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# Reinterpretation of G-banded Complex Karyotypes by Fluorescence In Situ Hybridization With Chromosome-Specific DNA Painting Probes and Alpha-Satellite Centromere-Specific DNA Probes in Malignant Hematological Disorders

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**In this study, we have performed fluorescence in situ hybridization (FISH) with chromosome-specific DNA painting probes 1, 2, 3, 4, 6, 8, and 12 and centromere-specific DNA probes 7, 10, 12, 17, 18, and X after G-banding on the same metaphase spreads from four patients with malignant hematological disorders to more precisely interpret their complex karyotypes. The findings demonstrated that the application of combined G-banding and FISH can more accurately explain complex karyotypes of hematological malignancies. FISH can detect not only the origin of marker chromosomes, but also the complex rearrangements that cannot be identified by routine banding techniques. This approach is very important to complement the cytogenetic analysis of malignant disorders and to evaluate the role of chromosome change in the development, progression, and prognosis of tumors. Am. J. Hematol. 55:69–76, 1997.** © 1997 Wiley-Liss, Inc.

**Key words: complex karyotype; G-banding; fluorescence in situ hybridization; leukemia; lymphoma**

## INTRODUCTION

Complex chromosomal rearrangements in human cancers have puzzled cytogeneticists for many years due to the limited resolution of routine banding techniques [1,2]. As a consequence, the banding pattern of rearranged chromosomes cannot be interpreted, resulting in marker chromosomes. Chromosomal deletions and rearrangements have been the significant determinants that have led to the identification of both oncogenes and tumor suppresser genes [3]. Therefore, alternative and complementary methods for the explanation of chromosomal aberrations would be very useful to more precisely study cytogenetic abnormalities of human cancers.

FISH analysis using complete lambda DNA libraries of single flow-sorted human chromosomes and repetitive centromeric DNA sequences permits the detection of DNA sequences both in metaphase and interphase cells [4,5] and evaluates not only numerical, but also structural aberrations [6–8]. In this study, we have applied a technique in which FISH can be performed directly after G-banding on the same metaphase spread. By using this combined FISH and G-banding technique, we have fully analyzed four cases of hematological malignancies that had complex karyotypes on routine cytogenetic studies.

Our results showed that the combination of FISH and G-banding permits a more precise evaluation of complex chromosomal rearrangements in malignant hematological disorders than conventional cytogenetic techniques alone.

## MATERIALS AND METHODS

### Cell Culture and Preparation

The materials used in this study were bone marrows from four patients with hematological malignancies. The metaphases were obtained from 72-hr non-stimulated

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cultures in RMPI 1640 medium with 6% FCS, penicillin:streptomycin (50 IU/ml:50 μg/ml) and 2 mM L-glutamine. Cells were cultured 24 or 48 hr, followed by synchronization with methotrexate block and thymidine or bromodeoxyuridine release.

### Giemsa Banding

The G-banding was performed according to the procedure described by Seabright [9]. After the G-banding and karyotyping, slides were destained in methanol:acetic acid (3:1, v:v) for 20 min. Air-dried slides were treated with RNase A (100 ng/ml in 2 × SSC pH 7.0) for 1 hr at 37°C, and washed three times in 2 × SSC (pH 7.0) for 2 min. Slides were then dehydrated in an alcohol series, air-dried, and treated with 0.6 μg/ml proteinase K in 20 mM Tris/2 mM $CaCl_2$ (pH 7.5) at 37°C for 7.5 min. Slides were washed three times in 2 × SSC (pH 7.0) at room temperature for 2 min, fixed in 3.7% formaldehyde in 50% 0.05 M $MgCl_2$/50% PBS for 10 min, washed in 2 × SSC at room temperature three times for 2 min, dehydrated in an alcohol series, air-dried, and taken through the hybridization procedure.

