



Advanced molecular cytogenetics in human and mouse

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Fluorescence *in situ* hybridization, spectral karyotyping, multiplex fluorescence *in situ* hybridization, comparative genomic hybridization, and more recently array comparative genomic hybridization, represent advancements in the field of molecular cytogenetics. The application of these techniques for the analysis of specimens from humans, or mouse models of human diseases, enables one to reliably identify and characterize complex chromosomal rearrangements resulting in alterations of the genome. As each of these techniques has advantages and limitations, a comprehensive analysis of cytogenetic aberrations can be accomplished through the utilization of a combination approach. As such, analyses of specific tumor types have proven invaluable in the identification of new tumor-specific chromosomal aberrations and imbalances (aneuploidy), as well as regions containing tumor-specific gene targets. Application of these techniques has already improved the classification of tumors into distinct categories, with the hope that this will lead to more tailored treatment strategies. These techniques, in particular the application of tumor-specific fluorescence *in situ* hybridization probes to interphase nuclei, are also powerful tools for the early identification of premalignant lesions.

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Identification of recurrent chromosome rearrangements plays a critical role in the diagnosis and prognosis of malignancy [1]. Leukemias and lymphomas are frequently characterized by recurrent chromosome breakpoints [2]. The identification of specific translocations in many of these malignancies has made the cloning of specific genes that undergo deregulation or are spliced together resulting in the production of a fusion protein possible. Both of these genetic alterations induce malignancy. In contrast, most solid tumors are defined by a specific pattern of chromosome gains and losses that are tumor type specific [2].

Conventional cytogenetics and molecular biology have each played an important role in the identification of such chromosomal aberrations. The advent of differential staining techniques has permitted more precise identification of chromosomes based on their banding patterns [3–5]. Since then, these techniques have been widely used to characterize cytogenetic abnormalities in tumor cells. The identification of either genomic imbalances or translocation

junctions has been used as an entry point for the identification of cancer-causing genes [1]. However, the sometimes poor quality of metaphase chromosomes, contamination of specimens by fibroblasts, selective growth of subclones not representative of the *in vivo* tumor, the inability to obtain mitotic cells and the sheer number of complex cytogenetic abnormalities have all complicated the accurate analysis of tumor genomes. Genetically engineered mice have also been increasingly employed as a model system for human cancer. Identifying and arranging mouse chromosomes into a karyotype, even in normal nontumorigenic cells, is complicated by the fact that mouse chromosomes are all acrocentric (i.e., the centromere resides at one end of the chromosome rather than interstitially as for most human chromosomes) and are relatively uniform in size. Thus, the above limitations in the use of conventional cytogenetic and molecular biologic techniques make it clear that further development of cytogenetic tools would facilitate the comprehensive identification of chromosomal aberrations.

CONTENTS

Fluorescence *in situ* hybridization

Spectral karyotyping

Comparative genomic hybridization

Expert opinion

Five-year view

Key issues

References

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The introduction of molecular cytogenetic techniques such as fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) and spectral karyotyping (SKY)/multifluor FISH (M-FISH) has helped overcome some of the limitations of chromosome banding techniques [6–10]. For example, FISH has permitted the identification of translocations that were not previously observed by banding [11]. FISH hybridization can also be applied to interphase nuclei [12], enabling one to screen large numbers of cells and identify copy number changes in a small subpopulation, thus providing the opportunity to identify early lesions [13]. Likewise, the application of CGH to solid tumors has led to the identification of recurring patterns of genomic imbalances, both for different tumors and for distinct tumor stages [14,15]. More recently, SKY has emerged as a powerful screening tool for interchromosomal structural aberrations and has aided in the characterization of complex tumor genomes [16–18]. This review will address these three techniques in more detail, discussing their practical application to understanding chromosome alterations in cancer as well as their inherent technical limitations. It is hoped that after completing this chapter, the reader appreciates the potential contained in the combined application of these advanced molecular cytogenetic techniques for deciphering the complex chromosomal rearrangements in cancer genomes.

