CHROMOSOME PAINTING AND ITS VERSATILITY IN MODERN DIAGNOSTICS

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ABSTRACT

The last two decades have seen the advent of a novel and versatile technique, well known as Chromosome Painting or Fluorescence In Situ Hybridization (FISH). During its maturation, various methodologies and modifications have been introduced to optimize the detection of DNA and RNA. The pervasiveness of this technique is largely because of its wide variety of applications and the relative ease of implementation and performance of in situ studies. Chromosome painting allows precise visualization of unique sequences, chromosomal sub-regions, or entire genome (DNA on metaphase chromosomes and interphase nuclei). It plays an increasingly important role in a variety of research areas, including cytogenetics, prenatal diagnosis, tumor biology, gene amplification and gene mapping. This review describes the applications of some FISH based techniques in human disease diagnosis.

Key words : *Chromosome painting, nucleic acid hybridization*

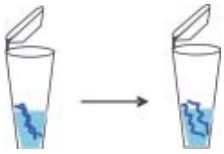
INTRODUCTION:

Fluorescence *in situ* hybridization (FISH) refers to the use of labeled nucleic acid sequence probes for the visualization of specific DNA or RNA sequences on mitotic chromosome preparations or in interphase cells (Plate 1). Exciting advances in FISH are changing the nature of cytogenetics, in both basic research and molecular diagnostics. Cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in tumorigenesis and the three-dimensional organization of the human genome. The high resolution that is achieved by these techniques, particularly by microarray technologies such as array comparative genomic hybridization, is blurring the traditional distinction between cytogenetics and molecular biology. The current review focuses on the advances that FISH has undergone to suit the requirements of present day diagnostics.

Plate - 1

Step 1: Denaturation

Conversion of double stranded DNA into single stranded form



Denaturation of labeled probe DNA

Denaturation of target DNA
(Interphase nuclei or metaphases on slide)

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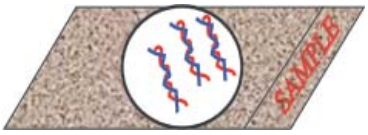
Step 2: Hybridization

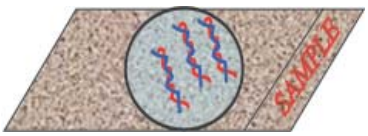
Binding of probe DNA to target DNA



Application of probe DNA to slide and overnight incubation at 37ºC

Step 3: Post hybridization washing and detection

Visualization of interphase nuclei / metaphases with bound probe



Washing of unbound probe DNA, application of counterstain and visualization using fluorescence microscopy

Probes and samples used for FISH:

As mentioned earlier, FISH uses fluorescently labelled probes for the visualisation of DNA sequences on metaphase spreads or interphase nuclei. Both numerical and structural aberrations can be determined. Probes can be for the whole chromosome, centromere, or locus specific. FISH probes for the entire genome are also often used. Interphase nuclei can be obtained from a range of clinical specimens including touch preparations, fine needle aspirates, bone marrow smears, and archival material (1).

Variations of FISH:

Interphase FISH

One application of FISH involves the hybridization of probes to interphase cells. This is extremely beneficial when it is not possible to prepare metaphase spreads as in the case of primary tumors. In addition, interphase FISH can be performed on paraffin-embedded, formalin-fixed tissue sections thereby allowing researchers to retrospectively analyze samples and correlate chromosome aberrations with biological and clinical endpoints. Interphase cytogenetics also allows one to precisely define the cell pool carrying chromosomal abnormalities, to identify whether aberrant

cells exist in clonal patches or as isolated events and to observe aberrations on a cell-to-cell basis rather than as a population (2).

Telomeric FISH (Q-FISH)

Subtelomeric probes are a relatively new addition to the arsenal of cytogenetic tests. This test is a collection of 41 different FISH probes that are used to identify rearrangements that cannot be seen by conventional cytogenetic methods (3). The subtelomeric probes target the regions right behind the ends of the chromosomes that enables to visualise if they are involved in rearrangements. Each of the probes is a different color so that the specific chromosomal segment can be identified. This is a very expensive and labor-intensive process. However, it can be used when a geneticist suspects a chromosomal abnormality and routine chromosomes are normal (4) or when there is chromosomal material of unknown origin.

