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Complete karyotype characterization of the K562 cell line by combined application of G-banding, multiplex-fluorescence in situ hybridization, fluorescence in situ hybridization, and comparative genomic hybridization

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Abstract

This study combines conventional cytogenetics, fluorescence in situ hybridization (FISH), multiplex-FISH and comparative genomic hybridization (CGH). In applying this multimodal approach on the human leukemia cell line K562, the chromosome composition was refined in detail and compared with data from the literature. A hypotriploid karyotype with a modal chromosome number of 67, and 21 unique marker chromosomes were identified. The classification of six markers was identical to published data and the composition of five further markers from the literature could be fully clarified for the first time. The composition of another five markers, which have been interpreted in divergent ways in different studies, were elucidated without doubt. Finally, five new markers of our study seem to have no equivalents in former studies, very likely due to limitations of conventional cytogenetics. The combinatory application of complementary techniques as shown in this study will be very useful to provide the basis of a refined genotype analysis on the chromosomal level.

Keywords: K562; M-FISH; CGH; CML

1. Introduction

The leukemia cell line K562 was derived in 1970 from a pleural effusion of a patient with chronic myelogenous leukemia (CML) in blast crisis [1]. CML is a clonal malignant disorder of the pluripotent hematopoetic stem cell, and progresses from a chronic phase through an accelerated phase to a blast phase [2]. The cytogenetic hallmark of CML is the Philadelphia translocation t(9; 22)(q34; q11), which reflects the rearrangement of the *ABL* and *BCR* genes [3–5]. The Philadelphia (Ph) chromosome [2,6–9] is visible in 90% of CML cases. In the remaining cases, complex translocations are present, with three, four or five chromosomes involved [10]. The *BCR/ABL* fusion gene is regulated by the *BCR* promotor [11]. The fusion protein is supposed to play a critical role in the neoplastic transformation of Ph-positive CML cases, due to its increased tyrosine kinase activity [12]. Furthermore, the *BCR/ABL* fusion protein induces CML in mice [13].

Because of the wide interest in K562, various cytogenetic investigations have been performed to characterize

Abbreviations: BSA, bovine serum albumine; CML, chronic myelogenous leukemia; CGH, comparative genomic hybridization; Cy3, Cy 5, Cyanine 3, 5; DAPI, diamidino phenyl indole; DIG-dUTP, digoxygenin-desoxyuridine triphosphate; dCTP, desoxycytosine triphosphate; DOP-PCR, degenerate oligonucleotide-primed polymerase chain reaction; FISH, fluorescence in situ hybridization; FITC, fluorescein isothiocyanate; M-FISH, Multiplex-fluorescence in situ hybridization; Ph, Philadelphia chromosome; PHA, phytohemagglutinin; RNase A, ribonuclease A; SSC, saline sodium citrat; TRITC, tetraetylrhodamine; WCP, whole chromosome painting; CEP, centromere probe.

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the chromosomes of this cell line in detail. Lozzio and Lozzio [1] have found a Ph-chromosome and a long acrocentric marker, interpreted as derived from a t(15; 17), with a hypodiploid karyotype in short time cultures, whereas long time cultures had shown a near triploid karyotype. In the following years, various cytogenetic analyses of K562 have been performed. Collins and Groudine [14] found a Ph positive hyperdiploid karyotype. Dimery et al. [15] classified the cell line as Ph positive and near triploid, whereas in several investigations the K562 karyotype has been characterized as Ph negative and near triploid [16–20]. One or two presumably Philadelphia-derived chromosomes, composed of amplified *BCR/ABL* fusion genes, were detected repeatedly [1,14,17–20]. Probably such a masked Ph chromosome was the reason for immortalization of the K562 cell line [20].

In the present work, the karyotye of K562 was analysed in detail by classical G-banding in combination with various molecular cytogenetic methods (FISH, M-FISH, and CGH), resulting in the refinement of karyotypes published earlier [17,18,20]. Multiplex-fluorescence in situ hybridization (M-FISH), a technique of detecting all 24 human chromosomes in different colors by 24 combinatorially labelled probes [21,22], provided valuable information about the chromosomes involved in the composition of K562 marker chromosomes. Fluorescence in situ hybridization (FISH) analysis by several whole chromosome painting probes (WCPs) and centromere probes (CEPs) was applied in confirmation experiments. The comparative genomic hybridization (CGH) map of gains and losses of chromosomal material was an efficient tool for corroboration of results achieved by the other methods. With the help of these three molecular cytogenetic methods, the G-banding karyotype could be clarified. The complete and definitive K562 karyotype was established.[1]

2. Materials and methods

2.1. K562 metaphase preparation

K562 cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM ACC 10) and cultured over a period of four to five years. Culturing of K562 cells, harvesting and preparation of slides was performed according to standard cytogenetic procedures. Harvested K562 cells were stored at −20°C in methanol–glacial acetic acid (3:1). For conventional and molecular cytogenetic analyses, they were dropped on microscope slides and air dried. Slide preparations were allowed to age at room temperature for 1 day, 2 days or 1 week before using in M-FISH, FISH or G-banding analysis, respectively.

