Fluorescence *in situ* Hybridization : A Rapid Analysis to Verify Chromosome Aberrations

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Abstract

We applied fluorescence *in situ* hybridization (FISH) to assess the presence of structural rearrangement and numerical chromosome aberrations in both metaphase chromosome and interphase nuclei. For this purpose, the biotinylated repetitive alpha-satellite DNA probes for chromosome 1, 18 and 8 (pUC1.77, L1.84 and pJM128) were used to identify tetraploid mosaicism, ring chromosome 18 and trisomy 8 mosaicism for pre-, post-natal and tumor diagnosis respectively. Utilizing this approach, we showed the usefulness of FISH for routine clinical cytogenetics in addition to chromosome banding techniques. The chromosome aberrations with unknown or unclear origin, detected by chromosome analysis, could be confirmed accurately and rapidly.

Key word : Fluorescence *in situ* Hybridization (FISH), Structural Chromosome Rearrangement, Numerical Chromosome Aberration, Mosaicism

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In conventional cytogenetic analysis, a typical investigation would involve chromosome banding studies for the identification of abnormal chromosomes(1,2). The interpretation of chromosome banding patterns requires a skillful cytogeneticist. Nevertheless it has been often technically difficult, especially the detection of minor structural changes and analysis of complex karyotypes. This

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method works efficiently for diagnosis of large chromosomal rearrangements or gain and loss of the whole or large segment of chromosomes where the quality of metaphase spread is good and the mitotic index is high.

The development of chromosomal FISH with specific DNA probes and the detection of signal by non-isotopic means has provided a powerful tool for the rapid analysis of human chromosome aberrations[3,4]. FISH could be used to detect numerical and structural abnormalities of both metaphase chromosomes and chromosomal domain in interphase nuclei[5-7]. Thus, FISH is an additional approach to the limitation of the traditional chromosome banding karyotype analysis in such cases where clear information of the aberration is not obtained. It also provides an opportunity to investigate cases in which the chromosome preparations have been difficult or impossible.

In this paper, we show that the FISH is readily applicable to clinical cytogenetic practice, by reporting the analysis of three diagnosis cases involving ring chromosome, trisomy and tetraploidy mosaicism in pre- and post-natal as well as in tumor diagnosis.

MATERIAL AND METHOD

Patients

Patient 1, a 1 year old boy who was transferred for routine cytogenetic investigation from the Pediatric Clinic. The patient had multiple congenital malformations of delayed development, hypospadias, abnormal bone structure, knock knee, mild hypotonia and no testis.

Patient 2 was a 18 year old female with ANLL, subtype M1 from the Division of Hematology, Department of Medicine.

Patient 3, a 35 year old expectant mother, was referred for prenatal diagnosis by amniocentesis at the Department of Obstetrics and Gynecology. Her indication was elderly prima gravida.

All three patients were admitted to Ramathibodi Hospital for investigations.

DNA probes

The following probes were used for FISH : pUC1.77 – satellite III repetitive DNA of chromosome 1[8], pJM128 – repetitive sequence of chromosome 8[9] and L1.84 – repetitive sequence of chromosome 18[10].

Preparation of cell materials

Metaphase chromosomes of lymphocytes were prepared employing the 72 hour short term culture technique[11]. After colchicine treatment, cells were treated with 0.075 M KCl hypotonic solution, fixed in glacial acetic acid-methanol and metaphase spread was made by standard procedure. For bone marrow culture, slight modification was used. Cells were treated with ethidium bromide for 2 hour after 24 hour culture and arrested with 8 min colchicine[12].

Prior to hybridization, slides were pretreated with 100 µg/ml RNAse A (Boehringer Mannheim) in 0.01 M HCl for 8 min at 37°C and postfixed in 1 per cent formaldehyde prepared in PBS/$MgCl_2$ for 10 min at room temperature.

Probes labeling and *in situ* hybridization

DNA probes were labeled by nick translation[13] in which dTTP were substituted by biotin-16-dUTP (Boehringer Mannheim). Aliquot of 100 µg of each DNA probe was precipitated in the presence of 10 µg salmon sperm DNA (Gibco BRL). The DNA was dissolved in 10 µl hybridization solution (50% formamide, 10% dextran sulphate, 2xSSC) and applied to metaphase chromosomes under coverslip. The coverslip was sealed with rubber cement. The denaturation of DNA probe and chromosome preparation was occurred simultaneously at 75°C for 5 min. The hybridization took place for 16 hour at 37°C[14].