RNA *In Situ* Hybridization (RISH)

In many situations, transcription of genes at the cellular level needs to be studied. Several groups have developed methods for FISH of RNA. This is a potentially important application of the FISH technique because it provides direct visual evidence of gene expression from a particular chromosome. House keeping genes, which are abundantly expressed, can be detected reliably. Further optimization and amplification of the signal can even allow detection of genes expressed at baseline levels (5).

Primed *In Situ* Labelling (PRINS)

The efficacy of FISH may be limited in specific applications by low-resolution sensitivity. The primed *in situ* labelling (PRINS) method is an alternative to *in situ* hybridization for chromosomal detection based on the use of chromosome-specific oligonucleotide primers. In this procedure, chromosomal identification is done by the *in situ* annealing of specific oligonucleotide primers, followed by primer extension by *Taq* DNA polymerase in the presence of labelled nucleotides. It has been demonstrated that the PRINS technique is more specific and considerably faster than classical FISH for chromosomal identification (6)

Fiber FISH (Dynamic Molecular Combing)

The term Fiber FISH refers to the common practice of fluorescence *in situ* hybridization (FISH) conducted on preparations of extended chromatin fibers. FISH on DNA fibers is useful in assessing the length of DNA probes, and to map probes relative to one another, as it can reveal even their degree of overlap. Thus, Fiber FISH has superior mapping resolution compared to interphase FISH. It can resolve DNA loci separated by a few kilobases and study loci as large as two megabases in a single experiment (7; 8).

Comparative Genomic Hybridization (CGH)

Comparative Genomic Hybridization serves as an important global screening test for chromosomal aberrations present within a tumor genome. This technique requires only genomic tumor DNA and metaphase preps from a normal donor, thus circumventing the preparation of high-quality tumor metaphase spreads. Tumor DNA extracted from archived, formalin-fixed paraffin-embedded tissue can also be used. This allows identification of chromosomal aberrations and facilitates the correlation of cytogenetic findings with histologic / histochemical information, clinical course, and prognosis. Analysis of small subregions of a histologically defined lesion is also possible (9). Once regions of gain or loss have been identified, these regions can be defined further using FISH or molecular genetic techniques (10). CGH coupled with microarray also known as array CGH has proved to be very informative in many clinical settings (11).

Combinatorial Binary Ratio Labeling (COBRA) FISH

Combinatorial fluorescence *in situ* hybridization (COBRA FISH) of the DNA of the 24 different human chromosomes with 5 fluorophores in conjunction with spectral or filter-based microscopic imaging has greatly advanced molecular cytogenetic analysis of chromosomes. Use of 5 fluorophores allows the identification of up to 31 different chromosomal targets on the basis of color combinations. (12) COBRA–FISH allows color discrimination of all of the p and q arms of each chromosome and permits detection and elucidation of intra and interchromosomal rearrangements (13)

Spectral karyotyping (SKY) FISH

Spectral karyotyping (SKY) is a molecular cytogenetic technique that allows differential visualization of all human chromosomes in distinct colors with a single hybridization and image exposure (14). After the chromosomes are classified and aligned in a karyotype table, interpretation and comparison of all aberrations is summarized in the karyogram.

Multiplex-FISH (M-FISH)

The M-FISH technology has the ability to identify the twenty-four different human chromosomes in a metaphase spread by the simultaneous hybridization of chromosome-specific DNA probes, each labeled with a different combination of fluorescent dyes. M-FISH differs from SKY only in that it is a filter-based system where separate images are acquired sequentially for each fluorochrome used. The individual fluorochrome files are then combined to generate the final image (15).

These techniques – M-FISH and SKY FISH, have combined the advantages of FISH with traditional chromosome banding techniques and spawned many variations resulting in diverse applications. They permit the detection of interchromosomal structural aberrations, such as translocations and insertions resulting in balanced as well as unbalanced rearrangements. SKY and M-FISH have the potential to identify cryptic translocations and clarify complex aberrations (marker and ring chromosomes), which are typically unidentifiable by conventional banding techniques. In addition, other aberrations such as double minutes can be better resolved, leading to the identification of critical oncogenes (16, 17).

Applications of FISH

FISH is generally used either to complement classic staining methods or a substitute for chromosome identification at metaphase or interphase. FISH has proved useful in several clinical settings to determine prognosis. Discrete information is obtained for each cell, which is an important advantage of the technique. In particular FISH demonstrates the qualities listed below for various diagnostic and/or prognostic applications:

Sensitivity: FISH can detect cryptic chromosomal deletions and rearrangements, not detectable by conventional means: submicroscopic microdeletions at the chromosomal level (18) and DNA level (19). FISH also helps in detection of single gene disorders - Duchenne's muscular dystrophy (20) and microduplications - mental retardation (21).

