Molecular Cytogenetics: techniques, developments and applications

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Abstract

There are many cytogenetic aberrations that are undetectable or unrecognizable by routine cytogenetics. The past decade has been an explosion in methodological advances in molecular cytogenetics technology. The cytogenetics techniques are changing from black and white to colors. Fluorescence *in-situ* hybridization (FISH) study has emerged as an extremely important tool for both basic and clinical research and diagnostic in leukemia and cancer. FISH can be used to identify chromosomal rearrangements, by detecting specific DNA sequences with fluorescently labeled DNA probes. Subsequently, newer FISH-based tests such as multicolor karyotyping, comparative genomic hybridization (CGH) and array CGH have a clinical potential as they enable resolution of complex karyotypic aberrations and global scanning of genomic imbalances, respectively. More recently, the cross-species array CGH analysis in cancer gene identification has also been demonstrated. This review summarizes the methodology and current utilization of these FISH techniques in unraveling chromosomal changes and highlights how the field is moving away from conventional method towards molecular cytogenetics approaches. Variant signal patterns of the clinical used FISH probes described here provide useful reference for clinical cytogenetics laboratories. In addition, the potential of the newer FISH developed tests in contributing information on genetic abnormalities will also be illustrated.

Key words: Molecular cytogenetics, Fluorescence *in-situ* hybridization, Multicolor karyotyping, Comparative genomic hybridization, Array CGH

Cytogenetics study is currently considered a mandatory investigation in newly diagnosed leukemia owing to its usefulness in disease diagnosis, classification and prognostication. The vast majority of recurrent cytogenetic rearrangements associated with leukemia are originally identified by conventional cytogenetics, which remains the standard laboratory test since this provides a global screen for abnormality and gives information on the total chromosomal complement. Although banding techniques represent the central theme at every cytogenetics laboratory, it is sometimes difficult to karyotype the tumor cells from a patient owing to unfavorable factors such as low specimen yield, low mitotic index, poor quality metaphases and other technical difficulties. In addition, it demands expertise and the interpretation of variant translocations or complex karyotypic configurations may challenge even the most experienced cytogeneticist. Therefore, cytogenetics analysis is not a standard diagnostic test in clinical laboratories. With the advent of fluorescence *in-situ* hybridization (FISH) technique, it is possible to detect both numerical and structural cytogenetic changes. It has managed to overcome many of the drawbacks of conventional cytogenetics. FISH assays are based

on the ability of single stranded DNA to hybridize to complementary DNA sequence. The procedure has evolved to the use of fluorescent labeled probes and is now a commonly employed procedure in routine diagnostic laboratory in the investigation of genetic changes in neoplastic cells. The provision of information for more accurate and specific diagnosis of malignant disorders has been a major contribution of molecular cytogenetics. Furthermore, the impetus for many of these FISH technology innovations has been the direct result of an increased understanding of the sequence, structure and function of the human genome, which has highlighted the intricate marvel of the DNA architectural blueprint housed within our chromosomes.[1,2] This review will summarize the development, current utilization, and the technical pitfall of molecular cytogenetics techniques in clinical and research laboratories. Furthermore, this article highlights how the techniques are moving away from conventional method towards molecular cytogenetics approaches.

1. Clinically useful FISH probe systems

Recently, there are a large number of already labeled commercial FISH probes of good quality available, rendering the technology accessible to clinical diagnostic laboratories. They also provide strong signal intensity with low background. The advantage of direct labeling for *in-situ* hybridization is that more than one probe may be used simultaneously, each labeled with different fluorochromes. Issues related to analytical sensitivity should be considered, especially with respect to disease monitoring for the post-treatment samples. It is advisable to subscribe to external quality assurance or proficiency testing programs, such as that operated by the College of American Pathologists (CAP), can also cater for laboratory performing FISH study. A molecular cytogenetics laboratory must establish standards for analysis and interpretation that comply with accreditation standards and that are appropriate for that laboratory.

In diagnostic laboratory, the most useful FISH probe systems are: 1) centromeric probes; 2) chromosome painting probes; and 3) locus specific probes for gene fusions. Centromeric probes hybridize to the alpha satellite repeats of chromosomes and are used for chromosomal enumeration. Changes in the copy number of chromosomes are determined, so that centromeric probes are applicable in demonstration of trisomy, monosomy and ploidy level abnormalities. Chromosome painting probes are designed to mark the entire chromosome of interest [Figure 1e], and are useful in deciphering cytogenetic aberrations that are difficult to resolve on morphological grounds, such as marker chromosomes of uncertain nature or complex changes.[3] However, small or cryptic rearrangements of <2-3 megabase (Mb) will not be uncovered using these probes. Locus specific probes hybridize to a unique sequence site in the human genome. Generally, they can be used in the detection of gene rearrangements as a result of chromosomal translocation, but also applied for the detection of gene amplification or deletion as well [Figure 1e]. Interphase analysis with FISH probes is used to detect amplification of *HER2* (v-erb-b2, erythroblastic leukemia viral oncogene homologue 2) in human breast cancer tissue sections, which identifies patients who might benefit from herceptin treatment.[4] In practical terms, FISH is considered the best approach for detection of *MYCN* (v-myc, myelocytomatosis viral related oncogene, neuroblastoma derived) amplification in childhood neuroblastoma [Figure 2a]. It can distinguish between *bona fide* low levels of the *MYCN* amplification from chromosome polysomy and copy number heterogeneity among tumor cells can be identified.[5] Interestingly, genetic heterogeneity of neuroblastoma might occur between primary tumor and bone marrow metastasis has also been documented.[5]

