Chapter 1

The Use of Molecular Cytogenetic Techniques for the Identification of Chromosomal Abnormalities

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Abstract

Chromosomal analysis is an increasingly important diagnostic procedure in numerous areas of clinical medicine that includes haematology, perinatology or obstetrics. Chromosomal disorders are viewed as a major category of genetic diseases, and sometimes the identification of abnormal chromosomes is not easily applicable. Just like the identification of the marker chromosome or the identification of the complex karyotypes is important in clinics for the evaluation of the patient prognosis as well as the treatment response, needless to say; fluorescence in situ hybridization (FISH) is the most suitable and rapid method in the above-mentioned situations. It gives chance to the rapid analysis of chromosomal aneuploidies in dividing and non-dividing cells. In this chapter, we will discuss the general principles of the chromosomal abnormalities and the molecular cytogenetic techniques that can help the identification of presence or absence of a particular DNA sequence or the evaluation of the number of organization of a chromosome or chromosomal region.

Keywords: FISH, chromosomal abnormalities, marker chromosome, molecular cytogenetics, cytogenetics

1. Introduction

A chromosome is the condensed version of the DNA, and it contains two sister chromatids. The critical parts are consisted of centromere, telomere and nucleolar organizing regions [1]. Depending on the mechanism, chromosomal abnormalities can be classified under two-major groups, numerical and structural abnormalities. The non-disjunction of chromosomes or anaphase lagging is the major cause of the numerical chromosome abnormalities. The structural abnormalities can be classified as balanced and unbalanced abnormalities. Balanced structural

abnormalities include translocations, insertions and inversions. Unbalanced structural abnormalities include deletions, duplications, marker and ring chromosomes [2, 3]. The identification of these numerical and structural abnormalities has an impact on the diagnosis of the syndromes, understanding of the phenotypic effects of chromosomal abnormalities, identification of the diagnosis and prognosis of haematological malignancies or solid tumours [3]. From that perspective, one can say that the identification of chromosome abnormalities has an important role in several conditions.

The diagnosis of chromosomal abnormalities is the most important factor in haematology, prenatal genetics and postnatal diagnosis of genetic conditions [4–6]. The identification of the chromosomal abnormalities in foetus is one of the most important thing in modern perinatology, or the identification of the BCR/ABL translocation in CML (Chronic Myeloid Leukaemia) is the most important diagnostic and prognostic factor in haematology. Chromosomal abnormalities involve the pathogenesis of several clinical conditions like infertility or hematologic malignancies and are important indicators for their diagnosis and prognosis [4, 5, 7]. There are a several methods that can be used to detect the genetic changes in genetic clinics include:

(a) conventional cytogenetics (karyotyping on cells derived from cell cultures using banding analysis; G-banding);

(b) molecular cytogenetics, e.g., fluorescence in situ hybridization (FISH), multicolour FISH, locus-specific FISH;

(c) molecular techniques to analyse DNA, RNA or proteins directly, e.g. the polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), quantitative real-time RT-PCR (RQ-PCR; qRTPCR), array CGH (comparative genomic hybridization), NGS (next generation sequencing), and microarray analysis [8].

Conventional cytogenetics is the golden standard and most comprehensive method to assess chromosomal abnormalities, especially numerical and structural chromosome aberrations. Technical issues like the need for fresh sample, difficulties in identification of masked or cryptic aberrations due to limited resolution by classic banding techniques have resulted in an increased use of molecular cytogenetic techniques, such as FISH, to identify specific abnormalities that are useful in either the diagnosis or management of hematologic disorders and are important tools for the identification of the cryptic translocations and sub-telomeric deletions in dysmorphic or mentally/developmentally retarded patients [8–12].

This chapter summarizes the structural abnormalities and the use of molecular cytogenetics as well as the identification of the chromosomal abnormalities.

1.1. Indications of the chromosome analysis in prenatal, postnatal ctyogenetics and haematological malignancies

Chromosomal analysis can be used as a golden standard for pre-natal and post-natal genetic diagnostic testing.