Specificity: By using a particular probe or probes, chromosomal material of unknown or uncertain origin can be identified - marker chromosomes (22) and chromosomal variants or polymorphisms (23).

Efficiency: FISH allows rapid screening of a large number of metaphases or interphases for a particular chromosome or other target sequence. Mosaicism (24) and chromosomal aneuploidies in prenatal samples (25) can be studied. FISH has also proved invaluable in monitoring residual disease status in patients with cancer (26).

Applicability: FISH allows interphase cells to be screened from a wide variety of tissues not directly accessible with conventional cytogenetics. Some of the studied samples are: human lung carcinoma tissue (27), endometrial tissue (28) and uncultured chorionic villus samples (29). FISH may also be applied to buccal smear samples (30) where venous blood is unavailable for cytogenetic analysis, or to blood smears, where an extremely rapid result is required (31). The FISH technique has also provided a great deal of information about chromosome behaviour at meiosis. FISH allows normal and abnormal chromosomes to be tracked through all stages of meiosis (32) Rapid, direct analysis of large numbers of the chromosomal complements of sperm, has been successfully performed using FISH by Chantot-Bastaraud *et al* (33).

Thus, FISH related applications allow information to be mined irrespective of whether a single locus needs to be studied or the entire genome needs to be scanned. The following section gives details on the impact of FISH on certain disciplines.

Cytogenetics

Classic cytogenetics has evolved from black and white to technicolor images of chromosomes as a result of advances in fluorescence in situ hybridization (FISH) techniques, and is now called molecular cytogenetics. Improvements in the quality and diversity of probes suitable for FISH, coupled with advances in computerized image analysis, now permit the genome or tissue of interest to be analyzed in detail on a glass slide. It is evident that the growing list of options for cytogenetic analysis has improved the understanding of chromosomal changes in disease initiation, progression, and response to treatment. The contributions of classic and molecular cytogenetics provided scientists and clinicians alike with new avenues for investigation.

Small, submicroscopic, genomic deletions and duplications (1 kb to 10 Mb) constitute up to 15% of all mutations underlying human monogenic diseases. Novel genomic technologies such as microarray-based comparative genomic hybridization (array CGH) allow the mapping of genomic copy number alterations at this submicroscopic level, thereby directly linking disease phenotypes to gene dosage alterations. At present, the entire human genome can be scanned for deletions and duplications at over 30,000 loci simultaneously by array CGH (approximately 100 kb resolution), thus entailing an attractive gene discovery approach for monogenic conditions, in particular those that are associated with reproductive lethality. (34)

Fluorescence In Situ Hybridization (FISH) showed three signals for chromosome X (green) and two signals for chromosome 18 (blue) confirming the karyotype results of a structural anomaly - dicentric X.

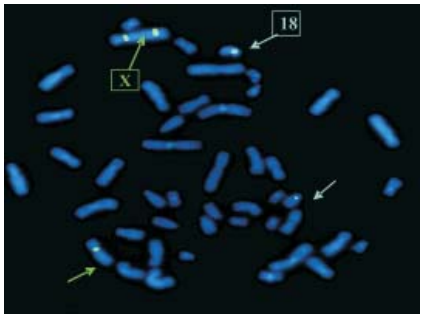


Plate – 2

Plate 2 shows the identification of a chromosome anomaly by FISH in a case of primary amenorrhoea referred to the Department of Human Genetics for cytogenetic investigation.

Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed three signals for chromosome 21 (orange) and two signals for chromosome 13 (green) indicating Trisomy 21 – Down's Syndrome.

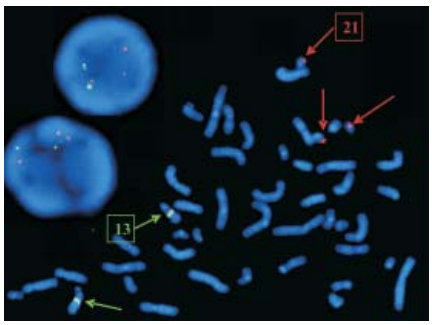


Plate – 3

Plate 3 shows the confirmation of Down's Syndrome – Trisomy 21 by FISH, in a paediatric patient referred to the Department of Human Genetics for cytogenetic investigation.