2.2. Comparative genomic hybridization (CGH)

CGH was carried out according to Du Manoir et al. [23] with modifications. High molecular weight genomic DNA was isolated from K562 cells and from peripheral blood of a normal male donor (DNA isolation kit for mammalian blood, Roche Diagnostics, Mannheim, Germany). Genomic DNA from K562 and normal control DNA were labelled with biotin-16-dUTP and digoxigenin (DIG)-11-dUTP (Roche Diagnostics, Mannheim, Germany) by nick translation (CGH Nick Translation Kit, Vysis, Downers Grove, USA), respectively. For hybridization, 500 ng of normal and test DNAs were combined with 50 μg human Cot-1 DNA (Gibco BRL, Gaithersburg, MD) and resuspended in 12 μl of hybridization mixture (50% formamide, 10% dextran sulfate, 2 × SSC, pH 7.0). Reference metaphase cells were prepared from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes of a normal male donor following standard cytogenetic procedures. The slides were denaturated in 70% formamide, 2 × SSC for 2 min at 72°C. Probe denaturation was performed at 72°C for 6 min. After a pre-annealing time of 30 min at 37°C, the probe was applied to the denatured metaphase preparation. The slides were incubated for 3 days at 37°C in a humid chamber. Post-hybridization washes were carried out with 50% formamide/2 × SSC at 42°C with agitation, followed by 0.1 × SSC at 60°C without agitation. After blocking with 3% BSA in 4 × SSC/0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO, USA), the biotin- and digoxigenin-labeled probes were detected using fluorescein isothiocyanate (FITC) conjugated to avidin and anti-digoxigenin FAB fragments, conjugated to tetraetylrhodamine (TRITC) (Oncor, Gaithersburg, MD), respectively [24]. Post-detection washes were performed 3 × 5 min with 4 × SSC/0.1% Tween 20 at 42°C, with agitation. For image acquisition a Leica DM RBE epifluorescence microscope (Leica, Cambridge, England) and a grey level CCD- (Charge Coupled Device-) camera (KOHU) were used. Image acquisition and analysis were performed by the cytovision software version 3.1 (Applied Imaging Corp., USA), implemented on a Dell Power Edge SP 590-2. For CGH analysis of the cell line K562, the mean ratio profile of 15 metaphase spreads was calculated.

2.3. G-banding analysis

Chromosome analysis was performed on G-banded metaphases and described according to conventional

[1] The authors wish to draw the reader's attention to an article being published during the process of reviewing and revision of this paper by Gribble et al. in Cancer Genetic Cytogenetic 2000; 118: 1–8.

cytogenetic nomenclature [25]. For image acquisition, a Leica DM RBE epiflourescence microscope (Leica, Cambridge, England) and a grey level CCD- (Charge Coupled Device-) camera (KOHU) were used. Image acquisition and analysis were performed by the cytovision software version 3.1 (Applied Imaging Corp., USA), implemented on a Dell Power Edge SP 590-2.

2.4. Multiplex-fluorescence in situ hybridization (M-FISH)

M-FISH analysis was performed according to Speicher et al. [21]. Twenty-four different chromosome colors were generated by combinatorial labelling of flow sorted chromosome pools. These chromosome pools were amplified and labelled by degenerate oligonucleotide-primed PCR (DOP-PCR) [26] with fluorescein derivative Fluor-X-dCTP, cyanine(Cy)3-dUTP, Cy5-dUTP (Amersham Life Science Inc., Arlington Heights, USA) and Biotin-dUTP or DIG-dUTP (Roche Diagnostics, Mannheim, Germany). The probe pools were composed as described by Eils et al. [27]. A combination of DNA from the five probe pools was precipitated with human Cot-1 DNA/salmon sperm DNA and resuspended in hybridization mixture (50% formamid, 15% dextran sulfate, 2 × SSC, pH 7.0). The K562 metaphase preparations were pre-treated with RNase A (Qiagen, Hilden) (1 μg/μl in 2 × SSC, 1 h, 37°C) and pepsin (Roche Diagnostics, Mannheim, Germany) (8.5×10^{-4}% in A. dest. pH 2, 4–10 min, 37°C). Slides were denatured in 70% formamid, 2 × SSC for 2 min at 70°C. After probe denaturation at 75°C for 7 min, pre-annealing was performed at 45°C for 30 min. The pre-annealed hybridization mixture was added to the denatured slides and allowed to hybridize for 48 h. Post-hybridization washes were performed 3 × 5 min with 50% formamide/2 × SSC pH 7.0 at 45°C, and 3 × 5 min with 1 × SSC pH 7.0 at 60°C, both with agitation. After blocking with 3% BSA in 4 × SSC/0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO, USA), the biotin- and digoxigenin-labelled probes were detected using Cy3.5 conjugated to avidin, and anti-digoxigenin FAB fragments conjugated to Cy5.5 (Amersham Life Science Inc., Arlington Heights, USA), respectively. Cy5.5 conjugated anti-digoxogenin was generated by usage of an anti-digoxigenin antibody (Roche Diagnotics, Mannheim, Germany) and a commercially available antibody-labelling kit (Amersham, Arlington Heights, USA). Post-detection washes were performed 3 × 5 min with 4 × SSC/0.1% Tween 20 at 45°C, with agitation. The air dried slides were counterstained with DAPI/Antifade (Oncor, Gaithersburg, USA). Image acquisition was done using a Leica DM RXA epifluorescence microscope (Leica, Cambridge, England) and a high resolution cooled CCD- (Charge Coupled Device) Sensys camera (Photometrics, Tucson, Arizona, USA). The computer system for image acquisition and analysis contained the Leica Q-FISH software version Y2.3.1.Beta (image acquisition) and Leica MCK version Y01.02.Beta (image analysis), on a Leica Q 550 CW computer.

