

# The importance of molecular cytogenetic analysis prior to using cell lines in research: The case of the KG-1a leukemia cell line

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**Abstract.** KG-1 and its less differentiated subline KG-1a are leukemia cell lines used in research in a number of laboratories. The karyotypes of the two lines were initially identical. In the following years, further analysis revealed that the cell lines had acquired additional karyotypical abnormalities and differed in the presence of certain typical chromosomal rearrangements. To obtain cytogenetic authentication prior to the use of the two cell lines, we analyzed their karyotype by combining DAPI- and CMA-chromosome bandings and a fluorescence *in situ* hybridization (FISH)-based approach by using BAC clones useful for the identification of chromosome regions of interest. Sequences of the *MYC*, *PLZF*, *RARA* and *BCR* genes, that are known to play a critical role in leukemogenesis, and certain BAC clones mapped to five known common fragile sites (CFS) were used for the FISH analysis. A telomeric probe (TTAGGG)<sub>n</sub> and a set of BAC clones were used to characterize the marker chromosome der(1) that was observed in the cell line KG-1a. The existence of notable differences between the karyotype of the KG-1a cell line previously described, and that described in this study, demonstrate that the use of established cancer cell lines should be preceded by cytogenetic and/or molecular characterization.

## Introduction

KG-1 and KG-1a leukemia cell lines have been used in research in numerous laboratories. KG-1, a cell line established in 1978 by Koefler and Golde (1), was derived from the bone marrow of a male individual with erythroleukemia that developed into acute myeloid leukemia (AML). The KG-1 cells are mostly at the myeloblast or promyelocyte stage of maturation.

KG-1a is a less differentiated subline of KG-1 developed after 35 passages. The karyotypes of the two lines were

initially identical (2). The comparative karyotypic analysis of the two cell lines performed in subsequent years by other authors using various cytogenetic and molecular techniques (3,4) revealed that the cell lines had acquired additional karyotypical abnormalities and, although sharing several identical structural aberrations, differed in the presence of certain typical chromosomal rearrangements. Particularly, Mrózek *et al* (4) used G-banding, spectral karyotyping (SKY) and fluorescence *in situ* hybridization (FISH) analyses to produce a detailed description of chromosome aberrations in the KG-1 and KG-1a cell lines. Comparative analysis of the two karyotypes is extremely useful for authentication of the two cell lines.

To obtain a characterization and cytogenetic authentication of the two cell lines prior to use, their karyotype was analyzed by combining DAPI- and CMA-chromosome bandings and a FISH-based approach. For FISH analyses a number of BAC clones useful for the identification of chromosome regions of interest were employed, including the *MYC*, retinoic acid receptor (*RARA*), promyelocytic leukemia zinc finger (*PLZF*) and breakpoint cluster region (*BCR*) genes. Evidence suggests that the *MYC* copy number gain has a role in human myeloid leukemia (5 and refs. cited therein). The *RARA*, *PLZF* and *BCR* genes are known to be involved in translocations generating fusion proteins that play a critical role in leukemogenesis (6). A number of BAC clones mapped to five known common fragile sites (CFS) were also used. CFS are preferential loci for double strand DNA breaks under stressful growing cell conditions. In cancer cells, CFS are frequently involved in recurrent chromosome rearrangements (7). A number of BAC clones mapped on chromosome 1 were used to characterize the marker chromosome der(1) that we observed in the cell line KG-1a. This study reveals the results of our analysis.

## Materials and methods

**Cell lines.** The analyzed KG-1 and KG-1a cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) approximately 20 years ago. The cells had not been maintained in culture for long periods of time but were refreshed a certain number of times. The cells were cultured in RPMI-1640 with 2 mM L-glutamine, 10% FCS at 37°C in 5% CO<sub>2</sub>.