- In prenatal diagnosis, prenatal chromosomal analysis is applied during the following conditions.
- Presence of structural chromosomal or genome abnormality in one of the parents.
- Previous child with de novo chromosomal aneuploidy or another genomic imbalance.
- Higher maternal ages.
- Positive family history.
- Abnormal findings during the maternal serum screening.
- Abnormal USG finding.
- Stillbirth or neonatal deaths [2].

Postnatally chromosome analysis is applied during the following conditions:

- Fertility problems.
- Suspected syndrome identification.
- Mentally or developmentally retarded patients.
- Problems of early growth and development.
- Family history.
- Neoplasm [2].

Prognostic and predictive chromosome analysis in haematological malignancies:

Chromosomal abnormalities are important for the chromosomal and molecular changes as well as the identification of specific hematologic malignancies and syndromes and have important therapeutic and prognostic impacts, which include [13]:

- t(15;17)PML/RARa is characteristic for acute promyelocytic leukaemia (APL), that is a unique variant of acute myeloid leukaemia (AML) treated with ATRA and arsenic dioxide.
- t(8;21) or inv(16) comprises the favourable risk group of AML.
- Deletions of 5q, monosomy 7, deletion of 7q, trisomy 8, deletion of 9q, trisomy 11, trisomy 13, and trisomy 1 are the unbalanced abnormalities in AML.
- Acute myeloid leukaemia with associated abnormalities of 11q23 has an intermediate survival. The *MLL* gene at 11q23 is involved in a number of translocations with different partner chromosomes. The more common translocations observed in childhood AML are t(9;11)(p21;q23) and t(11;19)(q23;p13).
- The complex karyotype in AML predicts a poor prognosis.

- t(9;22)BCR/ABL is typical for chronic myeloid leukaemia (CML), although it may be seen in AML, where it is associated with a poor prognosis.
- In MDS, the typical abnormalities are partial and complete chromosome loss, most commonly −5, 5q−, −7, 7q−, +8, 11q−, 13q−, 20q−, and −Y [6, 14, 15].

Understanding the role of chromosomal abnormalities in the pathogenesis of haematological malignancies led to the development of a selective treatment options and gives prognosis information [13, 16–20].

1.2. FISH development

The development fluorescence in situ hybridization (FISH) technique increased the resolution of visualization of the chromosome rearrangements which is at the submicroscopic level [12, 21]. The FISH is applied on metaphase chromosomes, interphase nuclei, fixed tissues or cells and solid tumour samples [22]. The procedure contains denaturation, hybridization, post hybridization washes, which removes unbound single-strand DNA and after washing, an anti-fade solution containing DAPI (4′,6-diamidino-2-phenylindole) is applied to the slide, and a coverslip must be added (**Figure 1**) [23]. For FISH analysis, epifluorescence microscopes with specific filters and for identifying fluorochromes, a charge-coupled device (CCD) camera that captures the images were needed. A huge range of probes can be used for the identification of the chromosome abnormalities, which includes whole-chromosome painting probes, chromosome-arm painting probes, repetitive centromeric, subtelomeric and locus-specific