2.5. Fluorescence in situ hybridization (FISH)

FISH analysis of K562 metaphases was performed using single copy probes, whole chromosome painting (WCP) probes and centromere probes (Chromosome Enumeration Probes, CEPs) (Vysis, Downers Grove, USA). All probes were directly labelled with spectrum green or spectrum orange. Applying the LSI™ *BCR/ABL* translocation probe, the localization of *BCR* and *ABL* genes in K562 metaphases were detected as spectrum green and spectrum orange signals, respectively. *BCR/ABL* fusion genes appeared as yellow fusion signals. The following combinations of WCP probes were hybridized to K562 metaphases: WCP 6 with WCP11, WCP 12 with WCP21, WCP 2 with WCP 19, WCP 19 with WCP 20, and WCP X. Eight FISH experiments were performed by combination of whole chromosome painting probes and centromere probes: WCP 5 with CEP 6, WCP 10 with CEP 10, WCP 10 with CEP 17, WCP 11 with CEP 6, WCP 11 with CEP 11, WCP 12 with CEP 12, WCP 19 with CEP, and WCP 9 with CEP 17.

The procedure used for each probe was as specified by the manufacturer (Vysis, Downers Grove, USA). In FISH experiments with combined application of whole chromosome painting probes and centromere probes, the hybridization buffer provided by the manufacturer for whole chromosome painting probes was used. In corresponding control experiments on normal metaphases, successful hybridizations were proved. In parallel to the *BCR* and *ABL* gene FISH, an additionally hybridization to Ph positive metaphases was performed. For image acquisition a Leica DM RBE epiflourescence microscope (Leica, Cambridge, England) and a grey level CCD- (Charge Coupled Device-) camera (KOHU) were used. Image acquisition was performed by the cytovision software version 3.1 (Applied Imaging Corp., USA) implemented on a Dell Power Edge SP 590-2.

3. Results

In this study, the cell line K562 has been analyzed by G-banding, M-FISH, FISH and CGH. M-FISH was crucial for the interpretation of several markers (see below). Furthermore, M-FISH enabled the detection of two marker chromosomes (M11, M13) which have not been identified by any conventional cytogenetic studies performed thus far. FISH experiments have been per-

formed to verify and specify G-banding and M-FISH results. CGH established the resulting effects of the rearrangements on the whole genome, thereby, in general, confirming the interpretation of the results of the former methods.

The combined analysis of K562 by G-banding, M-FISH, FISH and CGH revealed a hypotriploid karyotype with a modal chromosome number of 67 (range 64–69) and 21 unique marker chromosomes, one of them (M16) in two copies. This karyotype was found in 40 of 51 cells analysed by G-banding (Fig. 1). Two of the remaining 11 cells possessed one or two copies of an additional marker chromosome (M22), respectively. In these two cells M19 was not present. M22 could be characterized as an isochromosome i(1q23→1qter) and probably was derived from M19. These two cells are referred as side line. Nineteen K562 metaphase cells were investigated by M-FISH. Fifteen cells belonged to the stem line (a representative karyogram is shown in Fig. 2), two cells possessed the marker chromosome M22, and therefore belonged to the side line.

Three examples of FISH experiments are demonstrated in Fig. 3 and Fig. 4. The CGH result is shown in Fig. 5. In Table 1, the marker chromosomes of K562 are described and a comparison with published K562 karyotypes is given.

Subsequently, for each chromosome, the results of G-banding, FISH, M-FISH and CGH will be given.

Chromosome 1. Three normal copies of chromosome 1 were seen. M19 (see section concerning # 21) contained material from 1q, leading to an enh(1)(q23→qter) as shown by CGH.

Chromosome 2 existed as two normal copies, and as M1, a der(2)add(2)(q33). Chromosome 2 material also contributes to M17 (see section concerning # 21). These results are consistent with the CGH result of an enh(2)(q23→31). Single copy FISH experiments with *BCR/ABL* probes (Fig. 3) revealed that M1 was characterized by a fusion signal of these two genes at the telomeric region of 2q.

Chromosome 3. G-banding revealed two normal copies of chromosome 3. M9 and M10 (see # 10 section) both contain 3p21→3pter. Thus, the CGH mean ratio profile shows a dim(3)(p14→qter) and an enh(3)(p21→pter).

Chromosome 4. Three normal chromosomes 4 were seen.