### Probes and FISH

The alpha-satellite centromere-specific DNA probes 7, 10, 12, 17, 18, and X and whole chromosome painting probes 1, 2, 3, 4, 6, 8, and 12 were purchased from Oncor (Gaithersburg, MD) and IMAGENETICS (Napperville, IL), respectively. Painting probes for other chromosomes were commercially unavailable at the time of this study. Slides were denatured in 70% formamide in 2 × SSC (pH 7.0) at 70°C for 2–3 min, dehydrated in a cold alcohol series, and air-dried. The hybridization mixture was denatured for 5 min at 70°C and applied to prewarmed (37°C) slides under a coverslip. The hybridization was performed in a moisture chamber at 37°C for 16 hr. After hybridization, slides were washed three times at 45°C in 50% formamide/2 × SSC (pH 7.0), once in 2 × SSC, and once in 0.1 M PN buffer (0.1 M sodium phosphate, pH 8.0/0.1% NP-40). Hybridized probes were detected with fluoresceinated streptavidin, and signals were amplified with a second layer of fluoresceinated streptavidin after treatment with biotinylated anti-streptavidin. Nuclei were counterstained with propidium iodide (1 μg/ml) in an antifade solution, and photographs were taken with an epifluorescence microscope equipped for photography, using Kodak Ektachrome film 400 ASA or AGFAchrome 1000 ASA (Kodak, Rochester, NY).

We have evaluated the sensitivity and specificity of the individual DNA probes used in this study. Under the conditions used here we did not observe any cross-hybridization of DNA probes.

## RESULTS

### Case 1

The cytogenetic analysis from a 52-year-old man with B-cell lymphoma showed the following karyotype: 46-48, XY, 1q+, +6, 10q−, −12, t(14;18), + multiple markers(mar1-9) [40]. The constant anomalies were 1q+, +6, 10q−, and t(14;18). The presence of monosomy 12 and mar1-9 varied, but each one was found in at least two metaphases (Fig. 1A). FISH with chromosome-specific painting probe 1 resulted in staining of one normal chromosome 1 and an entire 1q+ as well as a segment of mar1, mar2, and mar6, respectively. Thus, the chromosome 1q+ may be interpreted as hsr(1)(q31) (Fig. 1B–E). The chromosome-specific painting probe 2 produced an entire staining of one chromosome 2 and the partial hybridization of the other chromosome, although G-banding had appeared normal for both (Fig. 1F,G). Therefore, the partially labeled chromosome 2 should be interpreted as der(2)t(2;?)(p21;?). The FISH with the painting probe 3 showed no extra rearrangement in addition to two normal chromosomes 3. The chromosome-specific painting probe 4 demonstrated hybridization of two normal chromosomes 4 in most metaphases that didn't contain the marker chromosome mar6, but the metaphases that contained the mar6 showed hybridization of a segment of this marker (Fig. 1H,I). Thus, the mar6 which was completely hybridized by chromosome-specific painting probes 1 and 4 could be explained as der(1)t(1;4)(?;?). The painting probe 6 showed the same results as the G-banding. The painting probe 12 resulted in an entire hybridization of a normal chromosome 12, mar5, and mar9 and a segment of mar7 in most metaphases (Fig. 1J,K). Therefore, mar5, mar7, and mar9 should be interpreted as de1(12)(q13), der(?)t(?;12)(?;q13), and del(12)(q15), respectively. The centromere-specific probe 10 showed the same result as the G-banding.

### Case 2

A 60-year-old female patient with acute nonlymphocytic leukemia (ANLL-M6) showed the following complex karyotype: 46, X, −X, −3, −4, −5, −14, −15, add(19)(p13), −20, +mar1-7[25]/46, idem, −8, +der(8)t(8;11)(q24;q13) [10]/46, XX [6]. The G-banding analyses of marker chromosomes showed that mar2 resulted from the deletion(10)(p13), mar5 originated from chromosome 18, and the other marker chromosomes could not be classified (Fig. 2A). The painting probe 3 produced staining of only one normal chromosome 3, and no extra rearrangement was found, confirming the G-banding result. FISH with painting probe 4 showed hybridizations of one normal chromosomes 4, an interstitial segment of add(19)(p13) as well as a segment of mar1. Moreover, an extra DNA sequence of chromosome 4 was found at the terminus of one chromosome 13 that