The initial design of locus specific probes in detecting chromosomal translocations employs the dual color single fusion system (S-FISH).[6] Typically, a probe labeled with one fluorochrome spans the 5'end to the translocation breakpoint of a gene and another probe labeled with a different fluorochrome span the 3' end of the breakpoint of the partner gene [Figure 2b]. In a metaphase or an interphase harboring the translocation, there is one signal each of the wild type allele and a

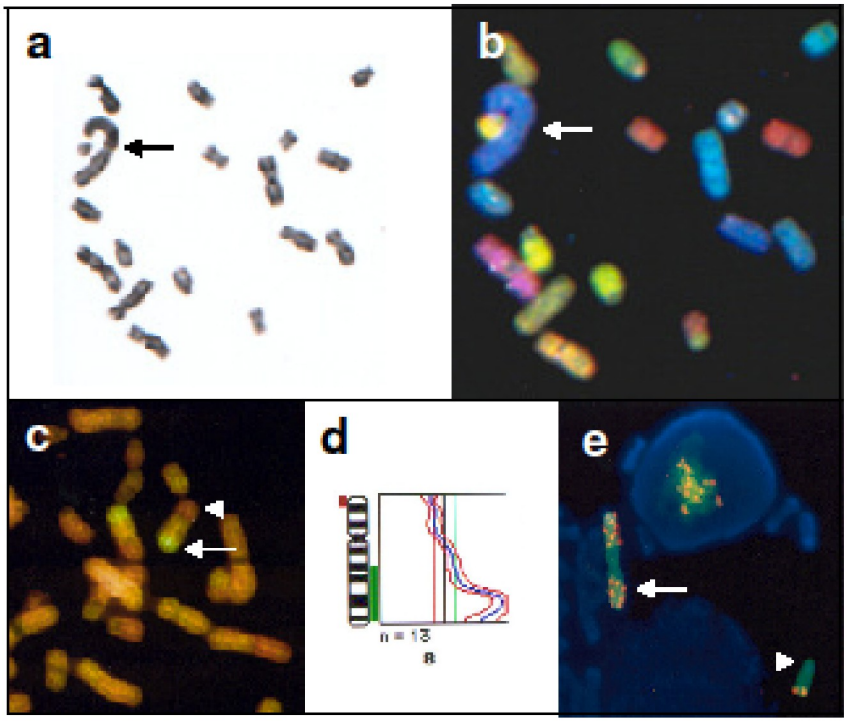

Figure 1. Cytogenetic characterization of a cancer cell line with various FISH-based approaches. (a) Partial G-banded metaphase showing a marker chromosome (arrow). (b) SKY image showing the derivative chromosome 8 (arrow). (c) In CGH analysis, showing amplified 8q sequences with green signal (arrow) and deletion of 8p sequences with red signal (arrowhead). (d) Average ratio CGH profile of chromosome 8, showing deletion of 8p and amplified 8q. (e) Metaphase FISH using whole chromosome painting probe (green) and *C-MYC* probe (red), which shows tandem duplication of *C-MYC* gene on both arms of the idup(8q) chromosome (arrow) and one *C-MYC* gene on the normal chromosome 8 (arrowhead).

fusion signal caused by juxtaposition of the fluorochromes as a result of gene fusion [Figure 2c]. However, the major drawback is false positive signal dual to close migration of two chromosomes or overlap signals by chance, especially in the detection of minimal residual disease and early disease relapse. In order to tackle this problem, the dual color signal fusion but extra signal (ES-FISH) system is subsequently developed.[6] The design is essentially the same as S-FISH but with a larger probe spanning upstream and downstream of the translocation breakpoint of one of the two genes involved in the fusion, so that an extra signal (diminished fluorescent intensity) is produced if the gene is disrupted, in addition to signals of the wild type alleles and the fusion signal [Figure 2d]. Recently, the advent of dual color dual fusion (D-FISH) probe represents a significant technological advancement in the monitoring of minimal residual disease and monitoring disease response to therapy.[7] Using the strict scoring criteria, and scoring at least 300 nuclei, it is now possible to further reduce the cut off level of false positive cells to 0.25%. When extended to the analysis of 6000 nuclei, the detection limit was improved to 0.079%. This system has gained popularity, in which large DNA probes span upstream and downstream of the translocation breakpoint of both fusion partners, so that in a positive metaphase or cell, there is one signal each for the wild type alleles and two fusion signals, one for the fusion gene and the other for the reciprocal product[6] [Figure 2e].