Chromosome 5. Was present in three normals and the marker M2 = der(5?)t(5; 6)(5pter→5p?11::?), leading to an enhancement in 5p. M-FISH was crucial for revealing the M2 composition out of chromosome 5 and chromosome 6 material. As in FISH, applying a CEP 6

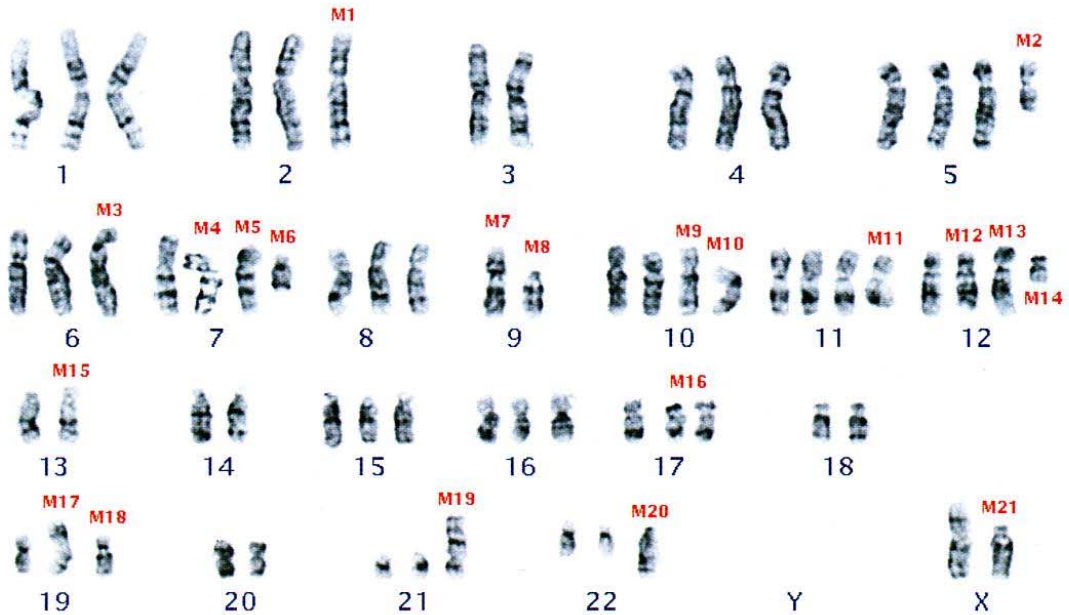

Fig. 1. G-banding karyotype of a representative K562 metaphase. This karyotype was found in 40 of 51 cells analysed. The descent of five markers, indicated by arrows, is uncertain as indicated: M2[1], M10[2], M11[3], M14[3], and M18[1] ([1]derivation unknown; [2]centromere originates from two different chromosomes, # 10 and # 17; [3]not derived from the chromosomes they are assigned to). For ease of interpretation, these markers are assigned to the chromosomes they are supposed to derive from or to the chromosomes they are composed of for the most part.

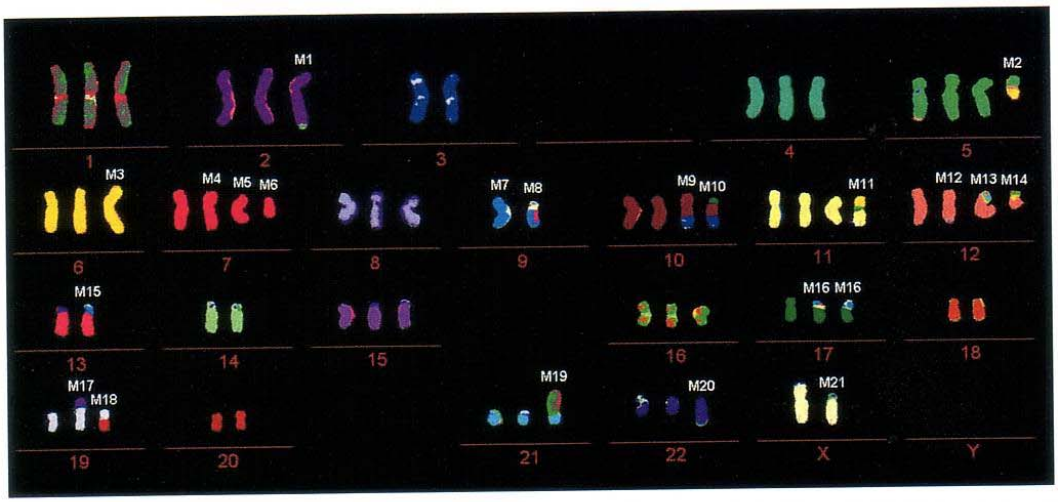

Fig. 2. M-FISH result of a representative K562 cell in classification colors, being in accordance with the stem line identified by G-banding. This karyotype was found in 15 of 19 cells investigated by M-FISH.

probe revealed a negative result this marker presumedly is a derivative 5.

Chromosome 6. Material of chromosome 6 was present in two normal copies, and in M2 (see section concerning # 5), M3, and M11 (see # 11 section). The characterization of M3 = dup(6)(pter → p12::p22 → qter) was performed by G-banding. The three markers led to the enh(6)(q23 → qter) and enh(6)(p21.1 → pter).

Chromosome 7. Chromosome 7 material was present in one normal copy and in the markers M4, M5, and M6. Their composition of chromosome 7 material was revealed by M-FISH. G-banding led to the detailed characterization of M4 = inv(7)(pter → p22::p11 → p22::p11 → qter) and M5 = del(7)(:p15 → qter). M6 was defined as der(7)rea del(7). Presumably M6 neutralizes the loss of chromosome 7 material caused by M5 and led to an enh(7)(q22 → qter).