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 (FIGURE 1). One methodology for labeling a nucleic acid probe, be it DNA or RNA, is enzymatically via either random priming or nick translation incorporation of a fluorescent molecule or immunogenic hapten-conjugated nucleotide analog. Direct chemical labeling can also provide excellent probes [301]. More recently, peptide nucleic acid (PNA) molecules have been developed, and these can also serve as probes [19]. The hybridization target can be either RNA or denatured, single-stranded DNA. Once the probe has been given sufficient time to anneal to its complementary target sequence, excess probe molecules are washed away and the hybridization pattern is visualized with a fluorescence microscope. Of note, hapten-labeled probes require detection with fluorescent-conjugated antibodies. A particular challenge of FISH is the ability to detect small

DNA target sequences (i.e., less than 1–3 kb). New signal amplification techniques, such as the use of tyramides [20–23] or rolling circle amplification [24–26], have been developed to increase signal intensities derived from small DNA targets previously undetectable by traditional approaches. However, these methods require considerable optimization and have not been widely used.

Probe types

The earliest applications of FISH utilized DNA probes specific for repetitive DNA sequences, such as those found at centromeres and other heterochromatic regions, as they generated a very intense signal due to the tandem arrangement of complementary sequences, and thus a very large target size [6,27,28]. The probes consisted of cloned genomic repetitive sequences. More recently, oligonucleotide or PNA probes have been designed that are specific for these genomic regions [19]. Unlike the aforementioned DNA repeat probes, a locus-specific probe (LSP) consists of a repeat-free labeled nucleic acid sequence specific to a single region of the genome. With the recent