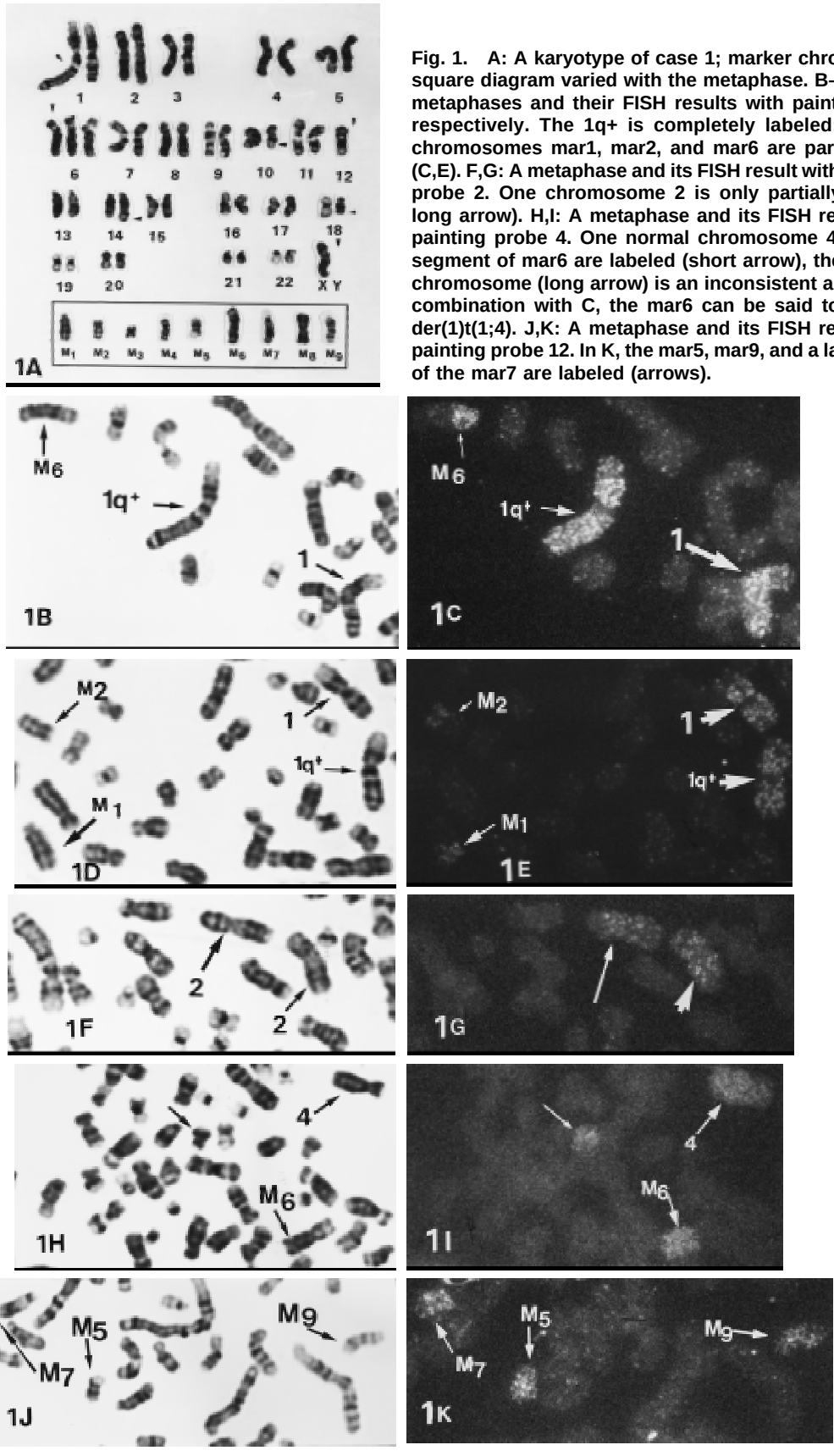



**Fig. 1. A: A karyotype of case 1; marker chromosomes in square diagram varied with the metaphase. B–C, D–E: Two metaphases and their FISH results with painting probe 1, respectively. The 1q+ is completely labeled; the marker chromosomes mar1, mar2, and mar6 are partially labeled (C,E). F,G: A metaphase and its FISH result with the painting probe 2. One chromosome 2 is only partially labeled (G, long arrow). H,I: A metaphase and its FISH result with the painting probe 4. One normal chromosome 4 and a large segment of mar6 are labeled (short arrow), the other small chromosome (long arrow) is an inconsistent anomaly (I). In combination with C, the mar6 can be said to result from der(1)t(1;4). J,K: A metaphase and its FISH result with the painting probe 12. In K, the mar5, mar9, and a large segment of the mar7 are labeled (arrows).**

