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A novel translocation t(8;18)(q13;q21) in acute monocytic leukemia evolving from constitutional trisomy 8 mosaicism

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Abstract

Constitutional trisomy 8 mosaicism (CT8M) has been considered as the first mutation in multistep carcinogenesis. We describe here a 38-year-old woman with a normal phenotype, who developed to acute monocytic leukemia with a novel translocation t(8;18)(q13;q21). Chromosome analysis and spectral karyotyping showed 47,XX,+8,t(8;18)(q13;q21)[20]. Fluorescence *in situ* hybridization (FISH) demonstrated that the breakpoint at 18q21 was centromeric to the *MALT1* and *BCL2* genes. FISH also revealed that trisomy 8 was detected in buccal mucosa cells, indicating that trisomy 8 was a constitutional abnormality. These results suggest that t(8;18)(q13;q21) had a crucial role in the development of leukemia as the second mutation following CT8M.

Keywords:

constitutional trisomy 8 mosaicism (CT8M); acute monocytic leukemia; t(8;18)(q13;q21); fluorescence *in situ* hybridization; buccal mucosa

1. Introduction

Constitutional trisomy 8 mosaicism (CT8M) occurs in approximately one in 35000 newborn children, and more than 80 certain cases of CT8M have been reported in the literature [1, 2]. Affected individuals often have mild to severe mental retardation and multiple phenotypic anomalies including facial dysmorphism, deep palmar and plantar creases, clinodactyly and scoliosis, although some patients with CT8M have normal intelligence and phenotypes [2, 3]. The mosaicism is usually investigated in phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes and cultured skin fibroblasts, but the distribution of the mosaicism is different in each tissue [2]. On the other hand, acquired trisomy 8 is commonly detected in the bone marrow cells of myeloid malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Trisomy 8 as a sole abnormality is observed in 11% and 6% of cytogenetically abnormal MDS and AML cases, respectively, and is considered to confer an intermediate prognosis [4].

Recently, it has been reported that CT8M could have a causative role in the development of hematological malignancies and embryonal childhood tumors, especially of myeloid malignancies [5-13]. Seghezzi *et al.* [11] summarized 11 cases of CT8M who developed to malignancies and defined CT8M as a genetically important first mutation in multistep carcinogenesis. However, subsequent mutations leading to myeloid malignancies with CT8M remain to be completely elucidated. We describe here an additional case of CT8M, who developed to AML with a novel translocation involving chromosome 8, t(8;18)(q13;q21).

2. Materials and methods

2.1. Case History

A 38-year-old woman was admitted to our hospital because of severe pneumonia and thrombocytosis in January 2006. She had no mental retardation and no physical anomaly. Peripheral blood showed hemoglobin 92 g/L, platelets 433 x 10^9/L and white blood cells 8.5 x 10^9/L with 1% myelocytes, 2% metamyelocytes, 9% band forms, 81% segmented neutrophils, 5% eosinophils and 2% lymphocytes. Bone marrow was hypercellular with 34.2% erythroblasts, 52% myeloid cells with normal differentiation, 8.0% eosinophils and 2.0% lymphocytes. Considering cytogenetic findings described in Results, we diagnosed the disease as reactive thrombocytosis due to pneumonia in a patient with CT8M. She was moved to the nearest hospital to her home for further treatment of pneumonia.

Six months later, she was readmitted because of rapid increase of white blood cells with monoblasts. Peripheral blood showed hemoglobin 122 g/L, platelets 136 x 10^9/L and white blood cells 93.5 x 10^9/L with 37% monoblasts, 33% promonocytes, 1% promyelocytes, 9% myelocytes, 5% metamyelocytes, 7% band forms, 6% segmented neutrophils, 1% basophils and 1% lymphocytes. Bone marrow was markedly hypercellular with 64.6% monoblasts and 20.8% promonocytes. These large-sized monocytic cells had subtly convoluted nuclei with prominent nucleoli and basophilic cytoplasm with vacuoles and fine azurophilic granules (Fig. 1A). They were positive for myeloperoxidase and α-naphthyl butyrate (ANB) esterase but negative for chloroacetate esterase stainings (Fig. 1B, 1C). The ANB positivity was totally inhibited by NaF (Fig. 1D). Therefore, we made the diagnosis as AML M5b in the French-American-British classification or acute monocytic leukemia in the World Health Organization classification. Unfortunately, surface marker analysis by flow cytometry could not be performed because of the admission on holiday. An induction therapy with daunorubicin and cytosine arabinoside was immediately started on admission, but she could not