Probe detection

The coverslip was removed and slide was washed 3 times for 5 min each in 2xSSC and subsequently 3 times for 5 min in 0.1xSSC at 42°C. The slide was preincubated with 3 per cent bovine serum albumin in 2xSSC at 37°C for 30 min, then incubated at 37°C for 30 min with strepavidin-Cy3 (Sigma). The unbounded antibody was removed by 3 washing steps with 0.5 per cent Tween-20 in 4xSSC at 37°C. All preparations were counterstained with 4', 6-diamidino-2-phenyllindole (DAPI) and mounted in anti-fade medium (20 mM Tris-HCl, pH 8.0, 90 per cent glycerol containing 2.3 per cent of DAPCO).

Microscopy

The slides were examined immediately after the anti-fade mixture was applied. The

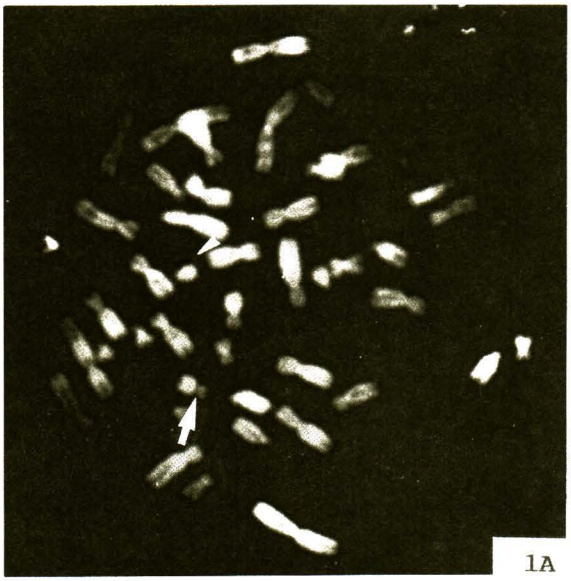


Fig. 1. FISH of patients using specific centromeric DNA probes.

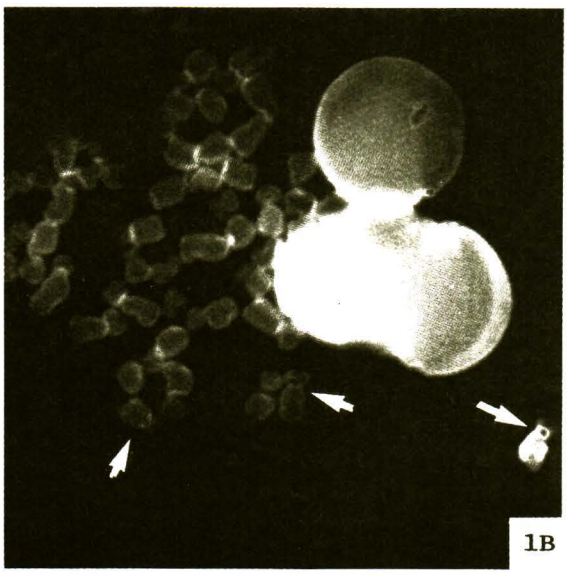
Fig. 1A. Hybridization of chromosome 18 specific probe–L1.84 on metaphase chromosomes of patient 1. Two hybridization signals were seen. One signal (arrow) was on the normal chromosome 18 and the other signal (arrowhead) was on the ring chromosome.

detected signals were recorded using fluorescence microscope (Olympus BX60) equipped with CCD camera and computerized image analyzer (Applied Image Cytovision) and printed by the digital image printer (Mitsubishi).

RESULTS

Patient 1 : By conventional G-banding, karyotype of the patient showed male pattern with a ring chromosome. This aberrant chromosome was suspected to be a ring of chromosome 18 since one chromosome 18 had not been seen – 46, XY, r(18)?. FISH using L1.84 DNA probe showed 2 signals of chromosome 18. One signal was hybridized on the normal chromosome 18 and the other appeared on the ring chromosome (Fig. 1A).

Patient 2 : Chromosome analysis from bone marrow cultures had been hampered by poor mitotic index and banding quality of the metaphase chromosomes, but nonetheless an extra chromosome 8 was detected in about 50 per cent of the analyzed metaphases. The karyotype of 46, XX/47, XX, +8 was reported. The result from FISH using the pJM128 probe confirmed the cytogenetic result of trisomy 8 mosaicism with 45 per cent trisomic cells (Fig. 1B, 1C).