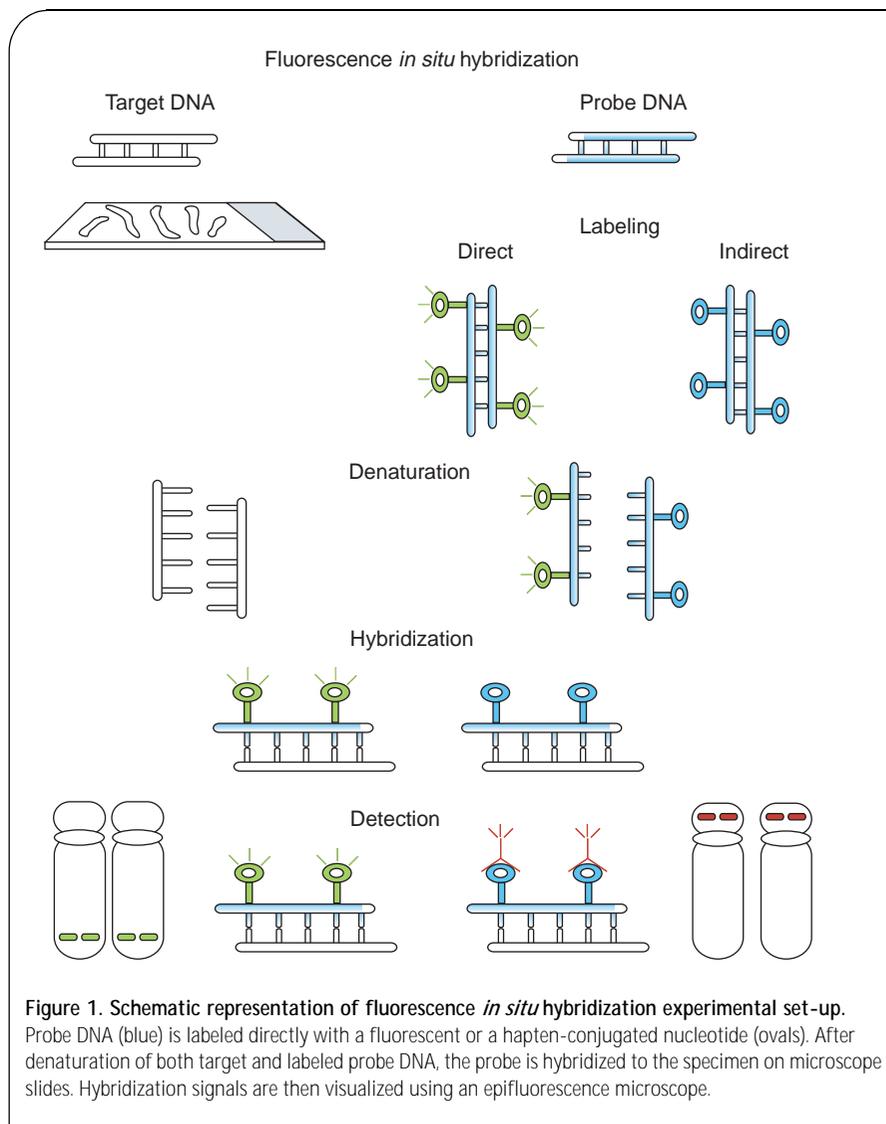


Figure 1. Schematic representation of fluorescence *in situ* hybridization experimental set-up. Probe DNA (blue) is labeled directly with a fluorescent or a hapten-conjugated nucleotide (ovals). After denaturation of both target and labeled probe DNA, the probe is hybridized to the specimen on microscope slides. Hybridization signals are then visualized using an epifluorescence microscope.

sequencing of genomes from numerous organisms, including human and mouse, the specific map location and availability of bacterial artificial chromosome (BAC) clones has greatly facilitated the acquisition of locus-specific FISH probes and systematic probe collections have been assembled [Ccap 2011].

FISH probes for larger genomic regions such as entire genomes [29], chromosomes [30,31], chromosome arms [32] and bands [33] are also often used. Whole chromosome painting (WCP) probes originally consisted of a chromosome-specific library derived by the cloning of a flow sorted human chromosome phage libraries [34–39]. These were first utilized as FISH probes in 1988 [40,41]. Another related FISH application involved the labeling of an entire human–hamster and mouse–hamster hybrid cell line genome (which contained one nonhamster chromosome) and hybridizing it back onto human or mouse metaphase spreads, respectively [42–45]. Chromosome-specific painting probes are now typically produced through degenerate oligonucleotide primed (DOP)-PCR labeling of flow-sorted chromosomes [30]. The use of differentially labeled WCPs in combination, which was first reported by Nederlof and colleagues [46], led to the development of SKY [8,9] and M-FISH [10] as a means of simultaneously delineating each individual chromosome in metaphase spreads in different colors. These methodologies are discussed in more detail later.

Applications & limitations

One application of FISH involves the hybridization of probes to interphase cells (FIGURE 2A). This is extremely beneficial when it is not possible to prepare metaphase spreads. For instance, one may wish to avoid potential artifacts associated with prolonged culturing of cells from a primary tumor or to screen a large number of cells in search of an extremely rare subpopulation [47,48]. Also, it is not always possible to establish primary tumors in tissue culture. 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 biologic and clinical end points [12,49]. Interphase cytogenetics also permits 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. The identification of numerical and structural chromosomal aberrations in interphase nuclei has the added benefit of enabling the simultaneous

