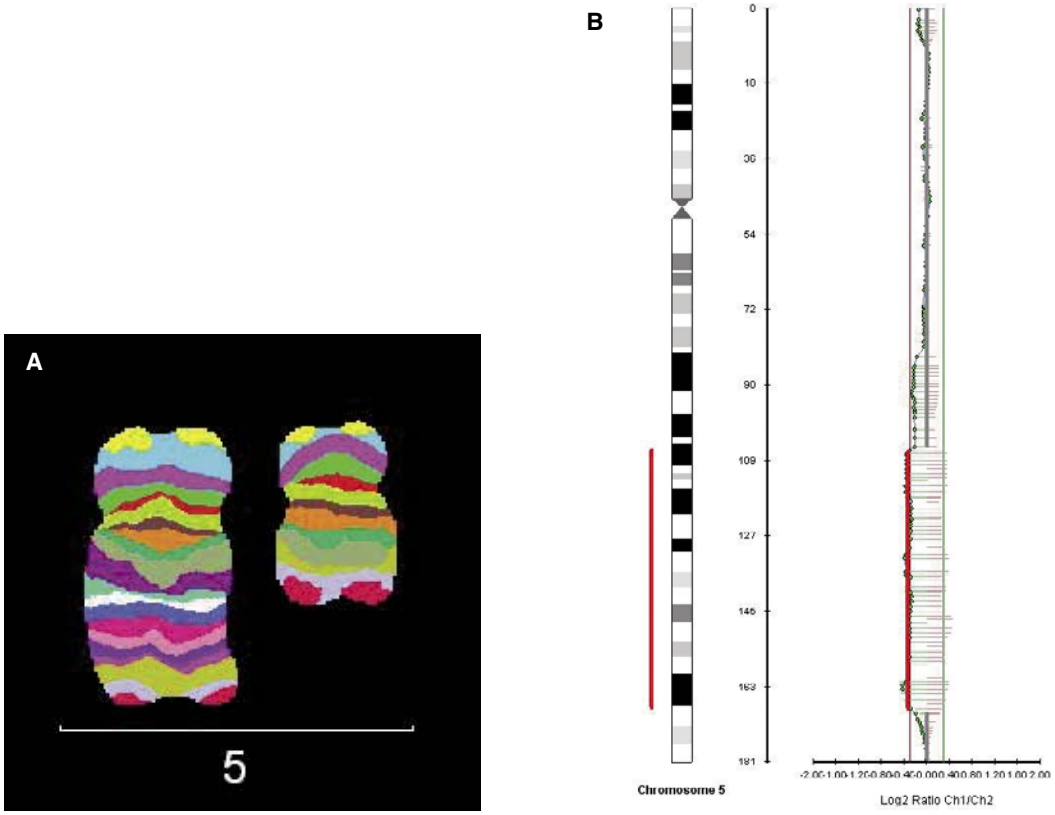

Fig. 3. Extent of deleted chromosome 5 in sample No. 4 as proved by mFISH (A) del(5)(q13.3q33.3) and aCGH (B) del(5)(q14.3q34) [84,45 Mb].

and oligonucleotide chips are more informative at the sequence level. On the other hand, data interpretation is more difficult for oligonucleotide-based array. However, well-designed aCGH is nowadays regarded as one of the most yielding techniques in the case of unbalanced chromosomal changes.

Some discrepancies between mFISH and aCGH results in our study can be explained by design and sensitivity of the methods used. Among other things, the results are influenced by the size of the pathological clone in bone marrow cells, low number of available metaphases analysed by mFISH and extent of deleted/amplified regions. It is also possible that aCGH-undetected chromosomal aberrations were part of balanced translocations.

We can conclude that mFISH is a very effective and reliable method to complement classical cytogenetic analyses and provides detailed information about chromosomal rearrangements and aberrations, but cannot determine their extent accurately. For precise specification of the size of the affected region, we have to use mBAND, which is capable of reaching the resolution potential of 500 kb. However, the results are limited by poor quality and/or low number of metaphases available.

For aCGH, DNA quality is fundamental and the results should be always confirmed by different available methods. Despite aCGH limitations, sensitivity of the method and requirement of no metaphase spreads represent the major advantages of microarray whole-genome studies.

The combination of the methods provides improvement in detailed analysis of abnormal karyotypes, determination of the extent and location of unbalanced aberrations. Our study proved that amplified DNA, which resolves limited quantity and quality of sample material, is suitable for aCGH, and these analyses supplemented with mFISH and mBAND have a strong potential to be used for specification and classification of tumour cells.

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