achieve a complete remission because of the resistance to chemotherapy. She died of disease progression in August 2006. Autopsy showed massive infiltration of leukemic cells into skin, liver, spleen, lung and heart as well as bone marrow.

2.2. Chromosome analyses and spectral karyotyping

Chromosome analyses were performed by the G-banding technique on short-term culture of the cells obtained from bone marrow. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [14]. Spectral karyotyping (SKY) was carried out with SkyPaint kit (Applied Spectral Imaging, Migdal Ha'Emek, Israel).

2.3. Fluorescence in situ hybridization (FISH) analysis

We used LSI MALT1 Dual Color, Break Apart Rearrangement Probe and LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probe (Vysis, Downers Grove, IL, USA) to characterize the 18q21 breakpoint of t(8;18)(q13;q21). The LSI MALT1 Probe consists of the 5' side 460 kb probe labeled with SpectrumOrange and the 3' side 660 kb probe labeled with SpectrumGreen. The expected pattern in a normal nucleus is two fusion (orange/green) signals. The LSI IGH/BCL2 Probe is a mixture of the IGH probe labeled with SpectrumGreen spanning 1.5 Mb containing the entire *IgH* locus at 14q32 and the BCL2 probe labeled with SpectrumOrange covering a region of 750 kb including the entire *BCL2* gene at 18q21. The expected pattern in a normal nucleus is two orange and two green signals. FISH analyses were performed on metaphase spreads of the bone marrow cells at the diagnosis of AML.

We also used CEP8 SpectrumOrange probe (Vysis), specific for the α-satellite region 8p11.1-q11.1, to examine the distribution of trisomy 8. FISH analyses with CEP8 probe were performed on 100 interphase nuclei of the buccal mucosa and bone marrow cells. Buccal mucosa cells were obtained by scratching the inner surface of the cheek. The cut-off values

for false positive of one signal and three signals were set at 7.0% and 2.0%, respectively.

3. Results

Chromosome analysis at the initial diagnosis of reactive thrombocytosis showed 47,XX,+8[19]/46,XX[1], whereas the karyotype at the diagnosis of AML evolved to 47,XX,+8,t(8;18)(q13;q21)[20] (Fig. 2A). Spectral karyotyping confirmed t(8;18)(q13;q21) with +8, and there was no additional cryptic abnormality (Fig. 2B). For further characterization of t(8;18)(q13;q21), we performed FISH analyses with the *MALT1* and *BCL2* probes on metaphase spreads. The *MALT1* gene is shown to be located about 5 Mb centromeric of the *BCL2* gene in chromosome 18q21 [15, 16]. Both the *MALT1* and *BCL2* signals were translocated to the der(8)t(8;18)(q13;q21) (Fig. 3A and 3B), indicating that the breakpoint at 18q21 in t(8;18)(q13;q21) was centromeric to the *MALT1* and *BCL2* genes.

At the initial diagnosis, trisomy 8 as a sole aberration was detected in almost all bone marrow cells, although there was no apparent hematological abnormality for possible diagnosis of myeloid malignancies. Then, to examine whether the patient had CT8M in spite of a normal phenotype, we also performed FISH with *CEP8* probe on interphase nuclei of non-hematopoietic cells. As shown in Fig. 4 and Table 1, trisomy 8 was detected in buccal mucosa as well as bone marrow cells. These results indicated that trisomy 8 was not acquired but a constitutional abnormality found in hematopoietic and non-hematopoietic cells as mosaicism.