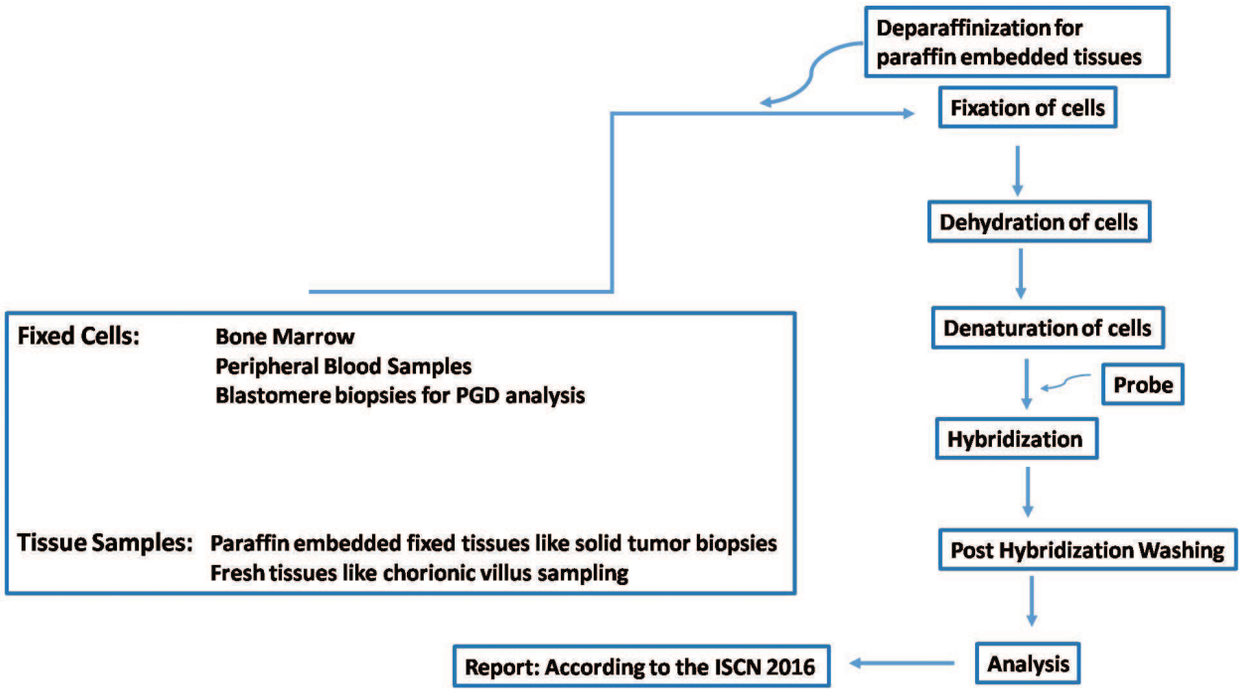

Figure 1. Algorithm for FISH analysis.

probes [24]. First, FISH probes obtained chromosome flow sorting [25] or microdissection [26] using universal degenerate oligonucleotide-primed PCR (DOP-PCR) [26].

There are different applications which used FISH-based methods like reverse-FISH [27], fibre-FISH [28, 29], M-FISH (multicolor FISH) [30], SKY (spectral karyotyping FISH) [31], flow-FISH [32], Q-FISH (quantitative FISH) [33], COBRA-FISH (combined binary ratio labelling FISH) [34], cenM-FISH (centromere-specific M-FISH) [35], podFISH (parental origin determination FISH) [36] and heterochromatin-M-FISH [37]. The most advanced FISH-based approaches included COBRA-FISH, M-FISH and SKY. These techniques give chance to the simultaneous visualization and the detection of all human chromosomes. These three FISH techniques use similar probes to be able to stain each of the 24 human chromosomes with a different colour [38].

Another high-resolution molecular cytogenetic technique for metaphase chromosomes, which gives chance to analyse chromosomes, is called multicolour banding (MCB). This technique involves the microdissection of chromosomal loci to obtain a set of probes that produce multi-colour pseudo-G-banding [39].