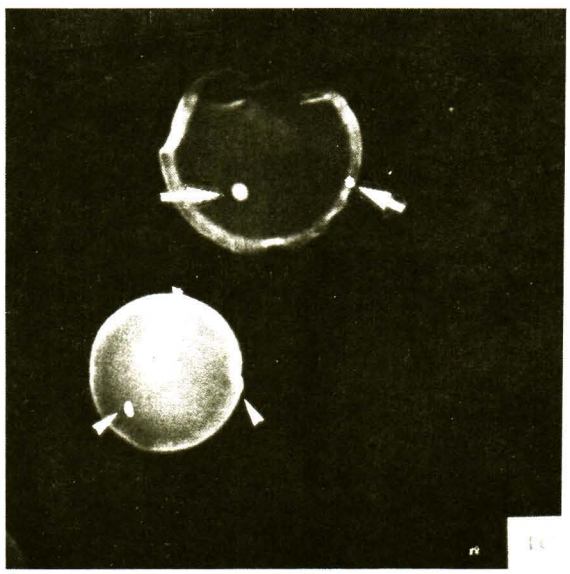
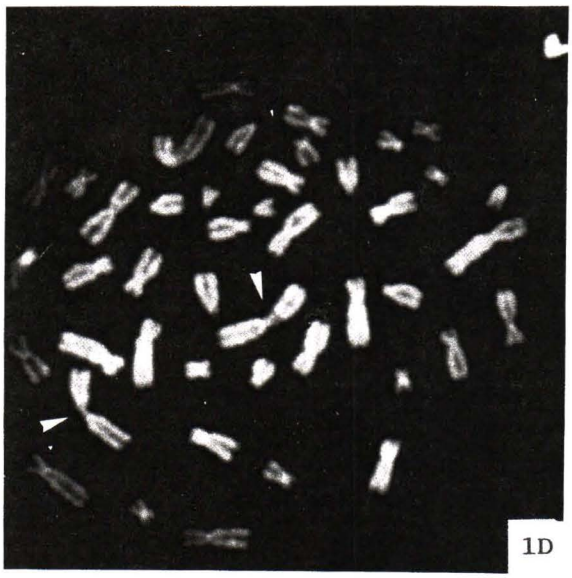


Fig. 1B, 1C. In patient 2, trisomy 8 mosaicism was detected by chromosome 8 specific probe–pJM128, three signals on chromosomes 8 (arrow) as well as three signals in one nucleus (arrowhead) and two signals in another nucleus (arrow) were seen.

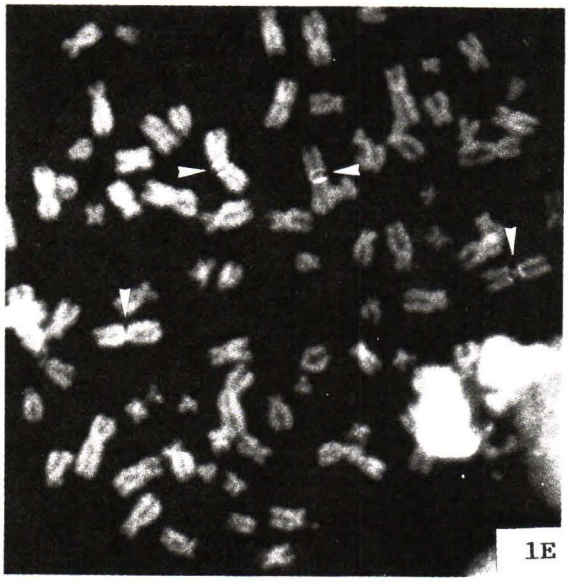
Patient 3 : Cytogenetic study of amniotic fluid cell culture showed 50 per cent mosaicism between normal cells, 46, XY, and tetraploid cells, 92, XXYY. In order to exclude pseudomosaicism due to culture artifact, FISH using pUC1.77 centromeric probe was done on interphase cells from fetal cord blood and 4 signals from 50 per cent of cells were determined (Fig. 1D, 1E, 1F).