4. Discussion

We have identified a novel translocation t(8;18)(q13;q21) in a patient with AML evolving from CT8M. This translocation has never been described in the literature to date [17]. The result indicates that CT8M was the first mutation in the multistep leukemogenesis and that t(8;18)(q13;q21) had a crucial role in the development of AML as a subsequent mutation. This case further emphasizes the association between CT8M and myeloid malignancies. In addition, FISH on interphase nuclei from buccal mucosa cells obtained by non-invasive procedure appears to be more practical for detection of CT8M than cell culture technique of skin fibroblasts or peripheral blood lymphocytes [18]. Even if patients of myeloid malignancies with trisomy 8 show no congenital anomalies, exclusion of CT8M by this method may be important to avoid erroneous use of trisomy 8 as a tumor marker.

To our knowledge, a total of 13 cases of hematological malignancies with CT8M, including seven cases of MDS, two cases of AML, two cases of atypical chronic myelogenous leukemia (CML), one case of idiopathic myelofibrosis and one case of acute lymphoblastic leukemia, have been reported; four of them had normal phenotypes [5-13]. Among these cases, only two had additional chromosome abnormalities besides trisomy 8, that is, 47,XY,del(5)(q?),+8 in juvenile CML and 46,XY,+8,der(17;18)(p10;p10),-18 in MDS [7, 9], whereas other 11 cases had trisomy 8 as a sole abnormality. It is likely that del(5)(q?) and der(17;18)(p10;p10) were the second mutations leading to myeloid malignancies with CT8M. In both cases, these two additional aberrations were detected in cells with trisomy 8. In the present case, t(8;18)(q13;q21) also appeared only in cells with trisomy 8 at the diagnosis of AML. These results indicate that leukemic cells could develop exclusively from the population of trisomic cells but not normal diploid cells, and confirm cytogenetically that patients with CT8M have an increased risk of myeloid malignancies.

Possible mechanisms of trisomy 8 contributing to myeloid malignancies include gene

dosage effects and duplication of mutated genes on chromosome 8. However, no evidence for cryptic abnormalities has been obtained in patients with constitutional as well as acquired trisomy 8 [4]. Therefore, it is unknown whether the translocation involving 8q13 was genetically associated with trisomy 8 in the present case. Rearrangements involving 8q13 are relatively rare cytogenetic aberrations in hematological malignancies. Besides inv(8)(p11q13) in AML and t(7;8)(q22;q13) in CML as a recurrent aberration, several sporadic translocations, such as t(8;12)(q13;p13) and t(8;20)(q13;q13), have been reported in AML [17, 19-21]. Among these abnormalities, the *TIF2* gene, encoding a nuclear receptor coactivator, was identified at 8q13 and shown to form a fusion with the *MOZ* gene at 8p11 in AML M4/5 with inv(8)(p11q13) [19]. This gene might be involved in t(8;18)(q13;q21), but unfortunately we could not examine the 8q13 breakpoint.

On the other hand, translocations involving 18q21 are frequently observed in lymphoid malignancies, especially t(14;18)(q32;q21) with *IgH/BCL2* in follicular lymphoma and t(11;18)(q21;q21) with *API2/MALT1* in MALT lymphoma [15]. We showed that the breakpoint at 18q21 in our case was centromeric to the *MALT1* and *BCL2* genes. In addition, several chromosome aberrations involving 18q21, such as t(18;21)(q21;q22), del(18)(q21) and t(5;18)(q33-35;q21), have been detected in myeloid malignancies [22-25]. The possible involvement of the *BCL2* gene was examined and discussed in cases with del(18)(q21) and t(5;18), respectively [23, 24]. However, the breakpoints at 18q21 in these cases have never been identified. The genes, located at 18q21 and proximal to *MALT1*, include *DCC* and *DPC4* tumor suppressor genes [16]. It is possible that these genes at 18q21 might be associated with t(8;18)(q13;q21), although genomic alterations of these genes have been rarely detected in myeloid malignancies [26, 27]. Characterization of t(8;18)(q13;q21) more minutely would contribute to the clarification of the mechanisms in multistep leukemogenesis from CT8M.