Prenatal Diagnosis

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. Numerical chromosome abnormalities are the major cause of inherited diseases with an incidence of 21% in spontaneous abortions. Of these, trisomies for sex chromosomes and chromosomes 13, 16, 18 and 21 account for 50% of chromosomally abnormal abortions. (35).

Prenatal diagnosis needs a rapid, accurate and overall genome analysis. FISH is a powerful tool for detecting some genetic diseases as well as microscopic or submicroscopic chromosome rearrangements in metaphases cells or interphase nuclei involving chromosomes commonly implicated in aneuploidies - 13, 18, 21, X and Y (36). Interphase FISH is very useful in urgent high-risk cases. The use of FISH overcomes the difficulties of conventional banding on metaphase spreads. The ability to generate accurate results in a few hours with FISH as compared to the two weeks typically needed for standard karyotype analysis has been instrumental in relieving the anxiety of many women, or in allowing them, their families and their physicians to make difficult decisions more swiftly. It is usually employed as an adjunctive tool to conventional cytogenetics (37).

Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed two signals for chromosome 21 (orange) and two signals for chromosome 13 (green) indicating no numerical anomalies associated with chromosomes 13 and 21.

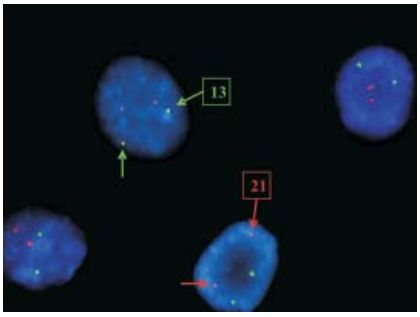


Plate – 4

Plate 4 shows human amniocytes from amniotic fluid probed by FISH in a case suspected as Down's Syndrome (Trisomy 21), referred to the Department of Human Genetics for prenatal diagnosis.

Preimplantation Genetics

Preimplantation genetic diagnosis (PGD) identifies genetic abnormalities in preimplantation embryos prior to embryo transfer. As mentioned earlier, the correlation between aneuploidy and declining implantation rates with maternal age demands screening of chromosome aneuploidies in human embryos by FISH using 13, 18, 21, X and Y probes should significantly reduce the risk of older patients undergoing in vitro fertilization (IVF) delivering trisomic offspring (35). PGD is being explored through polar body biopsy, biopsy of the single cell from the eight-cell embryo, and trophectoderm biopsy of the blastocyst (38). Interphase FISH using multi color, subtelomeric and centromeric probes is used to test single cells for structural or numerical chromosome abnormalities. It helps in identifying embryos free of specific genetic abnormalities (39). Results with PGD indicate a significant decrease in spontaneous abortions, from 81% before PGD to 13% after PGD (40).

Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed two signals for chromosome 21 (orange) and one signal for chromosome 13 (green) indicating that the blastomere biospied from the embryo was abnormal.

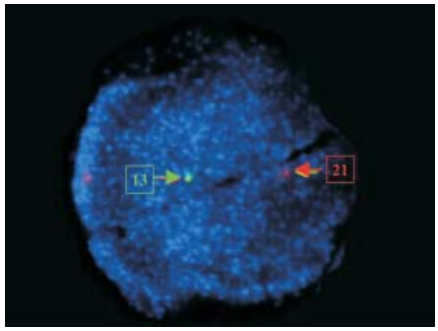


Plate – 5

Plate 5 shows FISH done on a blastomere obtained by embryo biopsy prior to IVF performed for research purpose.

Cancer Genetics

Chromosomal aberrations are found in cancer and tumor cell lines. Some of them are already characterized and correlated with specific syndromes and some of them have yet to be associated with a clinical outcome. Cytogenetic analysis is now a routine part of the diagnosis and management of a significant number of malignancies. Whilst conventional cytogenetics remains the most comprehensive method for assessing chromosome abnormalities, the technical difficulties associated with conventional cytogenetics in most cancers has resulted in increased use of FISH to identify specific abnormalities that are useful in either the diagnosis or management of these disorders. Aberrations such as aneuploidies, translocations, deletions, and gene amplifications are investigated in samples. This is accomplished using probes for centromeres, whole chromosome probes, and/or probes for specific aberrant sequences (41).