Strikingly, D-FISH system can easily identify chromosomal translocation variants with atypical signal patterns. Loss of DNA around the breakpoints of translocation has been observed in hematologic malignancies, notably deletions of

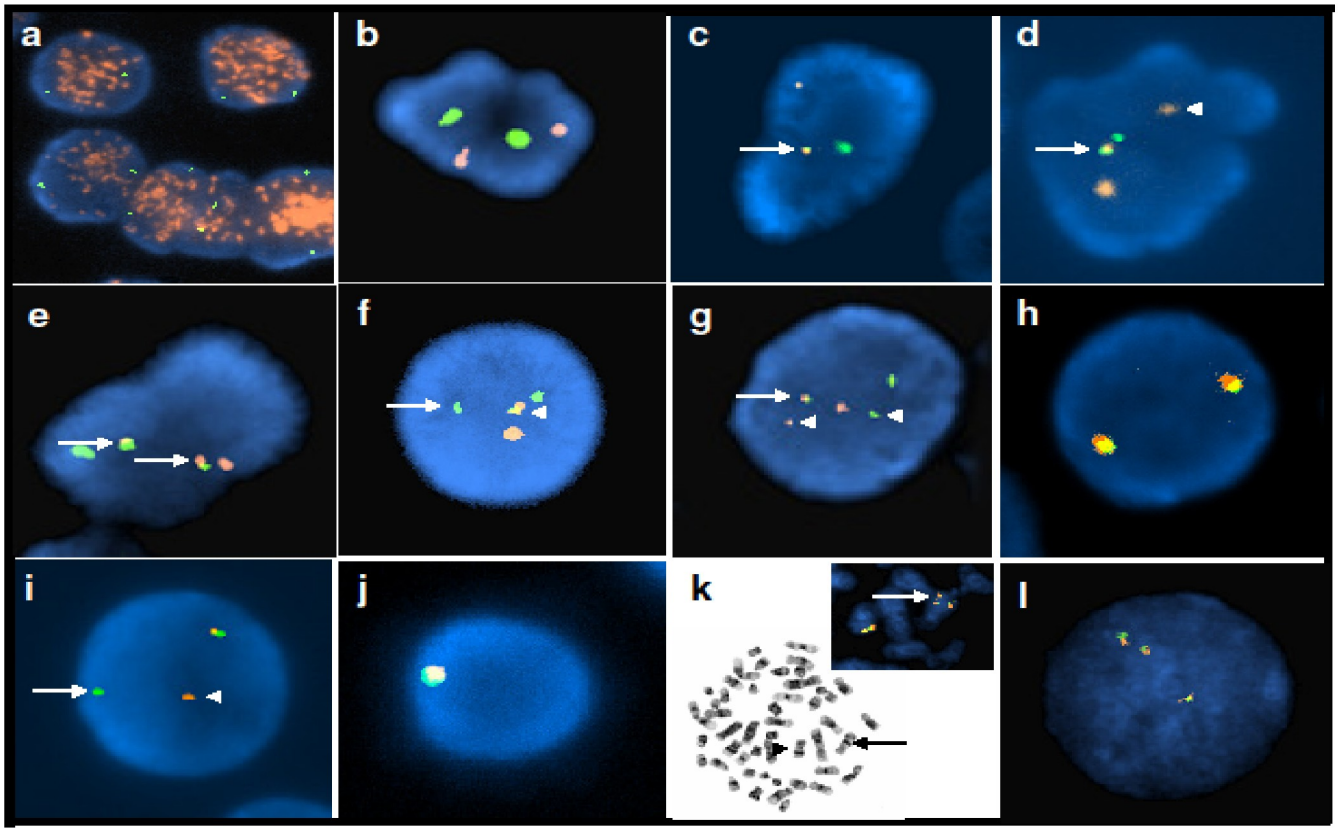

Figure 2. (a) *MYCN* amplification in neuroblastoma cells. The *MYCN* gene is labeled with a red fluorochrome, while the centromeric probe for chromosome 2 is labeled with a green fluorochrome. (b) Interphase FISH with *BCR/ABL* S-FISH probe, showing 2 green and 2 red signals in a normal cell. The *BCR* probe is labeled with a green fluorochrome, while the *ABL* probe is labeled with a red fluorochrome. (c) Interphase FISH with *BCR/ABL* S-FISH probe, showing a yellow fusion signal (arrow) in a Ph+ cell. (d) Interphase FISH with *BCR/ABL* ES-FISH probe, showing a yellow fusion signal (arrow) and extra red signal (arrowhead) in a Ph+ cell. (e) Interphase FISH with *BCR/ABL* D-FISH probe, showing two yellow fusion signals in a Ph+ cell (arrow). (f) Interphase FISH with *BCR/ABL* D-FISH probe, Ph+ cell harboring insertion of 5'*BCR* at *ABL* gene at 9q34 shows a yellow fusion signal (arrowhead) and a green 3'*BCR* residual signal (arrow). (g) Interphase FISH with *BCR/ABL* D-FISH probe, Ph+ cell harboring three-way translocation shows a yellow fusion signal (arrow) and two split 5'*ABL* and 3'*BCR* (arrowhead). (h) Interphase FISH with *MLL* break-apart FISH probe, showing 2 fusion signals in a normal cell. The 5'*MLL* probe is labeled with a green fluorochrome, while the 3'*MLL* probe is labeled with a red fluorochrome. (i) Interphase FISH using *MLL* break-apart FISH probe, *MLL* gene rearranged cell shows split signals: green signal 5'*MLL* (arrow) and red signal 3'*MLL* (arrowhead). (j) Interphase FISH using *MLL* break-apart FISH probe, deletion of one *MLL* allele or lose of chromosome 11 shows one fusion signal. (k) Metaphase showing normal chromosome 11 (arrow head) and dup(11)(q13q23) (arrow). Insert: Interphase FISH with *MLL* break-apart FISH probe, showing duplication of *MLL* gene on the chromosome 11 (arrow). (l) Interphase FISH with *MLL* break-apart FISH probe, showing *MLL* duplication (3 fusion signals).