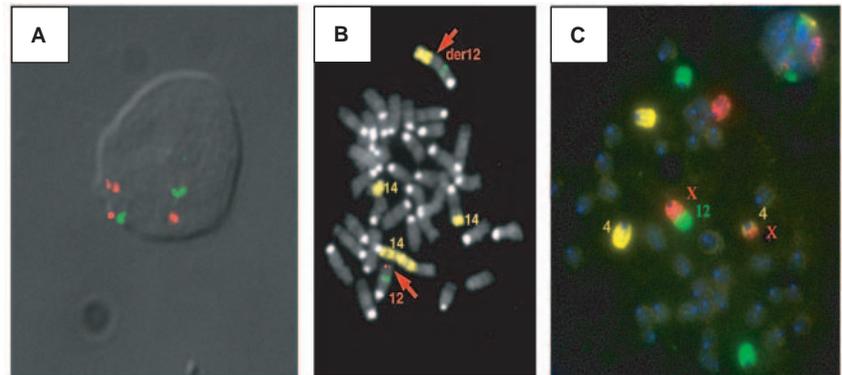


Figure 2. (A) Centromere-specific probes used for detection of chromosome copy number in interphase nuclei. Probes specific for the centromeres of two different chromosomes were hybridized to interphase nuclei. The green probe is present in two copies while the red probe indicates trisomy for this chromosome. The doublet observed for one of the green and one of the red signals is due to either the decondensation of centromere repeat regions of the genome in interphase or the fact that this particular cell is in S-phase and has already replicated these regions. (B) Locus-specific probes for mouse chromosomes. Bacterial artificial chromosome clones specific to single copy regions of the genome have been labeled with either Rhodamine110-dUTP (green) or digoxigenin-dUTP and subsequently detected with mouse antidigoxigenin followed by antimouse TRITC (red). Green signals represent hybridization to mouse chromosome 12D1 and red to band 12E. A chromosome 14 painting probe (yellow) was used to identify the translocation partner. The normal chromosome 12 (bottom arrow) shows hybridization with both chromosome 12 locus-specific probes (green and red) while the derivative 12 (top arrow) shows loss of the 12E region probe (no red signal) which occurred during the rearrangement with chromosome 14 (yellow paint). Also of note is the fact that there are no normal chromosome 14 in this metaphase, all having been either deleted (left) or rearranged (remaining three partially painted chromosomes). (C) Chromosome painting probes specific for mouse chromosomes 4 (yellow), 12 (green) and X (red). The chromosome painting enabled the identification of two translocations: T(X,12) and T(4,X).

assessment of chromosomal aberrations, cellular phenotype and tissue morphology [50,51].

Centromere-specific probes were first used to identify copy numbers of chromosome 18 in nuclei from normal cells and cells with a trisomy for chromosome 18 [12]. However, this approach only selects for a very small subset of chromosomes. From a strict cytogenetic standpoint, centromere-specific probes can only be used to score centromere copy numbers. Thus, locus- or chromosome region-specific probes are extremely valuable for assessing copy number changes involving chromosomal arms, a few chromosomal bands or specific genes. These subchromosomal copy number changes are often seen in solid tumors and exist as deletions, duplications, and double minute chromosomes on the cytogenetic level.

LSPs are also very useful in identifying or further characterizing structural aberrations (FIGURE 2B). For example, reciprocal translocations can be detected with LSPs that span the breakpoint region [52,53]. Such translocations can alter the expression of certain oncogenes and tumor suppressor genes, giving tumor cells a selective growth advantage. For example, tumor cells can be detected using LSPs for the *bcr/abl* translocation involving genes on human chromosomes 9 and 22 in chronic myelogenous leukemia [52] or the translocation of the *c-myc* gene to the immunoglobulin H locus in Burkitt's lymphoma [53]. Probe kits for the detection of above mentioned breakpoints and other relevant cytogenetic abnormalities are available from commercial sources.

WCP is useful for detecting translocations between non-homologous chromosomes (FIGURE 2C). WCPs hybridize along the entire length of the chromosome, and the hybridization of repetitive sequences is blocked by the addition of excess Cot-1 DNA. Painting experiments involving the use of only a few such probes are often used to confirm a suspected chromosomal rearrangement. Chromosome painting is also useful in the identification of translocations involving large genomic regions, while LSPs or collections of probes spread along the target in close proximity and can be used for more detailed mapping.

FISH probes have been widely used for telomere shortening detection. Among these are techniques such as primed *in situ* (PRINS) labeling or FISH with RNA-translated or nick-translated, double-stranded DNA (dsDNA) repeat probes. Long oligonucleotides have proven effective telomere FISH probes. FISH with short PNA telomere probes yields

detection efficiencies of almost 100% and, in combination with digital fluorescence microscopy, permit the assessment of repeat numbers at individual chromosome ends [54].