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Figure legends

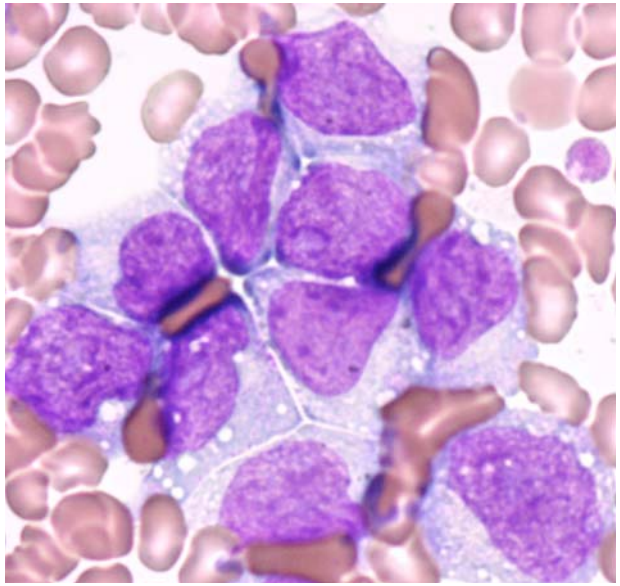
Fig. 1. Bone marrow smear at the diagnosis of AML (x1000). (A) Large monoblasts and promonocytes are shown (May-Grünwald-Giemsa staining). (B) Monocytic cells are positive for myeloperoxidase staining. (C) They are positive for α-naphthyl butylate (ANB) esterase but negative for chloroacetate esterase stainings. (D) The ANB positivity is inhibited by NaF.

Fig. 2. (A) G-banded karyotype of the bone marrow cells at the diagnosis of AML: 47,XX,+8,t(8;18)(q13;q21). Arrows indicate rearranged chromosomes.
(B) Spectral karyotyping of the metaphase spread after spectrum-based classification (left side, reverse DAPI; right side, SKY). Chromosomes were assigned a pseudocolor according to the measured spectrum. The karyotype is confirmed as follows: 47,XX,+8,t(8;18)(q13;q21). Arrows indicate rearranged chromosomes.

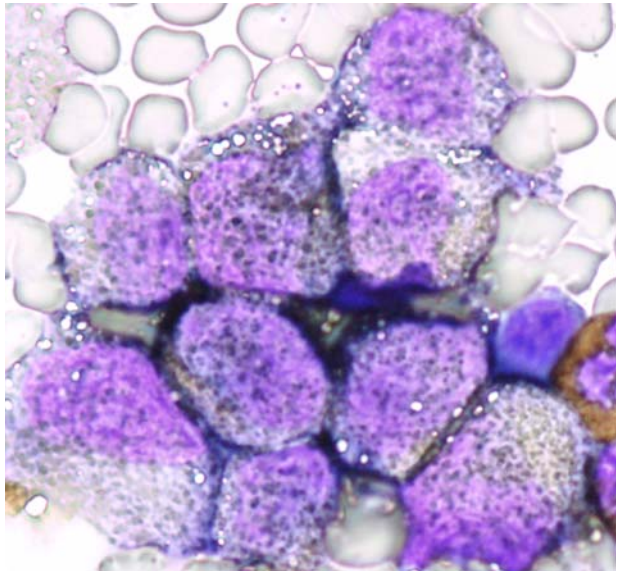
Fig. 3. FISH analyses with (A) *MALT1* and (B) *IgH/BCL2* probes on metaphase spreads.
(A) Arrows indicate *MALT1* (orange/green) signals on 1) normal chromosome 18 and 2) the der(8)t(8;18)(q13;q21). The *MALT1* signal at 18q21 is translocated to the der(8)t(8;18)(q13;q21).
(B) Arrows indicate 1) *IgH* signals on normal chromosomes 14 (green), 2) *BCL2* signal on normal chromosome 18 (orange), and 3) *BCL2* signal on the der(8)t(8;18)(q13;q21) (orange). The *BCL2* signal at 18q21 is translocated to the der(8)t(8;18)(q13;q21).