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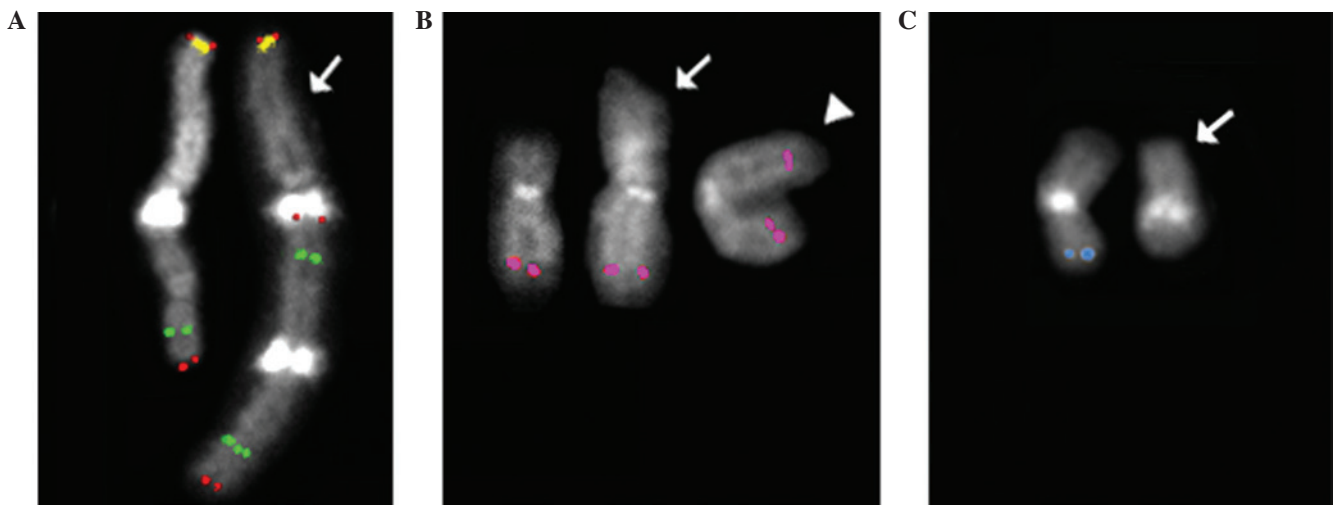


Figure 1. FISH experiments on chromosomes from KG-1a cells. (A) Chromosomes 1 and der(1) (arrow) are hybridized by a telomeric probe (red), BAC clones RP11-319A11 (yellow) and RP11-434B7 (green). The palindromic arrangement of the last clone and the interchromosomal telomeric signal can be detected on the chromosome der(1). (B) Chromosomes 8, der(8)t(8;12) (arrow) and idic(8)(p11) (arrowhead) are hybridized by the BAC clone RP11-440N18 (*MYC*). (C) Chromosomes 16 and del(16) are hybridized by the BAC clone RP11-264L1 (*FRA16D*). FISH, fluorescence *in situ* hybridization.

**Chromosome analysis.** Cell harvesting, DAPI- and CMA-chromosome bandings were performed using standard methods. The BAC clones used for the FISH analysis were: RP11-440N18 mapped at 8q24.21 that contains the entire *MYC* gene; RP11-1006G20 (11q23.2), the majority of the *PLZF* gene; RP11-1152A10 (17q21.2), the entire *RARA* gene; and RP11-434O9 (22q11.23), the first part of the *BCR* gene. BAC clones mapped to five known CFS were also used: RP11-158L8 (*FRA2G*, 2q31), RP11-48E21 (*FRA3B*, 3p14.2), RP11-425P5 (*FRA7B*, 7p22.1), RP11-36B6 (*FRA7H*, 7q32.3) and RP11-264L1 (*FRA16D*, 16q23.2). We also used a telomeric probe (TTAGGG)<sub>n</sub>, generated by PCR (8) and a set of BAC clones mapped onto chromosome 1 to characterize the marker chromosome der(1) that was observed in the cell line KG-1a: RP11-319A11 (1p36.32), RP11-79E5 (1q12), RP11-434B7 (1q32.3), RP11-385F5 (1q43) and RP11-438F14 (1q44). The BAC stabs were obtained from CHORI (Oakland, CA, USA), and a number of them were supplied by the Cytogenetic Unit of the University of Bari (Italy). FISH experiments were performed as described in a previous study (9).