derivative chromosome 9 adjacent to the Philadelphia (Ph) translocation breakpoint have recently been recognized in chronic myeloid leukemia (CML) using D-FISH system.[8] These deletions, found in around 15% of patients with CML, are large and occur at the time of the Ph translocation.[8] Thus, more recently a new method that incorporates an aqua-labeled probe for the *ASS* gene into the *BCR-ABL* D-FISH probe set has been introduced.[9] This tricolor D-FISH (TD-

FISH) method takes advantage of the *ASS* probe to distinguish between neoplastic and normal cells.[9] More recently, atypical FISH pattern in CML due to cryptic insertion of *BCR* to *ABL* gene at 9q34 has also been reported[10] [Figure 2f]. In addition, neoplastic cells with three-way translocation, involved 3-point break between three chromosomes, can also be identified using D-FISH probes [Figure 2g]. Using these atypical FISH patterns as an example, we have illustrated that, in clinical practice, atypical interphase FISH should not be interpreted in isolation, and should be integrated with information gathered through conventional cytogenetics, metaphase FISH, and if necessary molecular genetic studies.[11]

The advantage of FISH is for the detection of chromosomal translocations that are not amenable to polymerase chain reaction (PCR) detection due to widely distribution breakpoints such as *CBFβ* rearrangement, because FISH probes are much larger and hence there is better coverage of potential breakpoints over PCR analysis. Furthermore, for genes such as *MLL*, *TEL* and *RARα* that shows multiple translocation partners, the use of break-apart FISH probes gives important information on gene rearrangement, albeit unable to specifically incriminating the partner gene.[12] Typically, the dual color break-apart rearrangement probe labeled with one fluorochrome spans the 5'end to the translocation breakpoint of a gene and labeled with different fluorochrome spans the 3' end to the translocation breakpoint of a gene. The expected number of spots in a normal interphase nucleus is two fusion signals [Figure 2h]. In rearrangements involving the gene region the observed pattern will be one fusion signal and two split signals [Figure 2i]. The probe will also identify gene deletions as signal fusion with the loss of the other fusion signal, consistent with preservation of one allele and deletion of the other[13] [Figure 2j]. Furthermore, it will also identify gene amplification or duplication of the corresponding chromosome band, including the wild type gene[14] [Figure 2k]. The copy number of the fusion signal will be increased (>2) in these cases [Figure 2l].

Taken together, FISH has proven to be an essential tool that can be incorporated in most clinical cytogenetics laboratories. It is sensitive, rapid and critical complement to conventional cytogenetics.

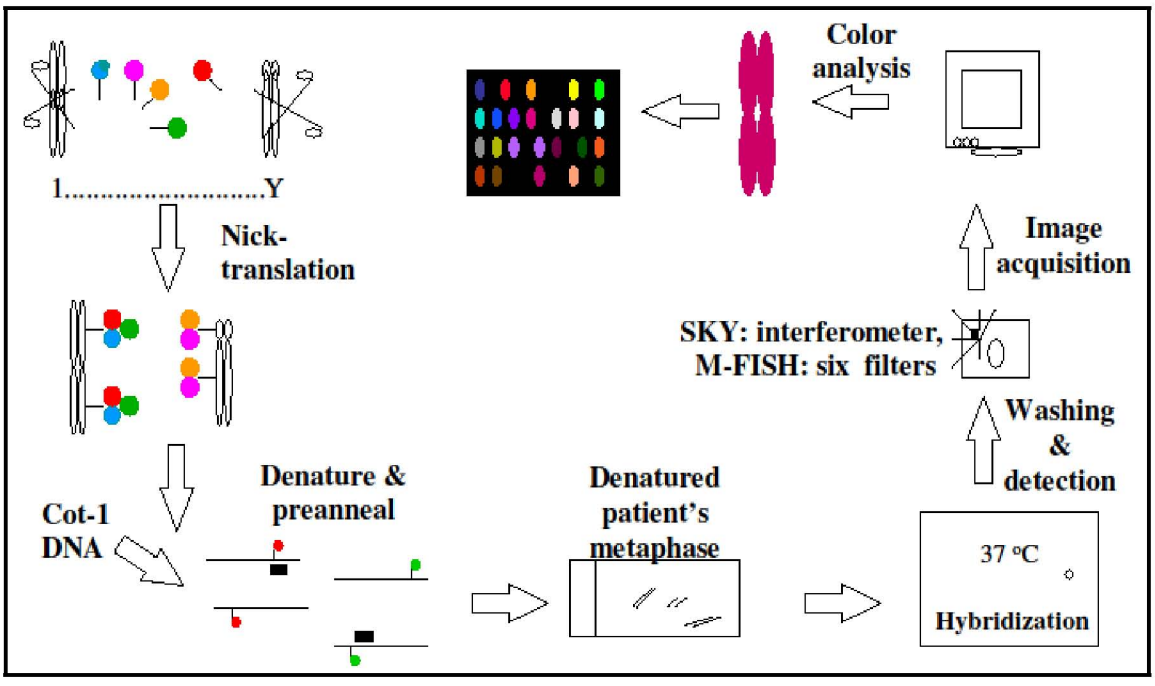

Figure 3. Flowchart summarizing the standard steps of multicolor hybridization *in- situ* hybridization.