1.3. The use of FISH analysis in clinical diagnosis

Fluorescent in situ hybridization (FISH) was used for mapping human genes [40–43], and today, this technology is utilized for the characterization of chromosomal rearrangements and marker chromosomes [25, 44], the detection of microdeletions [45], and the prenatal diagnosis of common aneuploidies [46, 47], the detection of prognostic or predictive chromosomal abnormalities in haematological malignancies in clinical cytogenetic laboratories. At the same time, numerous DNA probes have been commercialized, further promoting the wide-spread clinical applications of molecular cytogenetic. Many new FISH techniques have been developed, including primed in situ labelling (PRINS [48]), fibre FISH [29, 49], comparative genomic hybridization (CGH) [50], chromosome microdissection [51, 52], spectral karyotyping (SKY [31]), multiple colour FISH (M-FISH [30, 53]), colour banding [54], FISH with multiple sub-telomeric probes [55], and array-based CGH [56, 57]. With the current FISH techniques, deletion or rearrangement of a single gene can be detected, cryptic chromosome translocations can be visualized, the copy number of oncogenes amplified in tumour cells can be assessed, and very complex rearrangements can be fully characterized. Using interphase FISH, genomic alterations can be studied in virtually all types of human tissues at any stage of cell division, without the need of cell culture and chromosome preparation. In that case, FISH is a unique technique that gives way to identification of numerical or structural chromosomal abnormalities in 1–3 days. The biggest advantage of the FISH technique is that it is more cost effective and labour intensive than the quantitative PCR (q-PCR) or other molecular genetics techniques.

Depending on the suspected genetic abnormalities type, the FISH probes can be generally subclassified into the following categories:

(a) Centromere-specific probes.

(b) Whole chromosome (‘painting’) probes.

(c) Single-copy (locus-specific) genomic probes.

(d) Spectral karyotyping (SKY; multiplex metaphase FISH; multi-colour FISH).

(e) Translocation fusion probes [58].

2. Identification of the translocations

Translocation involves the exchange of chromosome segments between two chromosomes [2]. The balanced reciprocal translocation carrier individuals are clinically normal; they do have an increased risk for having children with unbalanced karyotypes secondary to meiotic non-disjunction of their translocation [1]. In addition to being inherited, reciprocal translocations can also occur as new or de novo mutations and can be disrupt the proto-oncogenes and can cause uncontrolled cell division and cancer development.

The identification of translocations is mostly used for the evaluation of the haematopoietic malignancies. There are two types of probes, which are used to detect translocations: (single- or dual-) fusion probes and break-apart probes. A dual-fusion probe consists of a pair of probes labelled with two different colours (fluorochromes), green (e.g. FITC) and red (e.g. rhodamine), directed against translocation breakpoint regions in the two different genes involved in a reciprocal translocation. Variant and complex patterns may also be identified and provide additional clinical information on the underlying chromosomal changes. One locus is adjacent to another locus like in a normal cell, but the second pair is separated. This implies some type of rearrangement, which separated two loci that are usually found together, and this kind of probes was called break-apart. Commonly used BA probes in hematologic malignancies include MYC-BA (Burkitt lymphoma; BL), ALK-BA (anaplastic large cell lymphoma; ALCL) and IGH-BA (lymphoma/MM) [8].

Whole chromosome probes (WCPs; chromosome 'painting' probes) consist of numerous overlapping probes that recognize and bind to specific nonrepetitive DNA sequences along the entire length of targeted chromosomes. WCPs can be used to identify marker chromosomes (rearranged chromosomes of unidentified origin) or translocations that are otherwise not evident or difficult to interpret with routine banding cytogenetics. Whole chromosome probes do not give information about the deletion or inversions [8].

3. Duplications

The presence of an extra genomic copy of a chromosomal segment, which causes a partial trisomy, is called duplication. A duplication can be derived as a de novo duplication or as a consequence of the unbalanced chromosomal organizations like isochromosomes, dicentrics, derivatives, recombinants and markers [1, 3]. When the duplicated regions contain genes, genomic rearrangements involving the duplicated sequences can result in the deletion of the region between the copies and thus give rise to disease like 22q11.2 duplication or

the 15q11-q13 microduplication [3]. Most cytogenetically detectable tandem duplications in humans appear to be direct [59]. The phenotypes of the duplications are typically less severe than those associated with comparable deletions. Same as the deletions, the locus-specific FISH analysis should be applied when the duplication is suspected.

4. Deletions

The autosomal chromosome deletions can be detected by conventional, high-resolution or molecular cytogenetic methods and produce monosomies that are generally associated with significant disorders [1]. Deletions are classified into two groups: interstitial and terminal deletions. Due to the haploinsufficiency of the regions or the continuous gene deletions, the phenotypes of these patients are highly variable [2].