Fig. 4. FISH analyses with *CEP8* probe on interphase nuclei of (A) buccal mucosa cells and (B) bone marrow cells at the initial diagnosis. Three *CEP8* signals (yellow) are detected in both tissues and indicated by arrows.

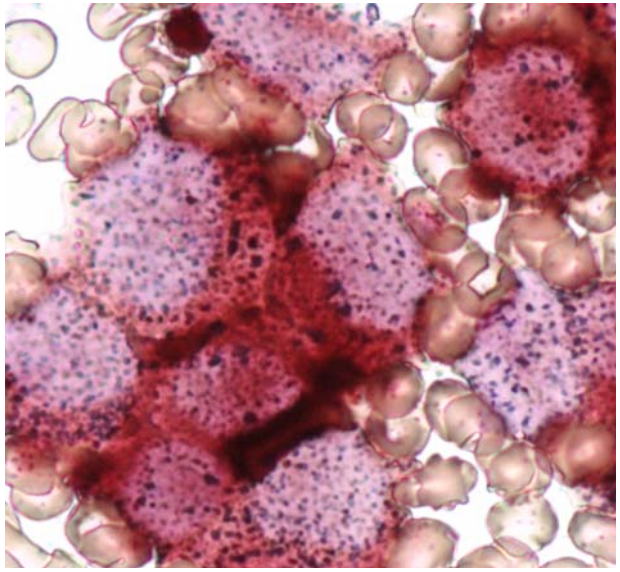
A



B



C



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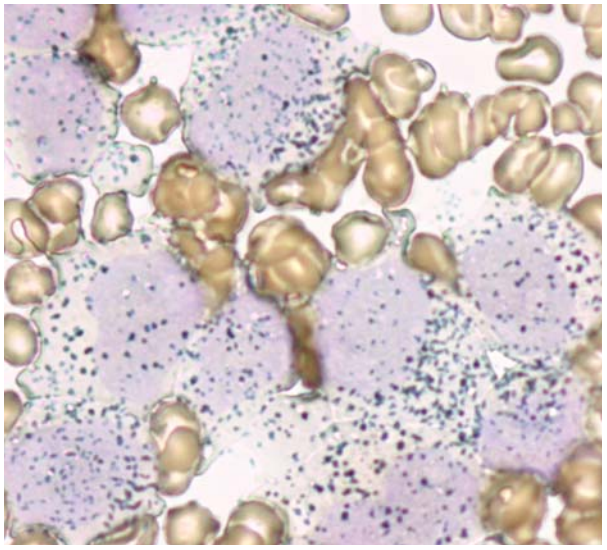