Spectral karyotyping

Methodology

SKY is a molecular cytogenetic technique that permits differential visualization of all human or mouse chromosomes in distinct colors with a single hybridization and image exposure (FIGURE 3) [9,55,56]. SKY utilizes a combination of Fourier spectroscopy with epifluorescence microscopy and charge-coupled device (CCD) imaging [57]. Human and mouse single chromosome painting probes are generated from flow-sorted chromosomes by DOP-PCR [58]. The probes are then PCR labeled through the incorporation of either haptenized or directly labeled nucleotides such that each labeling reaction

contains only one labeled dUTP (e.g., biotin-dUTP, digoxigenin-dUTP or Rhodamine 110-dUTP). Using five spectrally distinct fluorochromes, either alone or in combination, allows one to discern 31 different targets. The chromosome-specific probes are subsequently pooled together, repetitive sequences are suppressed with excess Cot-1 DNA in the hybridization mixture, and the probes are hybridized onto metaphase chromosomes. Hybridized chromosomes can be visualized using an epifluorescence microscope equipped with a single, custom-designed triple bandpass filter that permits the simultaneous excitation of all the fluorochromes. In addition, the entire emission spectrum of a metaphase, ranging between 400 and 800 nm, can be measured in a single exposure. A single image containing spectral information for each image point is acquired, and the fluorescent intensities in the green, red, and near infrared emission range are visualized in a standard red, green, blue (RGB) display image (FIGURE 4A) [8,9]. An image of the 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) counterstain is used for chromosome banding identification (FIGURE 4B). All pixels with the same spectral information are assigned a pseudocolor, which allows for the spectral classification of all chromosomes. After the chromosomes are classified and aligned in a karyotype table, interpretation and comparison of all aberrations is summarized in the karyogram (FIGURE 4C) [8,9]. M-FISH differs from SKY in that it is only a filter-based