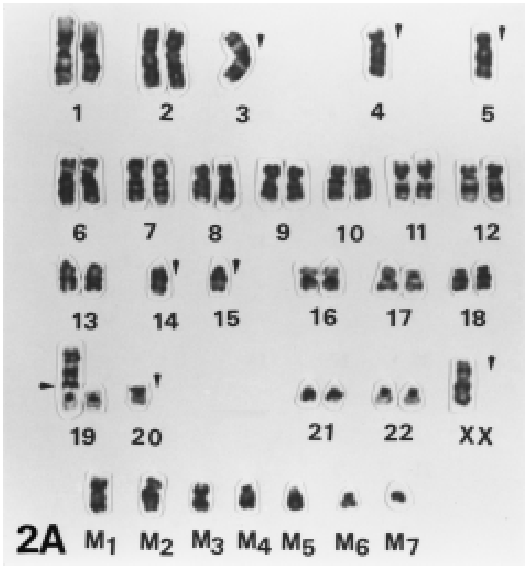
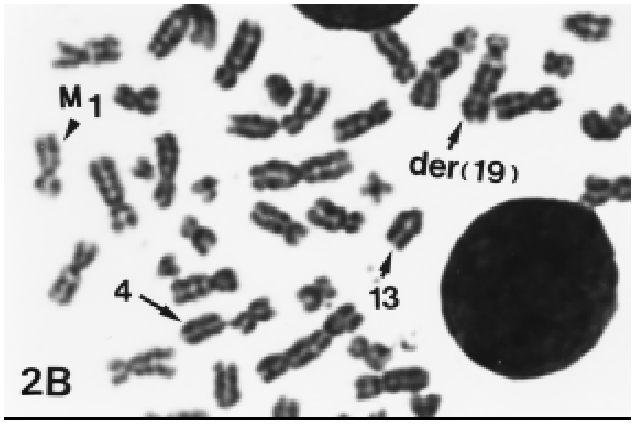
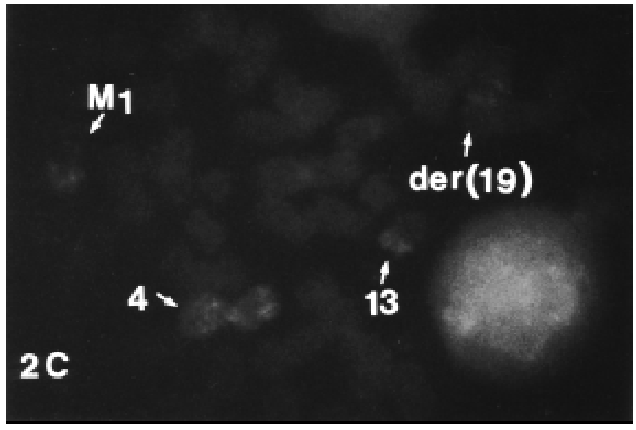
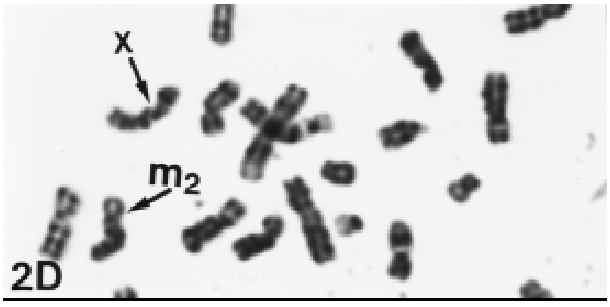
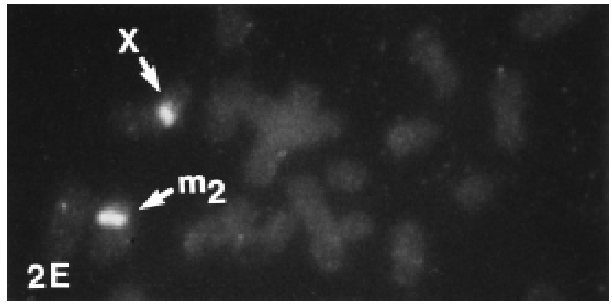



**Fig. 2. A: Karyotype of case 2; arrows indicate numerical and structural anomalies. B,C: A metaphase and its FISH result with the painting probe 4, respectively. One normal chromosome 4, a segment of the add(19), and the mar1 as well as one chromosome 13 are labeled (arrows C); we can also observe a break on the chromosome 4 (B). D,E: A G-banded metaphase and FISH with the centromere-specific X probe, respectively. The marker chromosome mar2 and one normal chromosome X are labeled (arrows, E).**