The deletions, which have a pathological significance, can be detected by routine methodology. Larger deletions have a more severe phenotype and associated with the major malformation than smaller ones. The gene continent of the deleted material is also important for the phenotypic severity of the patients and an important point in determining whether a specific deletion is viable [1]. The deletion of the chromosome segment can cause complex birth defects like Cri du chat syndrome, Wolf-Hirschhorn syndrome and DiGeorge Syndrome [2]. The locus-specific FISH analysis should be applied when the deletion is suspected. All stable chromosomes have telomeres at the end of the chromosomes. The sub-telomeric deletions were associated with the severe problems, which include mental retardation, developmental delay, and this terminal deletions cannot detect with the conventional cytogenetic techniques. The sub-telomeric FISH analysis is the appropriate technique to evaluate the abnormalities [10, 11, 21]. Some exceptions occur like loss of the short arm material from acrocentric chromosomes during the formation of Robertsonian translocations has no impact on phenotype [1].

5. Inversions

An inversion is an intrachromosomal rearrangement, which occurs when a single chromosome undergoes two breaks and is reconstituted with the segment between the breaks inverted. Two types of inversions occur: a paracentric inversion which both breaks occur in one arm and pericentric inversion which there is a break in each arm of the chromosomes [1]. The pericentric inversions can be easier to identify cytogenetically when they change the proportion of the chromosome arms as well as the banding pattern. The inversion does not usually cause an abnormal phenotype in carriers because it is a balanced rearrangement. The major problem of these patients is at risk for producing abnormal gametes that may lead to unbalanced offspring [2]. The breakpoints could be identified by visual inspection of the GTG image. However, further molecular cytogenetic analysis would be required to define the exact breakpoints. The locus-specific FISH is a suitable method, and also the break-apart FISH probes or m-banding is the useful molecular cytogenetics techniques for determining the inversions [60].

6. Complex chromosomal abnormalities

The complex chromosomal rearrangements (CCRs) involve two or more chromosomes, and at least three breakpoints are generally considered to be complex [61]. The greater the number of chromosome breaks and the higher the probability that an essential gene has been interrupted or that genetic material has been lost or gained during its formation. This CCRs are rarely seen in constitutional karyotypes and mostly seen in hematologic malignancies, and the identification of the structurally abnormal chromosomes is more important to be evaluated for the prognosis of haematological malignancies and important for the treatment response [62].

7. Identification of the marker chromosome

A marker chromosome is the extra structurally abnormal chromosomes in cytogenetics [2]. The precise characterization of marker chromosomes is important for prenatal and postnatal diagnosis and proper genetic counselling [63].

Mostly, the banding pattern of this abnormal chromosome does not permit for identification of the marker chromosome [62]. The chromosomal origin of marker chromosomes can be identified by using a combination of banding cytogenetics and molecular cytogenetic techniques including diverse fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (array CGH) (**Figure 2**) [63].

The small structurally abnormal chromosomes, which are called as supernumerary marker chromosomes (sSMCs), are generally equal or smaller in size than a chromosome 20 of the