## Results

**KG-1 cell line karyotype.** The karyotype of the KG-1 and KG-1a cell lines revealed a pseudodiploid modal number of chromosomes. The karyotype of the KG-1 cell line analyzed was the same as that described by Mrózek *et al* (4): 46,der(4)t(4;8)(q31;p21),-5,del(7)(q22q35), der(8)t(8;12)(p11;q13),+idic(8)(p11)x2,-12, der(17)(5pter→5p11::5q13→5q31::17p11.2→cen→17qter),der(20)t(12;20)(?;p13).

Therefore the *MYC* gene was present in six copies: one on a normal chromosome 8, four on the two copies of the idic(8)(p11) and one on the der(8)t(8;12); the *PLZF* and *BCR* genes were present in the normal number and chromosomal location and the *RARA* gene was present in two copies: one localized on a normal chromosome 17, the other on the short arm of the der(17)t(5;17).

**KG-1a cell line karyotype.** Differing from KG-1, the karyotype of the KG-1a cell line showed specific differences from that described by Mrózek *et al* (4): the presence, besides a normal chromosome 1, of a chromosome der(1) that has the duplication of the entire long arm (Fig. 1A); chromosome der(8;12) (Fig. 1B, arrow) instead of the der(8;22); only one normal copy of the chromosome 11 besides the i(11)(q10); a deleted chromosome 16q (Fig. 1C); two normal copies of chromosome 22 and lack of the chromosome Y. Accordingly, the description of the KG-1a karyotype observed (Fig. 2) is: 45-47,X,-Y,der(1)(pter→cen→q12::qter→q12::q12→qter), der(4)t(4;8)(q31;p21),-5,del(7)(q22q35),der(8)t(8;12)(p11;q13),+idic(8)(p11),i(11)(q10),-12,del(16)(q13~21), der(17)(5pter→5p11::5q13→5q31::17p11.2→cen→17qter), +der(19)t(14;19)(q13;q13.4),der(20)t(12;20)(?;p13).

Therefore, in the KG-1a karyotype the *MYC* gene was present in four copies: one on a normal chromosome 8, two on the idic(8)(p11) and one on the der(8)t(8;12) (Fig. 1B). We also observed that the *PLZF* gene was present in three copies: one on a normal chromosome 11 and two on the i(11)(q10). The *RARA* gene was present in two copies: one localized on a normal chromosome 17, the other on the short arm of the der(17)t(5;17). The *BCR* gene was present in normal number and chromosomal location on two chromosomes 22. Of note is that due to the presence of the chromosome der(1) bearing two copies of the long arm of the chromosome 1, three copies of the 54 genes indicated as involved in cancer (10) localized on the long arm of this chromosome are present (Fig. 3).

## Discussion

Koeffler *et al* (2) reported the presence of a large submetacentric chromosome (MAR-1) in the karyotype of the KG-1 and KG-1a cell lines, besides two normal copies of chromosome 1. Moreover, Furley *et al* (3) described the presence of a der(1) (mar 1) only partially identified and slightly