2. Multicolor FISH

Multicolor FISH is based on the simultaneous hybridization of 24 chromosome-specific composite probes [Figure 3]. Multicolor FISH is suitable for identification of subtle chromosomal aberrations, such as the translocation of telomeric ends, which is difficult to detect using conventional cytogenetics alone, and the identification of an unidentified chromosome (marker chromosome) and an unbalanced chromosomal translocation, which remain elusive after conventional cytogenetics analysis. Regarding to probe design, such chromosome-painting probes are generated from flow-sorted human chromosomes. Chromosome-specific unique colors are produced by labeling each chromosome library either with a single fluorochrome or with specific combinations of multiple fluorochromes. Two multicolor fluorescence technologies have been introduced, multiplex FISH[15] (M-FISH) and spectral karyotyping[16] (SKY). The difference between the two techniques is in the image acquisition process: they employ different methods for detecting and discriminating the different combinations of fluorochromes after *in-situ* hybridization. In SKY, image acquisition is based on spectral imaging system using an interferometer and a charge-coupled device (CCD) camera [Figures 1a, 1b]. This makes possible the measurement of the entire emission spectrum with a single exposure at all image points and simultaneously measures the intensity for each pixel in the image at many different wavelengths.[17] In M-FISH, separate images are captured for each of the six fluorochromes using narrow bandpass microscope filters. The images are subsequently merged by dedicated software.[18]

Although the accuracy of SKY is shown to be high, with an average misclassification error of 1.3%, the error of even a few pixels could lead to an incorrect cytogenetics conclusion.[19] Our previous study showed that single fluorochrome labeled whole painting probe were more sensitive than SKY probes in a case of acute promyelocytic leukemia with cryptic *PML-RARα* fusion.[19] There are two possible explanations. Firstly, different labeling methods may account for the difference in sensitivity. Whole painting probe is labeled with a single fluorochrome, whereas SKY probes are labeled with mixtures of five fluorochromes. Therefore, the resolution of whole painting probe may be better than the SKY painting probes. Secondly, the green and red fluorescence signals that are appropriate for their detection. Therefore, whole painting probe may be detected the interstitial insertion of a small chromosomal fragment or single gene into another chromosome with greater ease than SKY.[17] Nevertheless, the limitations to these techniques include the inability to detect intrachromosomal aberrations such as inversions, duplications and deletions.[18, 19] Furthermore, color blending can cause the formation of additional visible bands at sites where chromosome overlap and at translocation breakpoints. FISH analysis is subsequently required to characterize whether or not these bands represent small insertions or just the result of color blending.

More specific multicolor FISH tests have been developed to facilitate the identification of an intrachromosomal rearrangement, such as cross-species color segmentation FISH[20] (Rx-FISH) or by use of human overlapping microdissection libraries that are differentially labeled[21] (multi-color banding, mBAND). These two techniques provide precise information on intra-chromosomal rearrangements and exact break-point mapping. Rx-FISH consists of the combinatorial labeling of probe sets made from the chromosomes of two gibbon species (*Hylobates concolor* and *Hylobates syndactylus*) and their hybridization to human metaphases. The success of this cross species color banding depends on a close homology (>98%) between host and human conserved DNA, divergence of repetitive DNA, and a high degree of chromosomal rearrangement in the host relative to the human karyotype. Hybridization of human chromosomes with painting probes derived from both gibbons showed that, with the exception of human chromosomes 15, 18, 21, 22 and the sex chromosomes, each chromosome was differentiated in at least two and up to six segments. Rx-FISH relies on color combinations arising from three fluorochromes, which provides 7 colors instead of the 24 colors of M-FISH or

SKY. Although this number of colors means that many chromosomal regions share the same color, the distribution of colors gives unique "color bar code" banding patterns for each homologous chromosome pair. These unique banding patterns help to overcome the color limitations and at the same time provide a guide to the localization of chromosomal breakpoints. To improve the resolution of the color banding technique, human overlapping microdissection libraries that are differentially labeled can be used as probes. Currently, mBAND reveals a banding pattern with approximate 550 bands in the normal haploid human karyotype. The striking advantage of mBAND over Rx-FISH approaches that use individually labeled Yeast artificial chromosomes (YAC) or Bacterial artificial chromosomes (BAC) is obvious: in order to obtain the same number of color bands, with the unique mBAND technique the complexity of the probe cocktail as well as the number of fluorochrome combinations is at least three times less as compared to the Rx-FISH approach.