was interpreted as normal by G-banding (Fig. 2B,C). With these new findings, chromosome add(19) can be classified as der(19)t(19;4;?)(p13;?;?), mar1 as der(4)t(4;?)(?;?), and the rearranged chromosome 13 as der(13)t(4;13)(?;q32). The painting probe 8 produced the same result as the G-banding for the chromosome 8. The centromere-specific probe X resulted in two spots of centromeric fluorescence signals on one normal chromosome X and on the mar2 that was interpreted as del(10)(p13) by the G-banding. Consequently, mar2 should be classified as del(X)(p?) (Fig. 2D,E). The centromere-specific probe 18 identified two normal chromosomes 18. The mar5 that was interpreted as the ''+18'' by the G-banding did not hybridize to this probe. This finding suggests that the marker chromosome 5 did not originate from chromosome 18, but from an unknown chromosome. The painting probes 1, 2, 6, and 12 did not show any extra rearrangements in addition to the two normal chromosomes identified by G-banding.

### Case 3

The cytogenetic analysis from a 41-year-old male patient with ANLL-M1b showed the following karyotype: 44, X, −Y, −1, +i(1)(p10), der(2)t(2;3)(p?;p?), del(3)(p?), der(5)t(1;5)(q21;q15), −7, −12, del(17)(q21), −18,

+mar1, +mar2 [30] (Fig. 3A). The painting probe 1 resulted in complete hybridization to one normal chromosome 1, an i(1)(p10) and mar2 as well as a segment of der(5)t(1;5) (Fig. 3B,C). The painting probe 2 painted one normal chromosome 2 and most of the der(2)t(2;3)(p?;p?), as well as the p-terminus of the mar1 (Fig. 3D,E). The painting probe 3 showed staining of one normal chromosome 3 and a large segment of the del(3p), but a small p-terminal segment of del(3p) and der(2)t(2p;3p) weren't labeled. The der(2)t(2p;3p) and del(3p) identified by the G-banding, reinterpreted now by coupled G-banding and FISH, were considered as der(2)t(2;?)(p13;?) and der(3)t(3;?)(p13;?), respectively. The FISH results with chromosome-specific painting probe 4 and centromere-specific probe 7 were consistent with the G-banding, but we could not exclude the rearrangement between chromosome 7 and others due to the lack of a chromosome-specific painting probe 7. The painting probe 12 resulted in an entire hybridization of one chromosome 12 and a del(17)(q21) as well as a interstitial segment of der(5)t(1;5) identified by the G-banding (Fig. 3F–I). Therefore, der(5)t(1;5) and del(17) now should be classified as der(5)t(1;5;12)(1qter-1q21::12p?q?::5q12-5pter) and der(12), respectively. According to the above findings obtained from the combined G-banding and FISH techniques, the karyotype of this case was reinterpreted as: 44, X, −Y, i(1)(p10), der(5)t(1;5;12)(1qter-1q21::12p?q?::5q12-5pter), der(2)t(2;?)(p13;?), der(3)t(3;?)(p13;?), −7, der(12), −17, −18, +mar1[der(2)], +mar2[der(1)][30].

### Case 4

An 84-year-old female patient with chronic lymphocytic leukemia (CLL) transformed to ANLL was studied. The 62 analyzed metaphases from unstimulated peripheral blood culture revealed the following karyotype: 45, XX, 5q−, −10, −11, −12, −17, +mar1, +mar2, +mar3[62]. The marker chromosome mar1 was considered as a complex rearrangement der(17)t(1;3;17)(1pter-p12::3pter-p11::17qter-p11) according to the G-banding (Fig. 4A). FISH with chromosome-specific painting probes 1 and 12 and centromere-specific probes 12 and 17 were performed. The findings revealed that the large interstitial segment of the mar1 did not derive from 3pter-p11, but from chromosome, 12 (Fig. 4B,C). The terminal segment of the mar1 originated from chromosome 1pter-1p12, which is compatible with the G-banding (Fig. 4D,E). The centromere-specific probe 17 produced two spots of fluorescence signal from one normal chromosome 17 and mar1, respectively. The centromere-specific probe 12 also resulted in two signals from the mar1 and one normal chromosome 12, respectively. Thus, the mar1 can accurately be reinterpreted as a complex rearrangement der(17)t(1;12;17)(1pter-p12::12qter-cen::17cen-qter) according to the combined G-banding and FISH.

## DISCUSSION

Until now, the most characteristic chromosomal abnormalities of hematopoietic malignancies have been found by using banding techniques. However, the karyotypes of malignant hematological disorders are often very complex and usually cannot be fully characterized because of the limitations of routine banding techniques, thus restricting a better understanding of development and progression of tumors as well as an evaluation of prognosis.