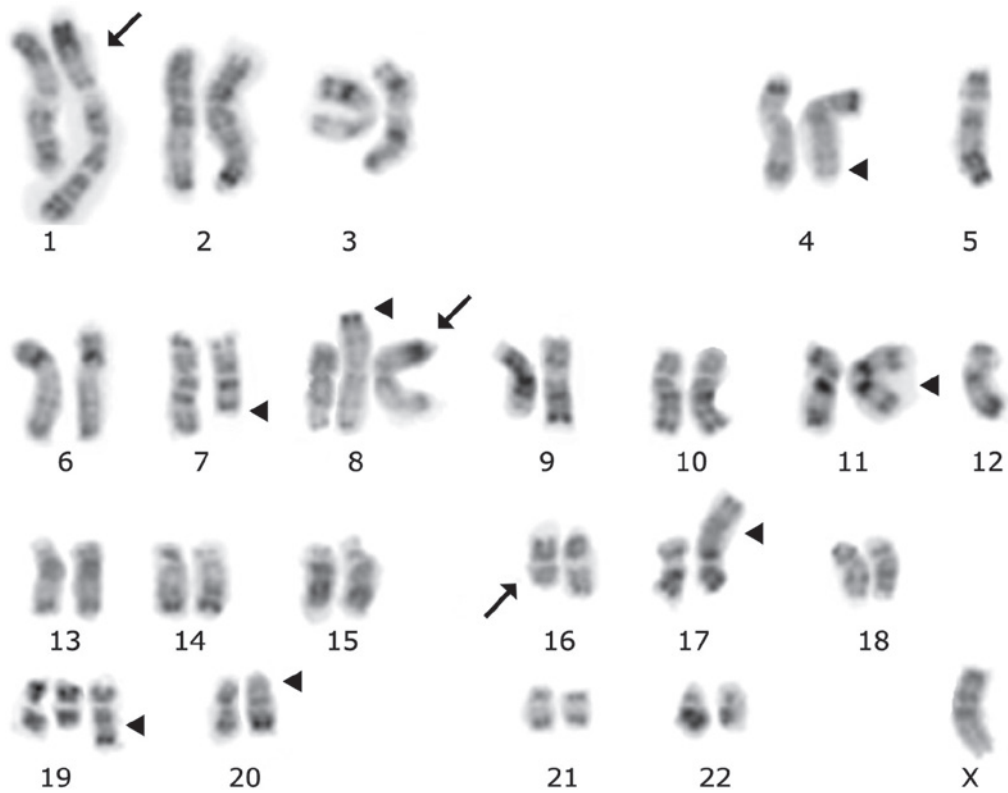


Figure 2. R-banding karyotype of the KG-1a cell line as mentioned in Materials and methods. The arrowheads indicate the chromosome rearrangements previously described by Mrózek *et al* (4). The arrows indicate the rearrangements only present in the KG-1a cell line described in the present study.

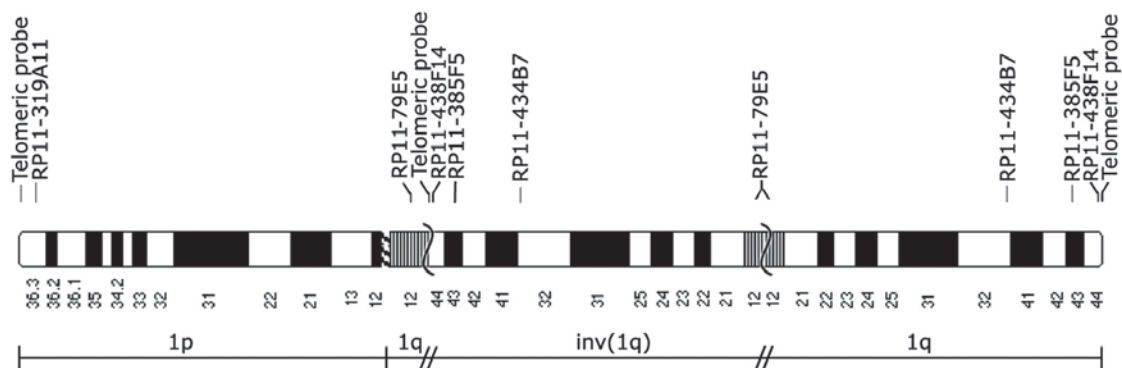


Figure 3. Map of the chromosome der(1) showing the BAC clones used as probes in the present study. The two curved lines indicate the breakpoint at the origin of the rearranged chromosome.

different in the two cell lines, besides two normal copies of chromosome 1. Therefore, it is likely that the KG-1 cell line examined by Mrózek *et al* (4) and by the authors of this study has lost the chromosome MAR-1 observed by Koeffler *et al* (2) and by Furley *et al* (3) and has maintained two copies of chromosome 1. Conversely, the KG-1a cell line examined by Mrózek *et al* (4) lost the chromosome MAR-1 and maintained the two copies of chromosome 1, whereas the KG-1a cell line examined in the present study has lost one normal chromosome 1 and maintained the der(1) described in the present study.

The differences between the KG-1a karyotype described by Mrózek *et al* (4) and the one described in the present study demonstrate again that although the established cancer cell lines maintained in culture or refreshed numerous times in different laboratories derive from the same original cell lines, they may have different genotypic characteristics due either to the selection of subclones or to intervening mutations. Therefore, the use of established cancer cell lines for research purposes in cancer biology, and in any other field, should be preceded by a proper cytogenetic and molecular characterization (11).

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