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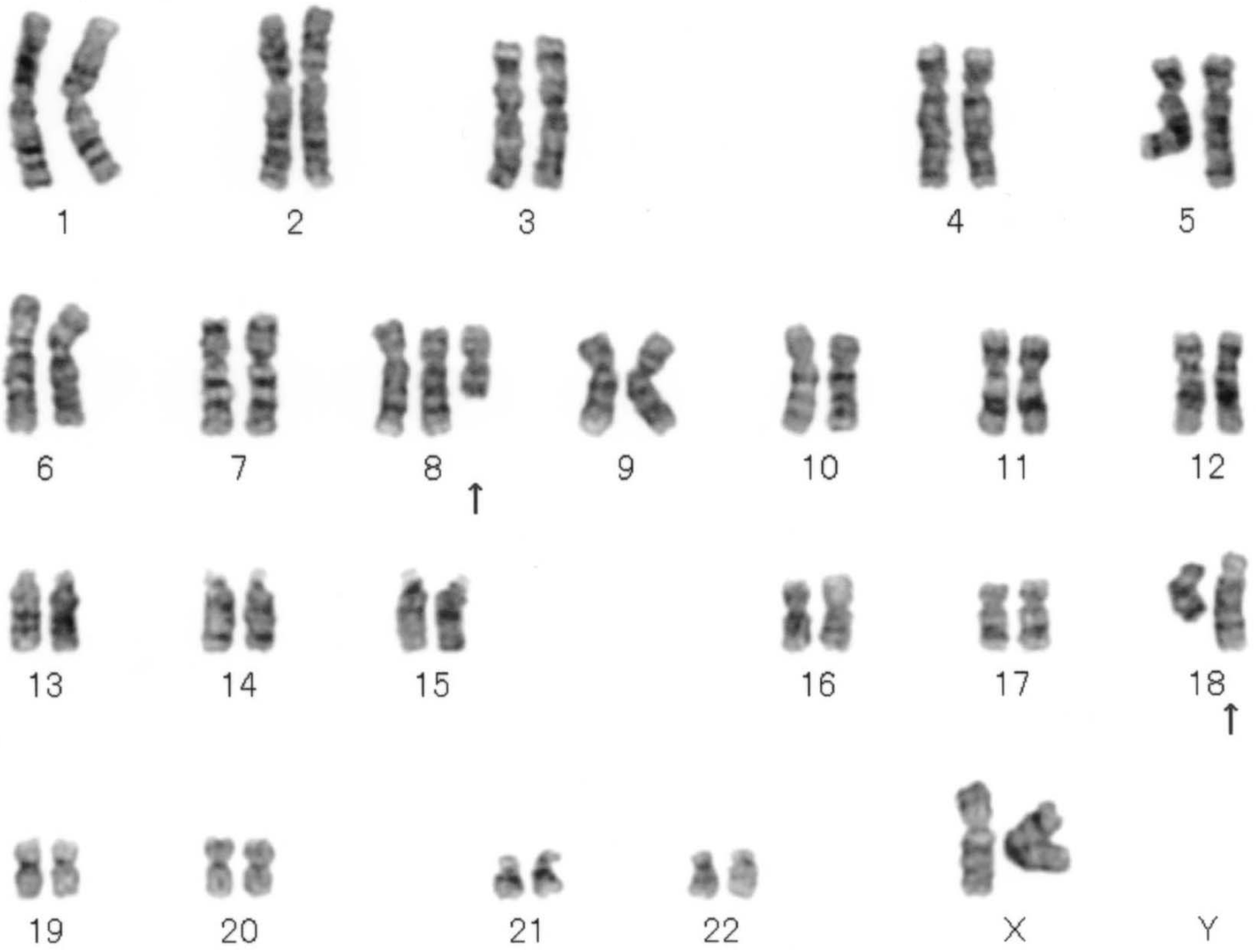

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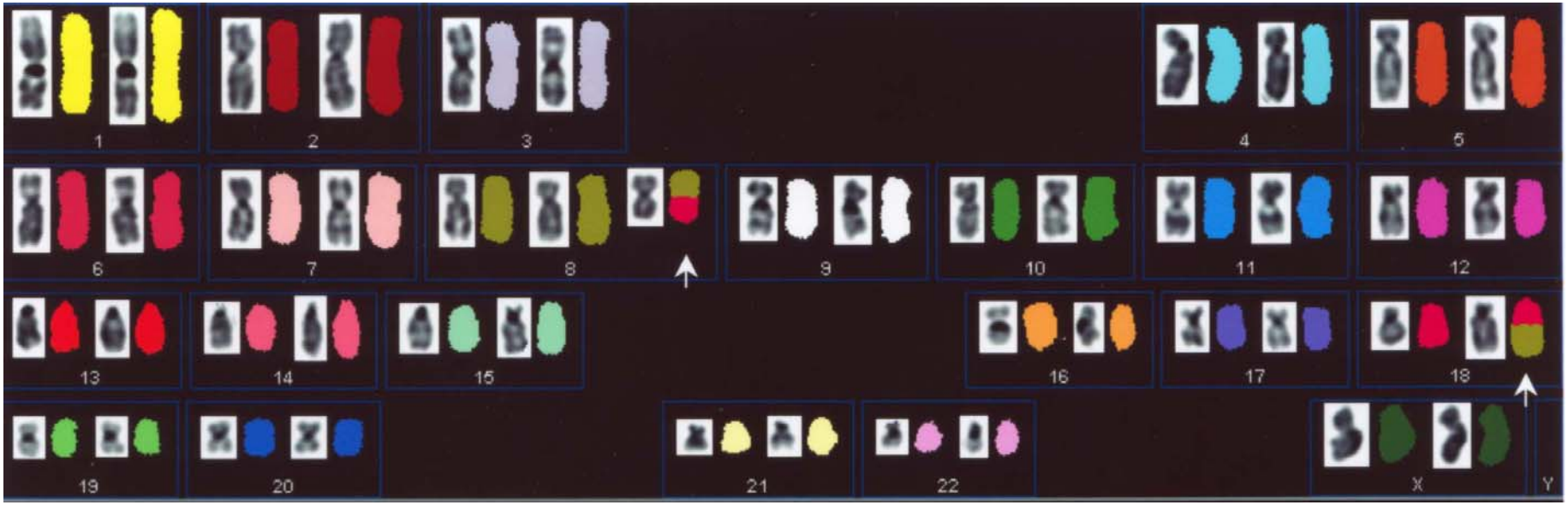