Chromosome 8. Three normal copies of chromosome 8 were seen.

Chromsome 9. There was no normal chromosome 9 present. There are five markers showing rearrangements of chromosome 9 material: M1 (see # 2 section), M7, M8, M15 (# 13 section), M16 (# 17 section), and M20 (# 22 section). FISH with *BCR* and *ABL* probes showed the contribution of these genes to M7 and M8 (Fig. 3). This FISH result led to the characterization of M7 as dup(9)(9q34::9p24 → 9qter). M1, M7, M15, and M16 resulted in the enh(9)(q22 → qter). M8 = del(9)(:p12 → qter) caused the dim(9)(p12 → pter).

Chromosome 10. Two normal copies of chromosome 10 were seen. M-FISH revealing the composition and G-banding leading to the breakpoints, M9 was characterized as der(10)t(3;10)(10pter → 10q23::3p21 → 3pter), and M10 was described as der(10;17)t(3;10;17)(17pter → 17q11::10p11 → 10q23::3p21 → 3pter). FISH experiments with WCP 10 plus CEP 10 probes and WCP 10 plus CEP 17 probes showed an involvement of both chromosome 10 and 17 centromere material in M10, respectively. Corresponding to the occurance of only two normal copies of chromosome 10, and M9 and M10, both containing 10q11.2 → 10q23, CGH demonstrated a dim(10)(q23 → qter) and an enh(10)(q11.2 → q22). The two normal copies of chromosome 10 and the marker M9 resulted in three copies of 10p. Therefore no gain or loss of this region was found.

Chromosome 11. Was seen as three normal copies and M11 = der(?)(t(6;11)(?::11q12 → 11qter). M11 was detected as marker by M-FISH and G-banding, confirmed by FISH with WCP6 and WCP11 probes. FISH with WCP11 plus CEP6 probes, and WCP11 plus CEP11 probes showed that M11 was neither a chromosome 6 nor a chromosome 11 derivative. M11 caused an enh(11)(q12 → qter).

Chromosome 12. Material was present in one normal copy and M12, M13, and M14. The chromosome 19 contribution to M12 = der(12)t(12;19)(12pter → 12q23-24::?) was confirmed by FISH with WCP12 and WCP19 probes. M13 = der(12)t(12;21)(21qter → q11::12p11 → 12qter) was detected as marker by M-FISH predominantly, confirmed by FISH using WCP12 and WCP21 probes (Fig. 4A). FISH by WCP12 and CEP12 probes revealed that M13 was a derivative 12 and that M13 resulted in a dim(12)(p12 → pter). The composition of M14 = der(?)t(12;19)(?::?) was shown by FISH. Accord-

ing to FISH analysis by WCP19 and CEP12 probes, M14 was not a chromosome 12 derivative.

Chromosome 13. Was present as one normal copy and M15 = der(13)t(9;13)(?::13p11 → 13qter), consistent with the loss of substantial parts of 13q. The chromosome 9 part of M15 was revealed by M-FISH.

Chromosome 14. Two normal copies of chromosome 14 led to a dim(14)(q).

Chromosome 15. Was present in three normal copies. However, CGH mean ratio profile showed a tendency for copy number gain of 15q.

Chromosome 16. Three chromosomes 16 were seen. From the CGH result, a distal 16q enhancement cannot be excluded.

Chromosome 17. Chromosome 17 material was involved in one normal copy, M10 (see # 10 section), and two copies of M16 = der(17)t(9;17)(?::17p11 → 17qter). M-FISH revealed the chromosome 9 contribution to M16, FISH with WCP9 and CEP17 probes showed the chromosome 17 derivation (Fig. 4B). Since the two markers M16 had lost chromosome 17p material, CGH showed a dim(17)(p11 → pter).

Chromosome 18. Two normal copies of chromosome 18 were seen by G-banding. However, the mean ratio profile indicated a diminuation only for 18q. Therefore, 18p sequences are possibly involved in several smaller rearrangements not suitable for detection by the methods applied.

Chromosome 19. Chromosome 19 was present in one normal copy and M12 and M14 (see # 12 section), M17 = der(19)t(2;19)(?::rea?dup19), and M18 = der(?)t(19;20)(19pter → 19p11::? → cen → ?::20q11 → 20 qter). M-FISH showed the composition of M17 and M18, FISH with WCP2 plus WCP19, and WCP19 plus WCP20 probes confirmed this. These results are consistent with the CGH analysis: M12 and M14 contained 19q material, M17 the whole chromosome 19 and probably a duplication in 19q, and M18 a copy of 19p. The resulting total sum revealed three copies of chromsome 19, leading to a mean ratio profile of 1.

Chromosome 20. Chromosome 20 material was involved in two normal chromosomes 20 and M18 (see # 19 section). Since M18 contained no chromosome 20p material, CGH showed a dim(20)(p11.2 → pter).

Chromosome 21. Was present in two normal copies and as part of M13 (see # 12 section) and M19 = der(21)t(1;21)(1qter → 1q23::21p11 → 21qter). The chromosome 21 contribution to M19 was shown by M-FISH. M13 and M19 resulted in an enh(21)(q11.2 → qter).