DISCUSSION

We have shown that the combined G-banding and FISH were a useful approach in delineating complex karyotypes for pre- and post natal as well as tumour diagnosis. The development of FISH, either using conventional fluorescence microscope or combined with computerized image processing[15], although it could not totally replace cytogenetics, had strongly enhanced cytogenetic resolution in confirmation of breakpoints and complex structural rearrangements. Interphase nuclei FISH using centromeric probes has been a promising and rapid application for the detection of numerical chromosome aberrations, since the preparation of metaphase chromosomes through cell culture is not necessary. It was, therefore, suitable for analysis of tumor samples by which the metaphase chromosomes had been difficult to obtain. Using specific DNA probe or multiple probes, the identification of marker chromosomes was more feasible as shown in this report.

Chromosome mosaicism, consisting of one normal and one abnormal chromosome complement, is encountered in around 1-2 per cent of all amniotic fluid cell cultures[16]. It may represent the true status of the fetus (true mosaicism) or may arise during cell culture (pseudomosaicism). In cases of suspected pseudomosaicism, without FISH



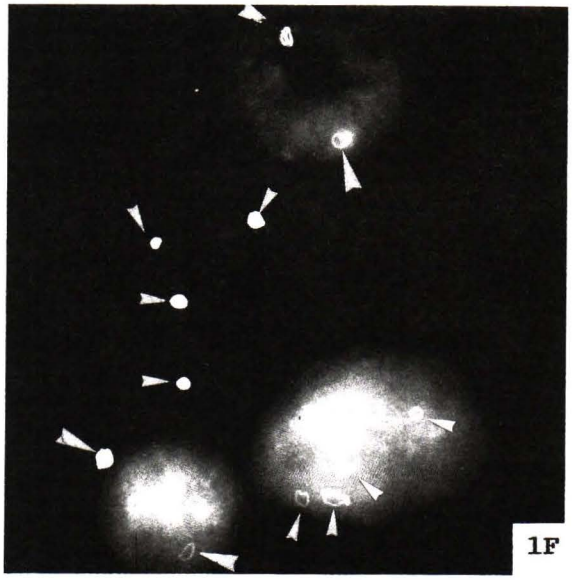


Fig. 1D, 1E, 1F. Two and four signals of chromosome 1 specific probe-pUC1.77 (arrowhead) were detected in diploid and tetraploid cells in patient 3.

technique, additional cultures either from amniotic fluid or fetal cord blood for chromosome analysis should be undertaken. These processes take at least 3 days to complete. The use of FISH with specific centromeric probes increases capabilities of rapid detection of such mosaicism in interphase nuclei, which is normally preceived necessary especially for prenatal diagnosis. It can, therefore, fulfill the requirement of routine clinical applications in aberration screening.

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ฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชัน : การวิเคราะห์โครโมโซมผิดปกติอย่างรวดเร็ว

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คณะผู้วิจัยได้ตรวจหาความผิดปกติของโครงสร้างและจำนวนโครโมโซม โดยการทำฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชันกับโครโมโซมระยะเมตาเฟสและนิวเคลียสระยะอินเตอร์เฟส สามารถตรวจพบเตตราพลอยด์โมสาอิค โครโมโซมวงแหวน 18 และโมสาอิคไตรโซมีของโครโมโซม 8 ด้วยการใช้ดีเอ็นเอชนิดอัลฟาแซทเทลไลต์ที่มีลำดับเบสซ้ำซ้อนจากโครโมโซม 1, 18 และ 8 (pUC1.77, L1.84 และ pJM129) ตามลำดับ ติดฉลากด้วยไบโอติน เป็นดีเอ็นเอติดตาม การวิเคราะห์วิธีนี้ใช้ได้ทั้งในการวินิจฉัยก่อนคลอด หลังคลอด และการวินิจฉัยมะเร็ง แสดงให้เห็นว่าวิธีฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชันมีประโยชน์มากต่อการวินิจฉัยทางเวชพันธุศาสตร์ ซึ่งเพิ่มเติมจากการวิเคราะห์แถบสีโครโมโซมที่ใช้กันเป็นประจำอยู่แล้ว ทำให้โครโมโซมผิดปกติชนิดที่ไม่ทราบที่มาแน่นอนเมื่อวิเคราะห์ทางเซลล์พันธุศาสตร์ ได้รับการวิเคราะห์ยืนยันอย่างแม่นยำและรวดเร็ว

คำสำคัญ : ฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชัน, โครงสร้างโครโมโซมผิดปกติ, จำนวนโครโมโซมผิดปกติ, โมสาอิคซึ่ม

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