Strikingly, distinct 3-dimensional organizations of chromatin in different tissue types should be addressed, as high-order chromatin arrangements are likely to have fundamental implications for development and cell differentiation. Therefore, more advanced and sophisticated software and hardware platforms to carry out multicolor 3D-FISH analyses in a high-throughput format.[21]

3. Comparative genomic hybridization (CGH)

CGH is a molecular cytogenetics technique based on quantitative two colors fluorescence *in-situ* hybridization[22] [Figure 4]. CGH allows, in a single experiment, to detect genetic imbalances in solid tumors or any desired test genome, and to determine the chromosomal map position of gains and losses of chromosomes or chromosomal subregions on normal reference metaphase preparations using a small amount of DNA. Briefly, tumor DNA (labeled green) and normal reference DNA (labeled red) are competitively hybridized to normal human metaphase spread. The reference DNA serves as a control for local variations in the ability to hybridize to target chromosomes. The relative amounts of tumor and reference DNA bound at a given chromosome are dependent on the relative abundance of those sequences in the two DNA samples. Digital image analysis gives a measurement of the ratio of green-to-red fluorescence along the chromosome on the reference metaphase spread reflect the

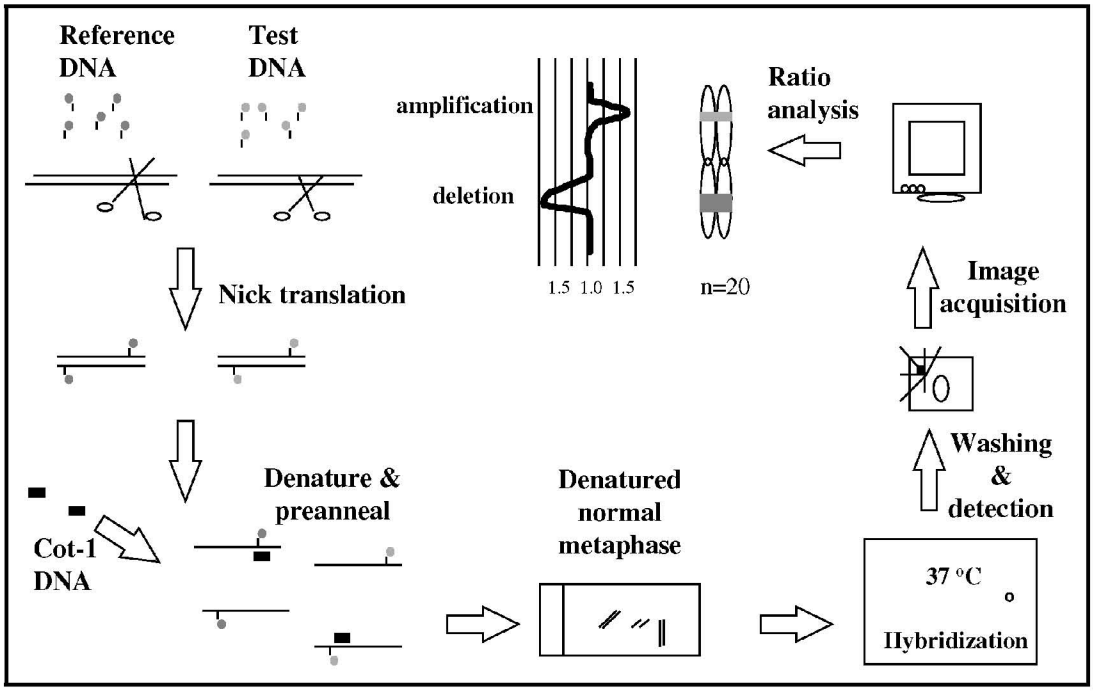

Figure 4. Flowchart summarizing the standard steps of comparative genomic hybridization (CGH).

copy number of corresponding sequences in the tumor DNA [Figure 1c]. If chromosomes or chromosomal subregions are present in identical copy numbers in both, the reference and the tumor genome, the observed fluorescence is a blend of an equal contribution of red and green fluorescence. If chromosomes are lost or chromosomal subregions deleted in tumor genome, the resulting color is shifted to red. A gain of a certain chromosome in the tumor would be reflected by a more intense green staining on the respective chromosome in the reference metaphase preparation [Figure 1d]. Subsequently, a genome-wide view of relative expression patterns within tissues according to chromosomal location a rapid approach, termed comparative expressed sequence hybridization (CESH) has been described.[23]

CGH has become one of the most widely used cytogenetics techniques in both basic research and molecular diagnostics. The distinct advantages of CGH is the fact that tumor DNA is the only requirement for this molecular cytogenetics analysis. Thus, archived, formalin fixed and paraffin embedded tissue can be used as well. It is applicable to cancer research especially for the low mitotic index of malignant cells and poor chromosome morphology and resolution. The scope of CGH has been extended to include the analysis of small amounts of DNA that have been obtained from small subregions of a specimen, such as microdissected tumor samples. This allows one to establish a correlation of the microscopic phenotype and the genotype in solid tumors. In addition, CGH offers a new experiment approach to study chromosomal aberrations that occur during solid tumor progression. The validity of CGH to delineate complex genetic changes in solid tumors has been investigated in several studies. However, the use of CGH is limited for the detection of chromosomal aberrations that do not involve genomic imbalances, such as inversions and balanced chromosome translocation. Currently, the possibility for the CGH technique to obtain resolution at the level of 2-4 Mb is noteworthy, provide that the experimental protocols are optimal. Amplifications of oncogenes and deletion of tumor suppressor genes are hallmark events of progressive malignancies. CGH technique has already made a significant impact on cancer cytogenetics, as a powerful tool for detection of chromosomal copy-number changes even in epithelial solid tumors where conventional cytogenetics techniques are pushed to elucidate tumor-specific genomic aberrations.[19] However, the constraints of CGH technique are its restricted resolution and escape from the detection of ploidy aberrations.