Recent studies demonstrated that the newly developed FISH method has provided additional resources to improve the analysis of chromosome abnormalities. It can verify the involved segments of the chromosomes not only in metaphase but also in interphase cells, and help interpret marker chromosomes that cannot be identified by banding techniques along [10–15]. However, the FISH method itself has some restrictions: first, its performance needs freshly made metaphase spreads; second, FISH results from unbanded slides must be interpreted conservatively regarding complex karyotypes. Therefore, a new technique that combined the G-banding method with FISH was used in the present study in order to more precisely interpret complex chromosomal rearrangements.

In case 1, the chromosome 1q+ was identified as hsr(1)(q31) after combined G-banding and FISH (Fig. 1B–E). The mar5 and mar7 were identified as del(12)(q13) and der(?)t(?;12)(?;q13), respectively. The 12q13 is a recurrent breakpoint in some solid tumors [16,17] and ANLL [18] but is less frequently reported in lymphoproliferative disease [19,20]. We have found involvement of the breakpoint 12q13 in a case with B cell lymphoma. Our results suggest that 12q13 is also a recurrent breakpoint in lymphoproliferative disease. Whether a gene or genes located in this region involved those diseases with the breakpoint of 12q13 needs to be investigated. We believe that with the increase of the application of the coupled FISH and G-banding, the more recurrent breakpoint can be discovered. This will facilitate the discovery of more oncogenes and a better understanding of the carcinogenesis.

The coupled FISH and G-banding method can distinguish small involved segments. For example, in case 3, a small interstitial segment of the der(5)t(1;5) identified by G-banding was verified as originating from chromosome 12 (Fig. 3H,I). Similarly, portions of chromosomes 1 and 2 were found in markers 2 and 1, respectively (Fig. 3B–E).

The coupled G-banding and FISH technique is able to identify chromosomes that were considered to be entirely lost by the G-banding technique. This is illustrated in case 2 where the sequences of the lost chromosome 4 were found in add(19)(p13), mar1, and one chromosome

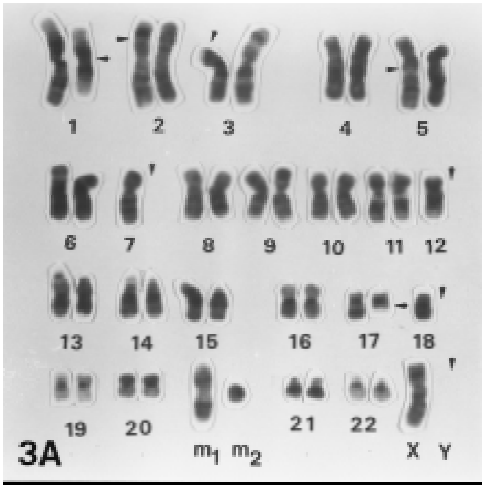
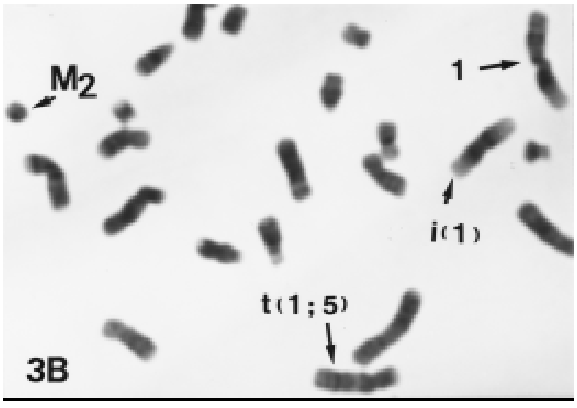
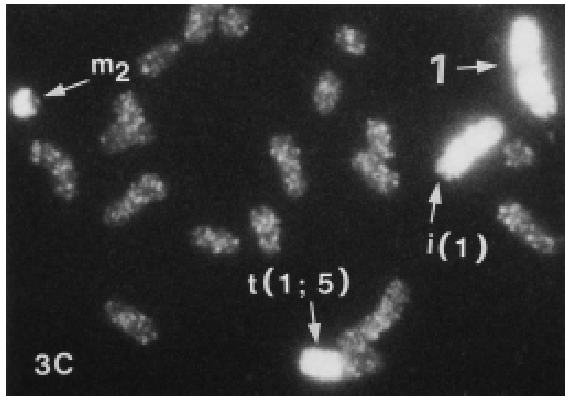
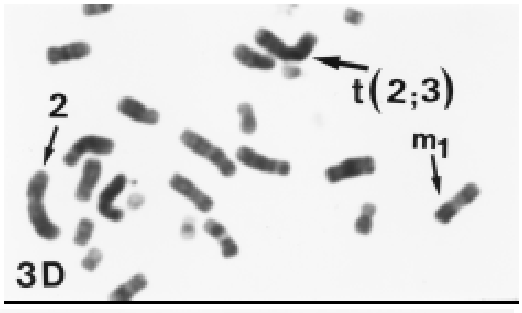
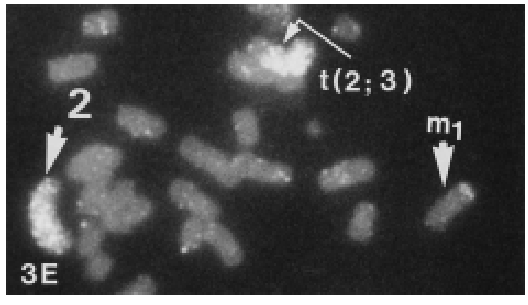