Chromosome 22. Chromosome 22 material was existent in two normal copies, and in M1 (see # 2 section) and M20. Single copy FISH with *BCR* and *ABL* probes revealed an acrocentric marker chromosome almost entirely composed of repeated *BCR/ABL* fusion genes (Fig. 3). The DAPI-banding pattern of this marker identified it as M20. The *BCR* gene amplification seen in M1 and in M20 was reflected by the amp(22)(q11.2), as detected by CGH. The dim(22)(q13) is in consistency with the occurance of only two normal copies of chromosome 22.

X chromosome. G-banding and M-FISH showed one normal X and M21 = del(X)(:p11 → qter), confirmed by FISH with a WCPX probe. Therefore, the CGH results showed a dim(X), especially for the p-arm. The mean ratio has to be interpreted as a diminuation of X, because the K562 cells originally have been derived from a female donor, whereas the reference DNA applied in our CGH experiments was of male origin.

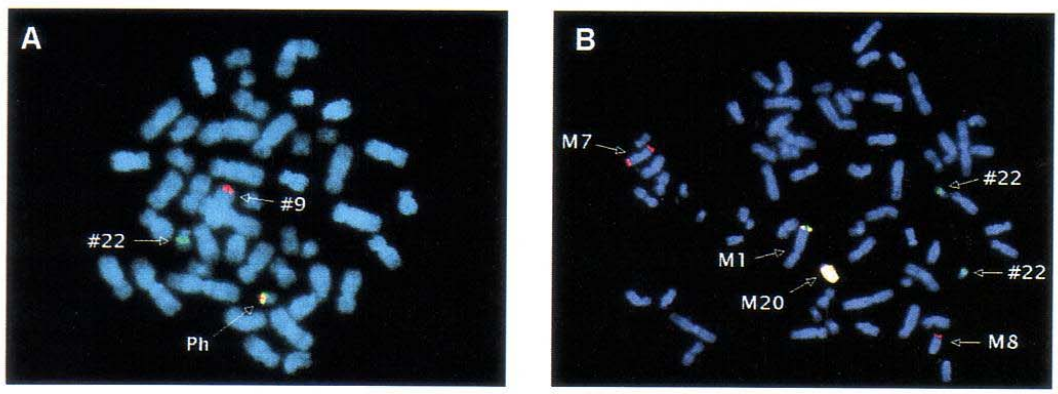

Fig. 3. FISH by *BCR* and *ABL* gene probes detected by spectrum green and spectrum orange, respectively. (A) As a positive control, hybridization to a metaphase cell of a CML case with a classic Philadelphia chromosome showed a yellow fusion signal. The normal chromosomes 9 and 22 showed red *ABL* and green *BCR* signals, respectively. (B) Hybridization to a metaphase of the cell line K562. M20 was composed of amplified *BCR/ABL* fusion genes and therefore showed multiple yellow fusion signals along the whole chromosome. Additional *BCR/ABL* fusion genes were located on a der(2) distal to band q33 (M1). M7 showed two red *ABL* signals at both telomers and M8 had one signal. The two normal chromosomes 21 showed green bcr signals.

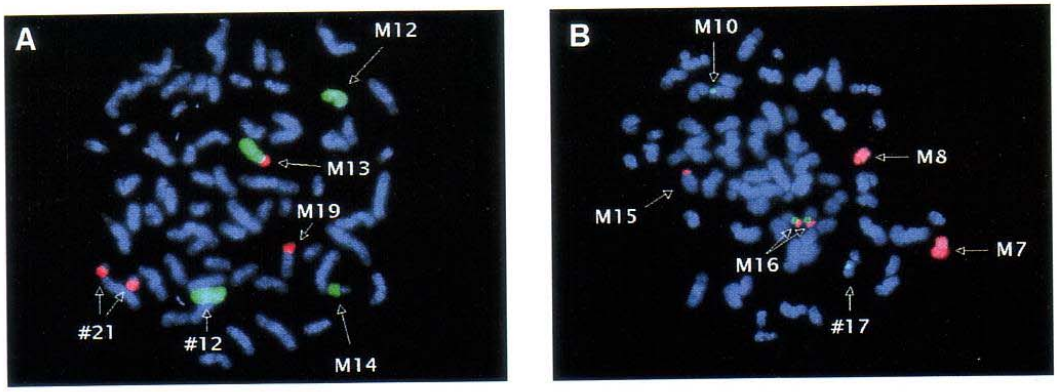

Fig. 4. Two examples of FISH experiments performed to confirm M-FISH results (A) and to reveal the origin of markers (B). (A) FISH with a spectrum green WCP 12 probe and a spectrum orange WCP 21 probe. M13 showed a red and a green signal and therefore was composed of chromosome 12 and chromosome 21 material. Moreover, the chromosome 12 component of M12 and M14 can be seen as well as the chromosome 21 component of M19. (B) FISH with a spectrum orange WCP 9 probe and a spectrum green CEP 17 probe. Red WCP 9 signals were seen on M7, M8, M15 and the two copies of M16. As green CEP 17 signals were present on M10 and M16, the origin of these markers from chromosome 17 was obvious.