4. Array CGH

The development of array CGH technology for 'molecular karyotyping' with a resolution of 100 kilobase (kb) to 1 Mb, tremendous technical advances in cytogenetics have changed clinical diagnostic and research approaches.[24] The concept and methodology of array CGH (also called matrix CGH) is essentially the same as its traditional predecessor except that the template against which the genomic comparison is performed is no longer a normal metaphase spread. Array-based CGH greatly improves the resolution of the technique by substituting the hybridization targets, the metaphase chromosome spread, with genomic segments spotted in an array format. In order to comprehensively assess the genome and to identify the focal genetic events occurring during tumorigenesis, a whole genome tiling path array CGH approach must be employed. The genomic segments can be BAC or P1 artificial chromosome (PAC) clones for hybridization targets immobilized on glass slides as arrays. DNA arrays consisting 2,000 to 4,000 BAC clones representing the sequenced genome at approximately 1Mb intervals have been developed.[25] Using overlapping clones, the resolution of the array was increased beyond the size of a single BAC clone and gains and losses of regions as small as 40-80 kb are detectable [Figure 5]. Oligonucleotide arrays are also used in copy-number detection. These arrays contain 25-mer oligonucleotides originally designed to assess human single-nucleotide polymorphisms (SNPs). This method has the advantage of measuring allelic loss of heterozygosity (LOH) alongside copy-number changes using the same platform and it allows a sensitive and specific detection of single copy number changes at the