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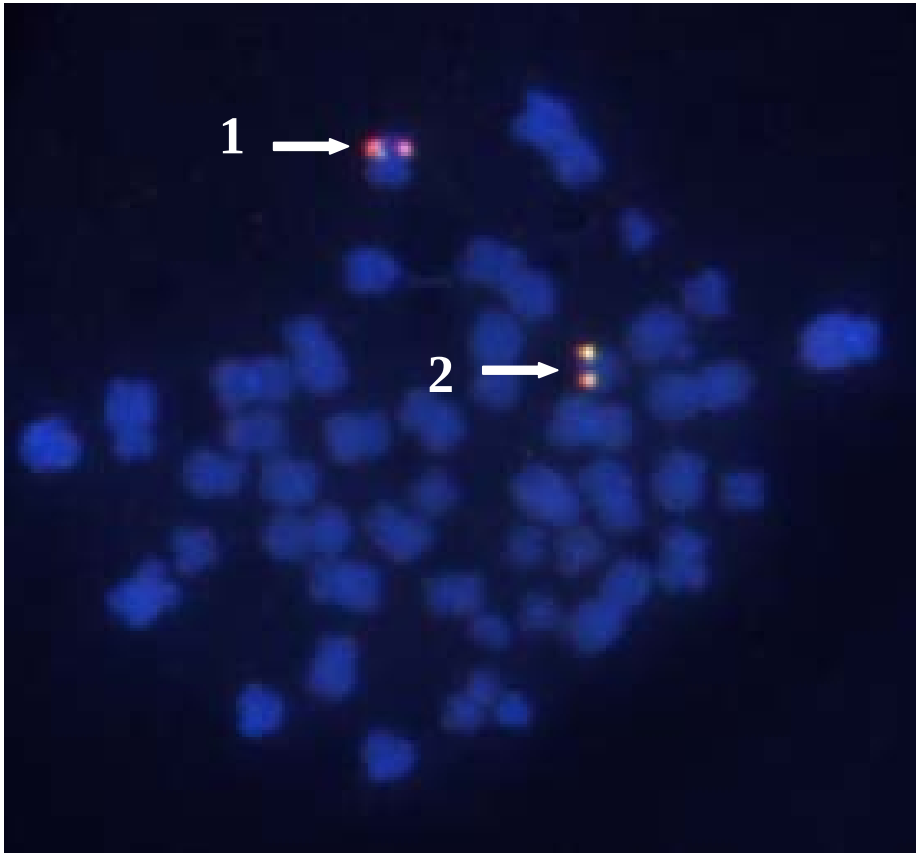