4. Discussion

K562 cells have been used in a broad variety of investigations. This cell line is a unique and reliable source of myelogenous leukemia cells of human origin in approaches studying immunotherapy of leukemia [28]. Furthermore, K562 is an important tool for the study of the differentiation of hematopoietic cells [29]. Since the initial publication [1], the K562 cell line has been applied in several thousand investigations. Various cytogenetic and molecular cytogenetic investigations have been performed to characterize the K562 cell line [1,14,17–20,30]. In this study, M-FISH was performed in combination with G-banding, FISH, and CGH achieving the complete karyotype characterization of K562.

We compared our data with K562 karyotypes published earlier [1,14,17–20] (see Table 1). M20, a marker consisting for the most part out of *BCR/ABL* fusion gene repeats, is of special interest. Lozzio and Lozzio found in K562 a Philadelphia chromosome and a long acrocentric marker, characterized by them as translocation chromosome t(15;17) [1]. This long acrocentric marker seems to correspond to our M20, as well as to the M3 Selden et al. have found by FISH with *c-ABL* and C_λ probes [17] and to the M3 Tkachuk et al. have characterized by hybridization with *ABL* and *BCR* probes [19]. In contrast to this, in three of the six publications dealt with in Table 1 two different marker chromosomes have been described presumably composed of *BCR/ABL* fusion genes [14,18,20]. The second Ph derived marker chromosome showed a similar banding pattern compared to the other *BCR/ABL* fusion marker Nevertheless, it appeared to be smaller [18,20]. The M2 and M3 of Collins and Groudine [14] seem to correspond to the two described acrocentric markers. In several studies, statements on the extent of the *c-ABL* or *BCR/ABL* amplification were made. Southern blot analyses revealed a 4- to 8-fold amplification of the *c-ABL* sequence [14] or a 9-fold copy number of the *c-ABL* oncogene per haploid genome [17]. Interphase FISH showed 8–16 hybridization pairs per nucleus [19] or a 22- to 24-fold amplification of the *BCR/ABL* fusion gene [20]. A high amplification of the *BCR/ABL* fusion gene (range from 8 to 24) appeared to be consistent with our data. However, due to technical reasons, a visual estimation of the *BCR/ABL* dosage did not seem to be sufficiently exact.

In Table 1, the K562 marker chromosomes found in this study were listed and compared with karyotypes described in the literature [17,18,20]. Sixteen of our 21 marker chromosomes seem to have counterparts in other publications. Eleven of these 16 markers were characterized in former studies similar to our interpretation (M1, M3, M4, M7–M10, M15, M16, M19, and M20. The classification of six of these markers in the literature was confirmed by our results (our M1, M3, M4, M7, M8, and M20). The composition of M9, M10, M15, M16, and M19 have been identified fully in our study. Interestingly, the two copies of M16 found in our study and the correspondent markers found by Wu et al. [20] seemed to be classified as normals by other authors. Five of our markers (M2, M14, M17, M18, and M21) seemed to be present in other investigations [17,18,20], however, they were interpreted in several different ways. Especially due to the use of M-FISH and FISH, we were able to elucidate their composition without doubt. For the markers M5, M6, and M11–M13 we found no corresponding markers in the litera-

ture. M11–M13 probably were not found in other investigations because of the limitations of conventional cytogenetics.

Thus, the combined use of conventional cytogenetics, M-FISH, FISH and CGH in this study revealed a karyotype refinement of the cell line K562.

The M-FISH technique was proven as an effective tool for marker characterization. Nevertheless, even this method has its limitations, as can be seen regarding the K562 marker M20. Whereas FISH analysis revealed the composition of M20 out of *BCR/ABL* fusion gene repeats, according to M-FISH the marker consisted of chromosome 22 material exclusively (Fig. 2). Presumedly, the chromosome 9 contribution has been failed to be detected because of the small size of the involved *ABL* gene sequences. This is an example of the different conventional and molecular cytogenetic methods being complementary.

Our CGH findings were partially consistent with a CGH investigation of the K562 cell line performed earlier. The CGH result of Rodley et al. [30] is corresponding with our findings, concerning the gain of chromosomal material in 1q, 3p, 5p, 6p, 9q34, 16q, 21q, 22q11.2 and the chromosomal losses in 3p, 9p, 10q, 13q, 14q, 17p, 22q and X. In other parts the CGH results of Rodley et al. differ from ours, e.g. regarding our findings of gains in 2q and 11q, which have not been found by Rodley et al., and the balance of chromosome 8, which was in contrast to the diminuation in 8p found by Rodley et al. Nevertheless, our CGH findings of gain in 2q and 11q and balance for chromosome 8 matched the findings by the other methods applied in our study.