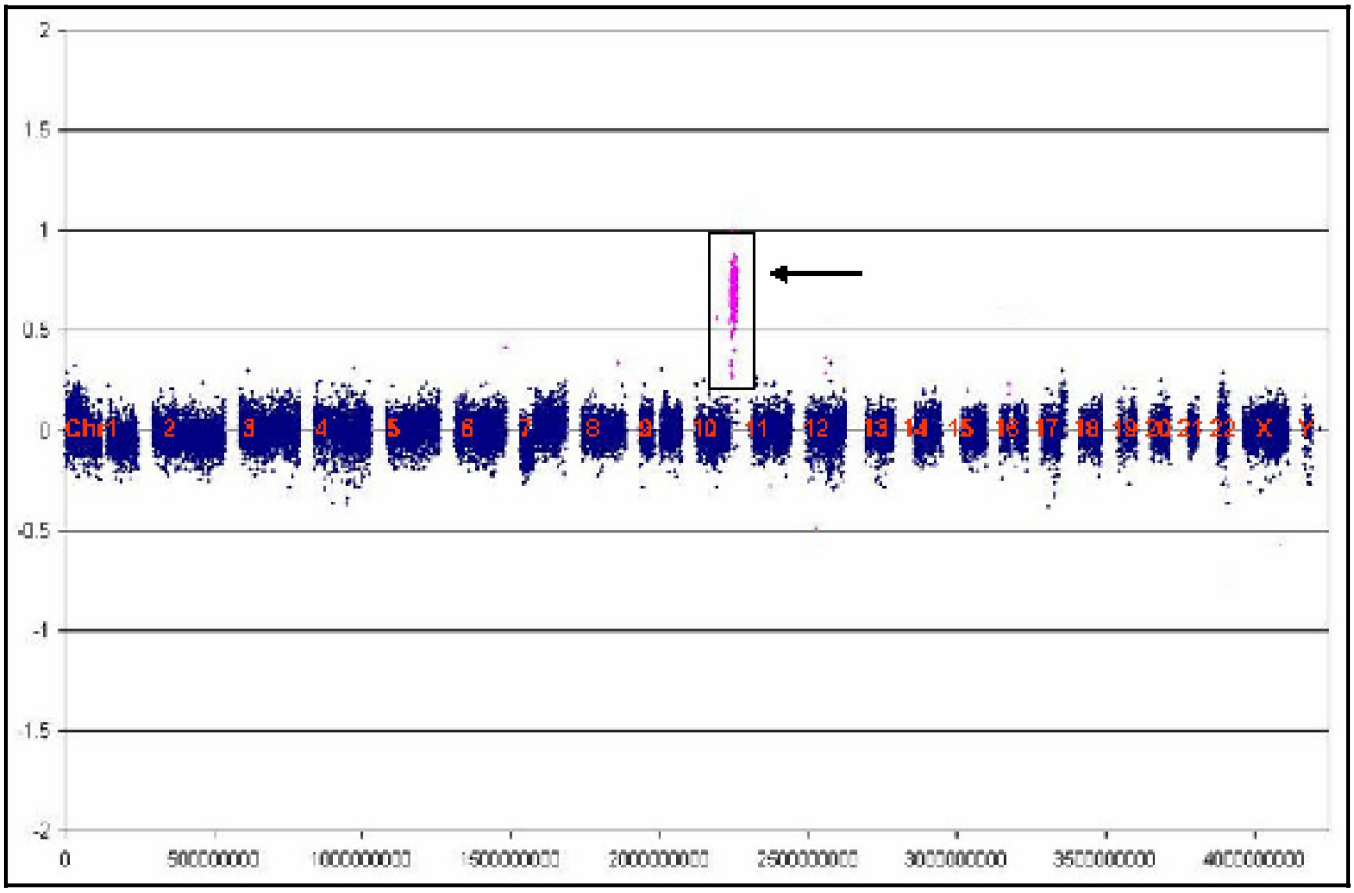

Figure 5. Using 1Mb whole genome tiling path array CGH approach, the patient shows amplification of 150 BAC clones in the long arm of chromosome 10 from 116383658.5 to 134016426.5 in the genome (arrow). Amplification or deletion of other chromosomal subregions is not detected.

submicroscopic level throughout the entire human genome.[25] More recently, copy number changes at a resolution of individual exons have been identified.[26]

Array CGH technology has much greater multiplexing capabilities than targeted FISH studies and offers much higher resolution for overall genomic screening than conventional cytogenetics studies; array CGH allows the recognition of deletions and duplications in the genome in a single experiment. Array CGH has been used for a variety of approaches. Some of the applications pertinent to cytogenetics laboratories, such as determination of general polymorphisms, characterization of acquired genetic changes, use in prenatal diagnostics, identification congenital genetic defects, and evolutionary characterization. More recently, ultra-high resolution array painting facilitates breakpoint sequencing of the derivative chromosome has been demonstrated, and therefore the precise breakpoint region can be easily mapped.[27] Furthermore, array CGH has provided important insights into aspects of normal genomic variation. Array CGH is one of these technologies that has recently revealed a newly appreciated type of genetic variation: copy number variation (CNV), in which thousands of regions of the human genome are now known to be variable in number between individuals.[28] Some of these copy number variable regions have already been shown to predispose to certain common diseases, and others may ultimately have a significant impact on how each of us reacts to certain foods, microscopic infections, medications, and other aspects of our ever-changing environment. Therefore, before applying array CGH in a diagnostic setting, a better knowledge of polymorphisms present in general populations required. More recently, cytogenetically balanced translocations are in fact frequently associated

with segmental gain or loss of DNA in prostate cancer cell lines have been reported.[29] It reveals that imperfectly balanced translocations in tumor genomes are a phenomenon that occurs at frequencies much higher than previously demonstrated.[29]

The resolution and coverage of array CGH are dependent on the density of the array used. An array covering the entire genome at very high resolution would have potential disadvantages in clinical and research use, however, more array probes are likely to generate a higher number of false positives. Large arrays are more expensive to quality control, fabricate, and interrogate. Large and very high resolution arrays are likely to generate information that may be difficult to interpret. Alterations in regions of the genome that do not have established clinical relevance will be burdensome to the clinical cytogeneticists for useful interpretation. Furthermore, this technique will not detect balanced rearrangements and low-level mosaicism for unbalanced numeric or structural rearrangements, and it does not exclude mutations in any gene represented on the array clones.

The complexity of genomic aberrations in most human tumors hampers delineation of the genes that drive the tumorigenic process. More recently, cognate mouse models recapitulate these genetic alterations with unexpected fidelity have been demonstrate.[30] These results indicate that cross-species array CGH analysis is a powerful strategy to identify the responsible genes and assess their oncogenic capacity in the appropriate genetic context.[30]

5. Future Prospects and concluding remarks

In the past decades, innovative technical advances in the field of cytogenetics have greatly enhanced the detection of chromosomal alterations and have facilitated the research and diagnostic potential of cytogenetics studies in constitutional and acquired diseases. The filed of molecular cytogenetics has expanded beyond the use of FISH to other techniques that are based on the principle of DNA hybridization. The considerable gap in resolution conventional cytogenetics techniques (5-10 Mb pairs) and molecular biology techniques (base pairs) has been to a large extent by FISH, which allows the assessment of genetic changes on chromosome preparations. Some noteworthy innovations that have altered the landscape of clinical and research investigations include the use of various targeted FISH techniques, the use of multicolor FISH to identify the chromosomal alterations unresolved by karyotyping, and yet another major advancement of chromosomal CGH which offers genome wide screening by determining DNA content differences and characterizing the chromosomal imbalances even when fresh specimen and chromosome preparations are unavailable. More recently, the development of array CGH, which allows the detection of much smaller genomic imbalances, involves the use of an ordered set of defined nucleic acid sequences derived from various sources, immobilized on glass slides.

Currently, array CGH approach is poised to revolutionize modern cytogenetic diagnostics and to provide clinicians with a powerful tool to use in their increasingly sophisticated diagnostic capabilities. More recently, the cross-species array CGH studies described testify to the notion that genetically tractable mouse models represent an invaluable tool not only to identify new cancer-causing genes but also to assess the context-dependent vulnerability of tumors to multitarget intervention strategies.[30] However, all gains and losses identified on the array CGH should be validated by FISH or molecular confirmation analysis. Taken together, the goal of the molecular cytogenetics laboratory is to identify the type of techniques that are most useful and informative for a particular study, prepare quality experimental materials, and perform a thorough analysis to arrive at an interpretation useful for research and diagnostic works.

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