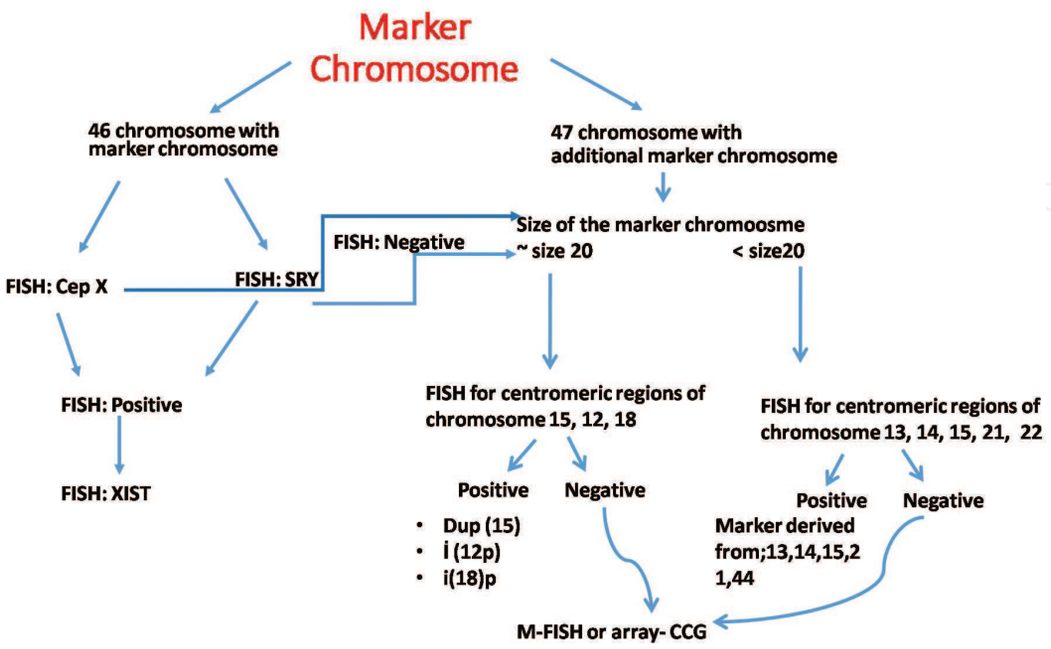

Figure 2. The possible algorithm chart for the identification of the marker chromosome.

same metaphase spread [64], and the chromosomal origin can be identified by conventional banding techniques, and molecular cytogenetic techniques are necessary for their characterization. **Figure 2** could be used for the identification of the marker chromosome.

Due to the effect of two or more chromosomal abnormalities, the conventional cytogenetics is limited to the identification. The m-FISH or the SKY is the best molecular cytogenetics choice to identify these CCRs.

8. Oncogenic amplifications

Amplification refers to the increasing copy number of a gene. Amplification causes gene upregulation of gene expression. The oncogenic amplification has described several solid tumours, which are often associated with progression, therapeutic response and prognostic markers of the cancer [65].

The oncogenes coded proteins, which have a role on control cell proliferation and programmed cell death. These oncogenes are activated by mutation, gene amplification and translocation. The oncogenic amplification mostly occurs in metastatic and low-differentiated tumours and reflects the genetic instability of solid tumour cell [66]. MYC, EGFR and RAS gene families are frequently amplified oncogenes in solid tumours. MYCN amplification in neuroblastoma or Her-2 amplification has been demonstrated, and the locus-specific probes allow accurate enumeration of each locus within individual nuclei [65]. Also, dual colour break apart rearrangement probes were used for the identification of these gene rearrangements [67].

9. Final remarks and conclusion

FISH techniques do not require mitotically active cells for evaluation of the chromosomal abnormalities, do not require culturing and allow disease monitoring in haematological malignancies. FISH can be applied on fixed, fresh tissue and on paraffin-embedded materials like paraffin-embedded solid tumours. It allows the analysis of a large number of cells. The SKY, M-FISH or COBRA-FISH techniques provide an overall evaluation of the whole genome. On the other hand, FISH analysis is not a screening test and cannot detect small intragenic mutations, deletions or insertions. Because it requires chromosome-specific FISH probes and generally less sensitive than the molecular genetics techniques.

Instead of array-CGH technology, in developed countries, the cytogenetic testing is the first line test in the diagnostic investigation detection of novel or rare chromosomal abnormalities like microdeletions, microduplications or trisomies. With the increased technology, array-based analysis like array comparative genome hybridization can help easily assess the relative copy number of genomic DNA sequences in a comprehensive, genome-wide manner, but the main disadvantage in aCGH is that it cannot detect translocation or balanced abnormalities, and in these conditions, FISH is important to determine the nature of the abnormality and its risk of recurrence.

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