In conclusion, there are conformations and differences between our characterization of the K562 karyotype and the CGH results of Rodley et al. [30] and

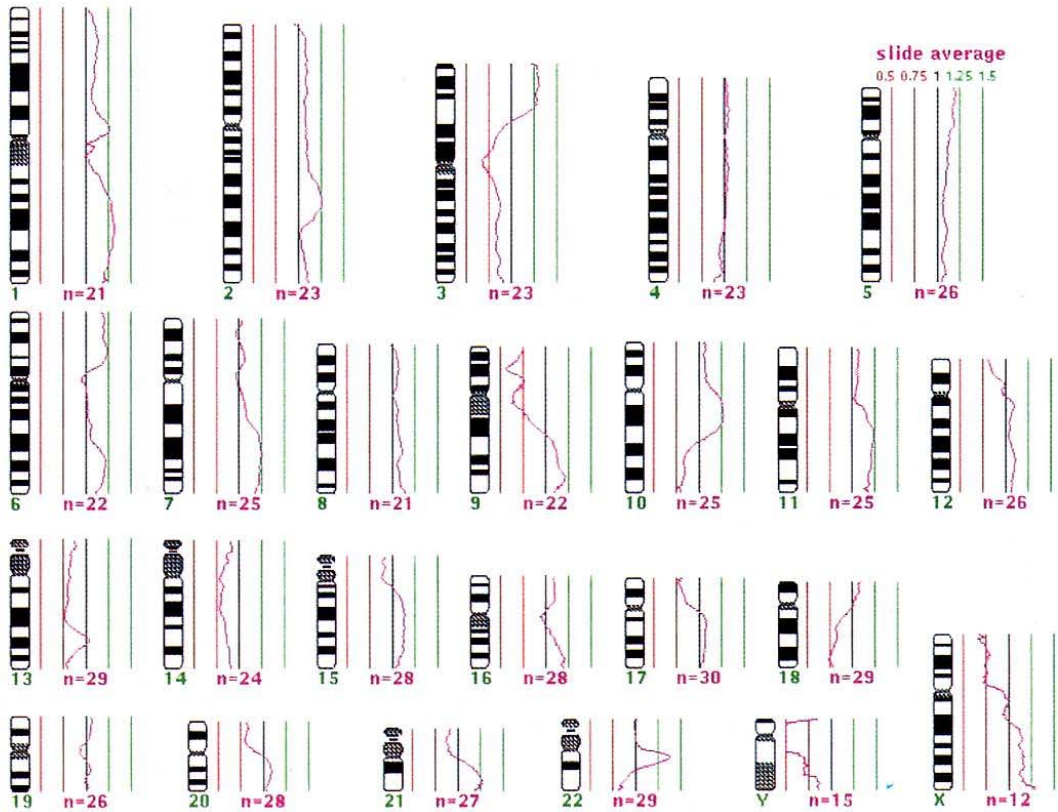

Fig. 5. CGH map of chromosomal gains and losses. Average ratio profile. Due to the hypotriploidy of the cell line the CGH result reflected deviations of the chromosome copy number from the triploid state. There were copy number gains in 1p13, 1q23→qter, 2q23→q31, 3p21→pter, 5p, 6p21.1→pter, 6q23→qter, 7q22→qter, 9q22→qter, 10q11.2→q22, 11q12→qter, 15q, 16q22→qter, 21q11.2→qter, and 22q11.2, and losses of chromosomal material in 3p14→qter, 9p12→pter, 10q23→qter, 12p12→pter, 13q12→q22, 13q32→qter, 14q, 17p11→pter, 18q12→qter, 20p11.2→pter, 22q13, and X.

Table 1
Marker chromosomes of the K562 cell line. Comparison with published data

Marker	Publications containing the marker	
	Similar interpretation	Different interpretation[a]
M1 = der(2)add(2)(q33)	[17,20]	
M2 = der(?)t(5;6)		[17,18,20]
M3 = dup(6)(p12→p22)	[17,18,20]	
M4 = inv(7)	[18,20]	
M5 = del(7)(p15)		
M6 = der(7)rea del(7)		
M7 = dup(9)(q34)	[17,18,20]	
M8 = del(9)(p12)	[17,18,20]	
M9 = der(10)t(3;10)	[20]	*[18]*
M10 = der(10,17)t(3;10;17)	[20]	*[17,18]*
M11 = der(?)t(6;11)		
M12 = der(12)t(12;19)		
M13 = der(12)t(12;21)		
M14 = der(?)t(12;19)		[17]
M15 = der(13)t(9;13)	[17,18]	[20]
M16 = der(17)t(9;17), ×2	[17,20]	
M17 = der(19)t(2;19)		[20]
M18 = der(?)t(19;20)		[20]
M19 = der(21)t(1;21)	[17,20]	*[18]*
M20 = *BCR/ABL* fusion marker	[14,17–20]	[1]
M21 = del(X)(p11)		[20]

[a] Quotations of publications in which the marker has not been characterized at all were printed in italics.

K562 karyotypes published earlier [17,18,20]. The different K562 karyotype characterizations differ partly regarding their set of markers, however they share many cytogenetic features such as at least one acrocentric marker chromosome composed of amplified *BCR/ABL* fusion genes, a great number of other markers, and a near triploid chromosome number. Thus, the K562 karyotype has remained relatively stable over a period of nearly three decades of subculturing. It is probable that there exist different sublines of K562, which differ slightly in their marker set.

The definite karyotype of the human leukemia cell line K562 is important for further investigations of the normal and pathological hematopoesis. In addition, it is very helpful to determine this cell line's identitiy. For example, Green et al. [31] checked on the cell line's identity by cytogenetic analysis before using it in studies on induced myeloid differentiation. Most important, knowledge about other consistent chromosomal rearrangements and genomic imbalances will be the basis for understanding of progression in leukemia. Furthermore, our study shows the unprecedented power of M-FISH analysis, in combination with FISH, CGH and G-banding, for resolving structural and numerical chromosomal aberrations.

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