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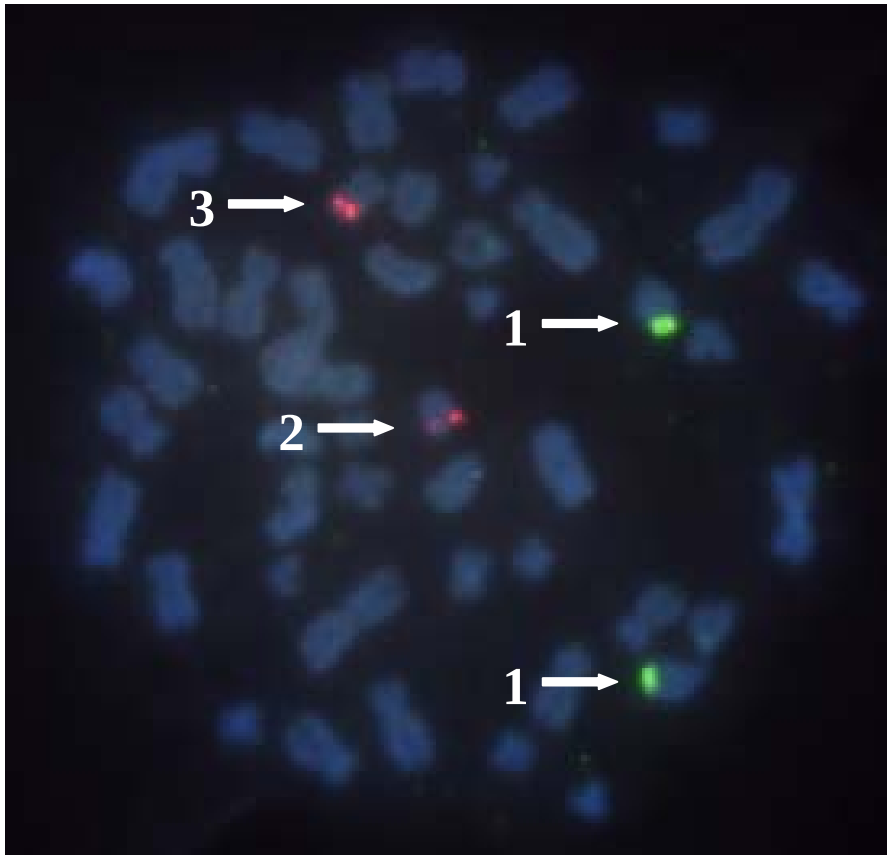

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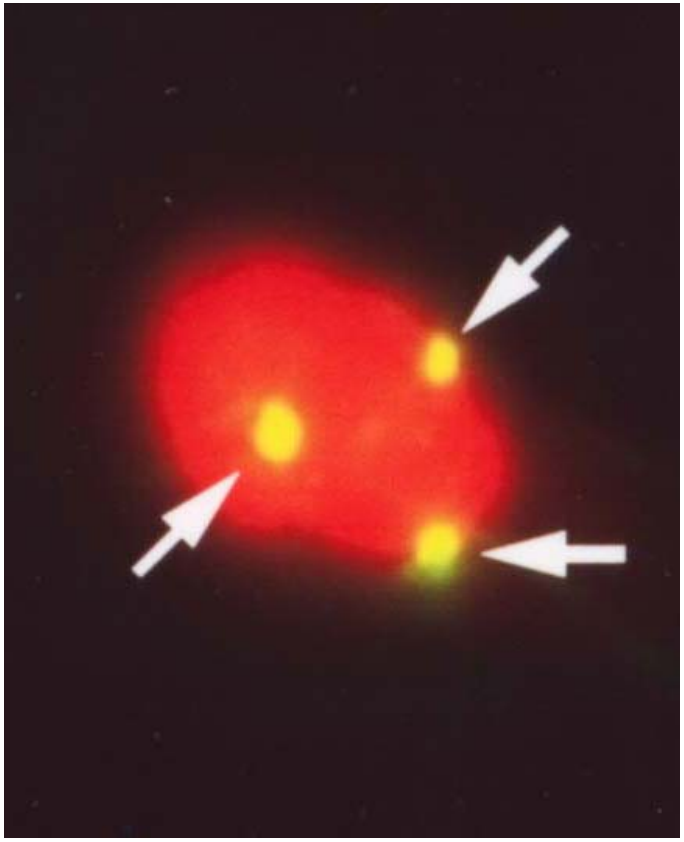

B