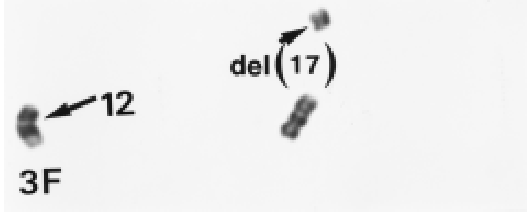

**Fig. 3. A: A karyotype of case 3. Arrows indicate numerical and structural anomalies. B,C: A G-banded metaphase and FISH with the painting probe 1, respectively. One normal chromosome 1, an i(1)(p10) and mar2, as well as a large segment of the der(5)t(1;5) are labeled (arrows, C). D,E: A metaphase and its FISH result with the painting probe 2. A large segment of t(2;3) and a small segment of the mar1 are labeled as is one normal chromosome 2 (arrows, E). F–G, H–I: Two metaphases and their FISH results with the painting probe 12, respectively. DNA sequences of the chromosome 12 were detected on the der(5)t(1;5) and del(17) identified by the G-banding (arrows, G,I).**

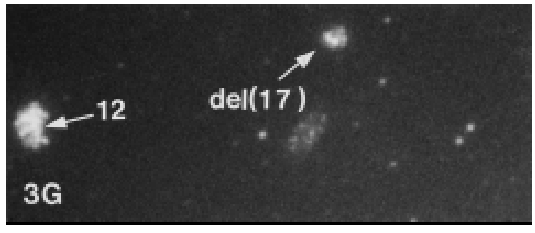


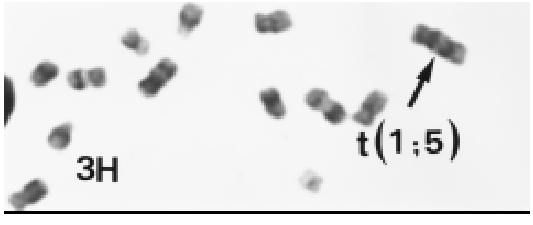



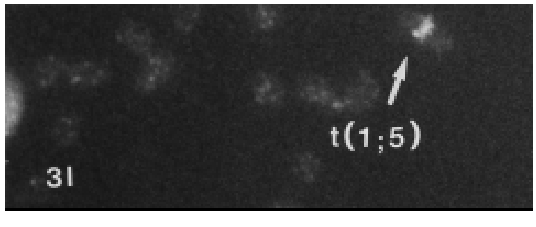



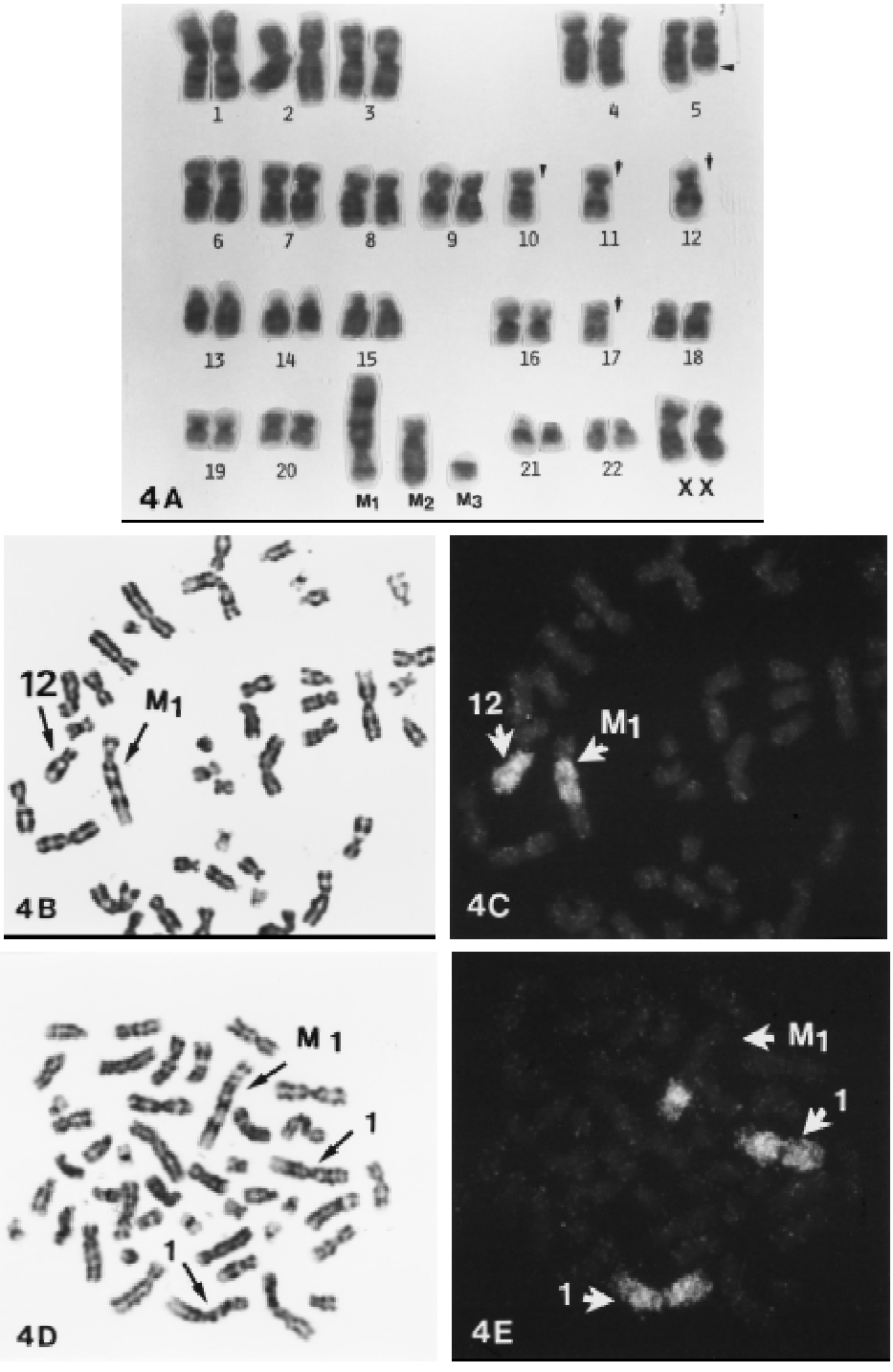



**Fig. 4. A: A karyotype of case 4. B,C: A metaphase and its FISH result with the painting probe 12, respectively. D,E: A metaphase and the FISH result with the painting probe 1, respectively.**

13, which was interpreted as normal by the G-banding (Fig. 2B,C). We didn't study any cases with apparently normal karyotypes of FISH, therefore, we can not address the significance of the combined G-banding and FISH to reveal occult abnormalities.

In summary, FISH after G-banding helped identify the origins of marker chromosomes in all four cases described here. It is obvious that the application of FISH combined with classical banding patterns has greatly facilitated the interpretation of complex rearrangements, but some of them still remain unidentifiable because of the usage of only whole chromosome DNA painting probes and centromere-specific DNA probes. The rapidly increasing availability of region- and band-specific

probes will further elucidate complex rearrangements [21,22]. The recently developed microdissection technique can be performed on banded chromosomes [23,24]. With this technique, we can PCR amplify DNA from rearranged chromosomes that cannot be identified by routine banding techniques or by the combined G-banding and FISH technique. FISH using labeled PCR fragments or DNA libraries can then be performed on banded metaphase spreads obtained from a healthy control. In the near future, with the increased utilization of this new technique, we will be able to more completely describe the complex rearrangements of human cancers.

## ACKNOWLEDGMENTS

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