In chronic lymphocytic leukemia (CLL), genetic analyses by FISH and DNA sequencing have greatly improved the understanding of pathogenic events and prognostic markers (42). The combination of metaphase and interphase analyses and the investigation of specific structural aberrations by FISH have definitely made tumor diagnostics much rapid and accurate

Plate 6 shows the identification of the bcr-abl gene fusion as identified by FISH and typical of Chronic Myeloid Leukemia (CML), in a patient referred to the Department of Human Genetics for cytogenetic investigation.

Fluorescence In Situ Hybridization (FISH) of interphase cells showed a fusion signal (yellow) confirming the presence of Philadelphia chromosome in a patient referred for chronic myeloid leukemia (CML) with green / red signals representing normal chromosomes – 22 / 9.

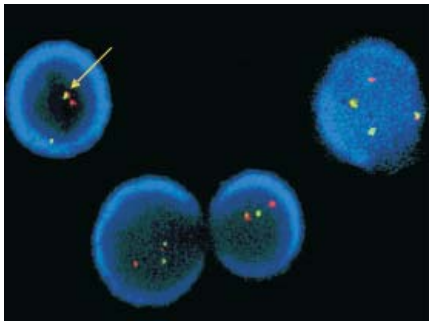


Plate - 6

Radiation biodosimetry

In almost every instance involving accidental, unexpected or suspected radiation exposure, biodosimetry comes to the picture. Biodosimetry involves the identification and scoring of certain biomarkers specific to and induced by radiation. To be useful, a biomarker for exposure and risk assessment should employ an end point that is highly quantitative, stable over time, and relevant to human risk (43). Biodosimetry is usually performed by enumerating the number of unstable chromosome aberrations – Dicentric Chromosomes and Centric Rings in peripheral blood lymphocytes of exposed individuals (44) Radiation induced unstable chromosomal exchanges are eliminated from the body within 1-3 years depending on the exposure condition. As a result, there is a considerable uncertainty in this dosimetry for past exposures (45, 46).

This is circumvented by scoring stable chromosomal exchanges such as translocations. Studies of the Japanese A-bomb survivors and patients receiving radiotherapy have shown translocations to persist in peripheral blood lymphocytes many years after exposure and repeated cytogenetic analyses have also indicated that the frequencies of cells with translocations remain unchanged (47). Thus, they are potentially a better indicator of cumulative dose.

The advent of Chromosome Painting (FISH) has revolutionized biodosimetry in simplifying the analysis of translocations. FISH assay not only makes the identification of translocations very easy, but also increases the sensitivity by its ability to score events, which conventional banding may fail to detect. The usefulness of this technique is demonstrated by its ability to resolve Complex chromosomal aberrations associated with high radiation doses, which are quite cumbersome and time-consuming to deduce by routine conventional cytogenetic techniques such as Banding by Trypsin and Giemsa (GTG Banding) (48).

Dual color FISH with whole chromosome and pan-centromere probes facilitates rapid detection of translocations. This approach allows analysis of translocations for assessment of genetic damage at long times after exposure or as a result of chronic exposure during a long period of time. Multi-color FISH with locus specific probes allows assessment of the frequency of cells carrying specific aberrations known to be associated with tumorigenesis, analysis of the series of genetic changes that occur during tumor evolution and correlation between genotype and phenotype (49).

The metaphase shown below shows complex chromosomal aberrations involving chromosome 1 (green) and chromosome 3 (orange), an indication of high doses of radiation exposure.

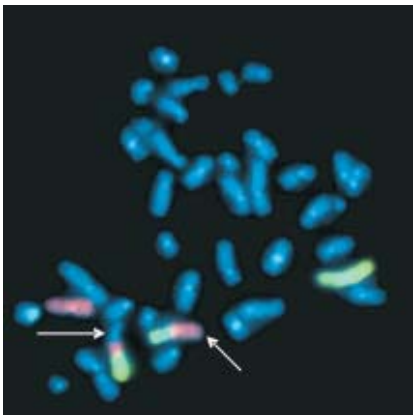


Plate – 7

Plate 7 shows some of the complex aberrations that have been identified by FISH (Whole Chromosome Painting) in peripheral blood lymphocytes exposed to gamma radiation.

FISH - past, present and future

Although the basic principles of FISH have remained unchanged, high sensitivity detection, simultaneous assay of multiple species, and automated data collection and analysis have advanced the field significantly. Efficiency and sensitivity have been improved by combining methodologies. In the future, this technique is likely to have significant further impact on live-cell imaging and on medical diagnostics.

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