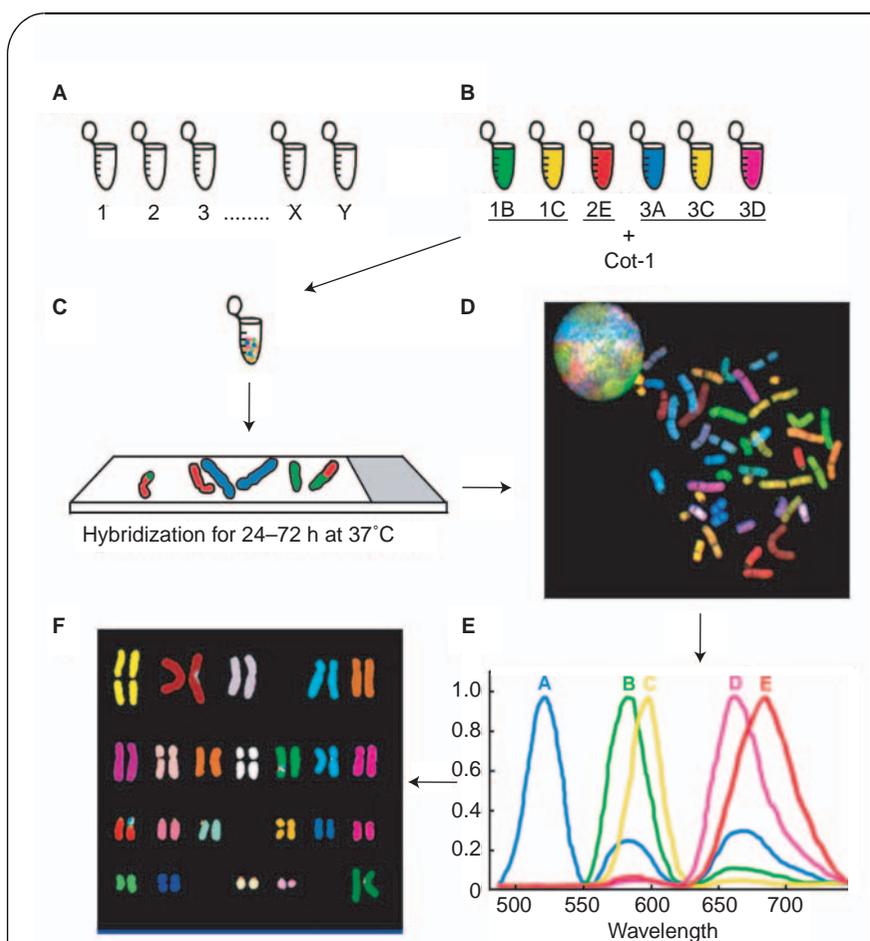


Figure 3. Schematic representation of spectral karyotyping hybridization experimental set up. (A) Flow sorting is used to isolate individual chromosomes. (B) Each chromosome is then labeled with a unique combination of fluorescent dyes or haptens. (C) The hybridization cocktail (a combination of these differentially labeled chromosome painting probes and Cot-1 DNA to suppress the hybridization of repeat sequences) is hybridized to tumor metaphase preparations. (D) After washes and detection of the hapten-labeled probes, the metaphases are imaged using a fluorescent microscope. (E) A spectrophotometer in the imaging system generates a spectral signature for each pixel in the image. (F) Each pixel is assigned a classification color based on its spectral signature. Each normal chromosome is therefore a single color.

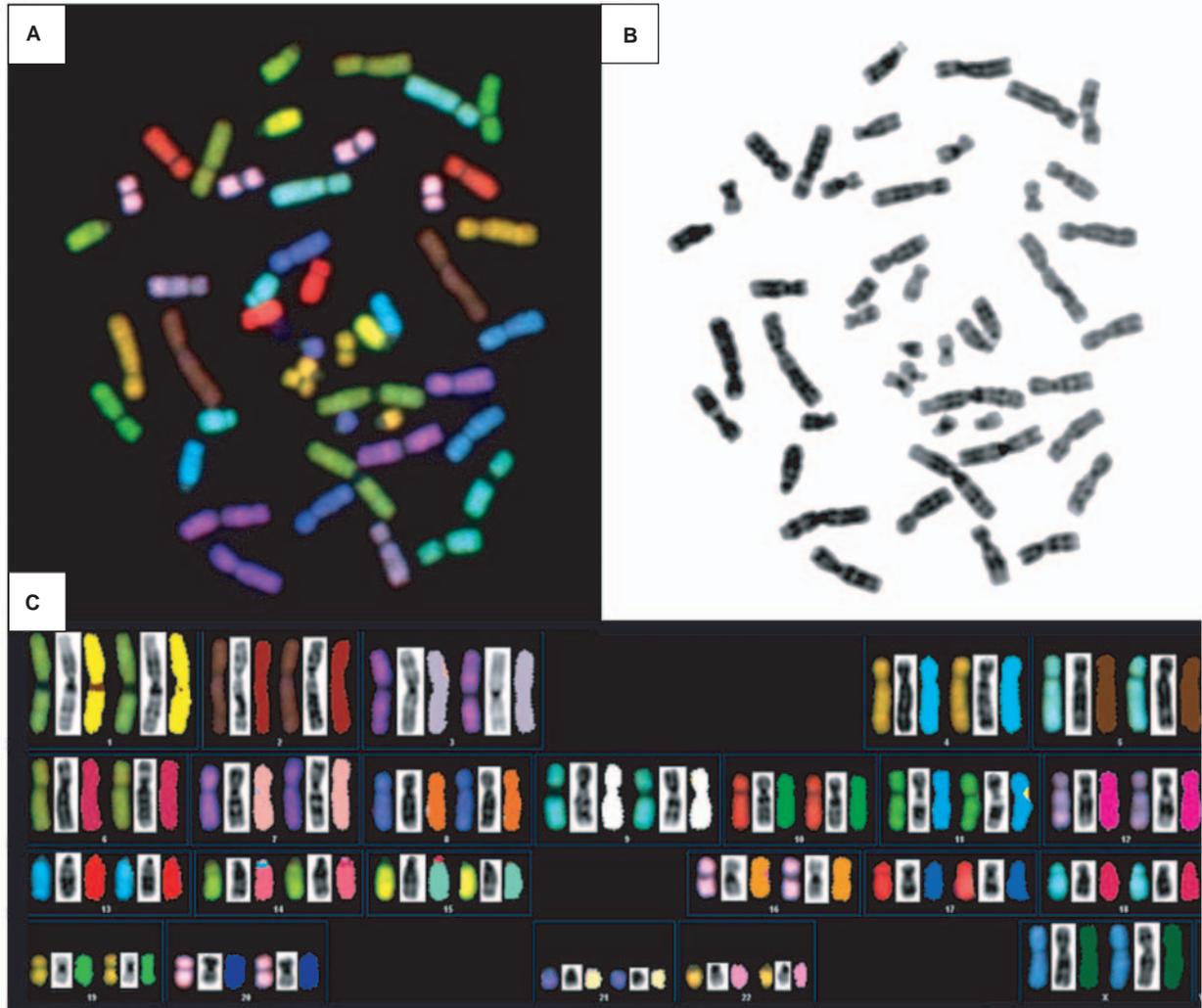


Figure 4. Spectral karyotyping of a normal human female metaphase. (A) Metaphase spread depicted with red, green and blue colors as determined by spectrophotometric analysis of each pixel in the image. (B) Inverted DAPI banding of chromosomes. (C) Karyogram containing spectral, DAPI and classification of each chromosome.

DAPI: 4,6-diamidino-2-phenylindole-dihydrochloride.

system where separate images are acquired sequentially for each fluorochrome used. The individual fluorochrome files are then combined to generate the final image.

Applications & limitations

SKY combines the advantages of FISH with traditional chromosome banding techniques. SKY permits the detection of interchromosomal structural aberrations, such as translocations and insertions resulting in balanced as well as unbalanced rearrangements. It therefore facilitates the identification of cryptic translocations and the clarification of complex aberrations [59–67]. SKY also enables the identification of material such as marker and ring chromosomes, the components of which are typically unidentifiable by conventional banding techniques [68–71]. In addition, other aberrations such as double minutes can be better resolved, leading to the identification of critical oncogenes [72,73].

Intrachromosomal alterations resulting in small deletions or duplications and para- or pericentric inversions do not result in changes in chromosome size or spectral signature of the aberrant chromosome and therefore cannot be detected by SKY. In addition, very small marker chromosomes or double minute chromosomes cannot always be unambiguously classified. Ultimately, a combination of molecular cytogenetic methods and banding techniques will result in the most comprehensive analysis of tumor metaphases.

Comparative genomic hybridization

Methodology

CGH utilizes the hybridization of differentially labeled tumor and reference DNA on normal metaphase chromosomes to generate a profile of DNA copy number changes in tumor genomes along the chromosome length (FIGURE 5) [7,29]. Total genomic DNA from a tumor specimen is isolated using

standard procedures. Each genomic DNA is then differentially labeled; the test DNA with biotin-dUTP and the reference with digoxigenin-dUTP, using a standard nick translation reaction. Alternatively, direct labeling with fluorochrome-conjugated dUTP may be used. The labeled genomes are pooled in equal amounts with excess Cot-1 DNA and subsequently hybridized to normal metaphase chromosomes. Following a 48–72 h incubation, biotin-labeled tumor DNA is detected with avidin-FITC resulting in green fluorescence and anti-digoxigenin and TRITC-labeled secondary antibodies are used to detect the control DNA in red fluorescence [29,74].

Fluorescent images of chromosome metaphases are acquired using an epifluorescent microscope (FIGURE 6A). The quantitative measurement of fluorescence intensity values based on digital image analysis is essential for accurate CGH analysis [75,76]. A CCD camera and fluorochrome-specific optical filters are used to acquire the FITC and TRITC fluorescence (for review [77]). If the tumor karyotype is normal, the observed fluorescence reflects an equal contribution of both the red and green fluorescence and thus the chromosomes will appear yellow. A gain of tumor DNA will be visualized as green staining of the corresponding region on the chromosome. Loss of a chromosome or deletion of a chromosomal subregion will shift the resulting color towards red (FIGURE 6B). Specialized software is used to measure fluorescence intensity values, segment the chromosomes along their axes and identify and orient each chromosome [78]. Fluorescence ratio profiles are calculated along the axis of each chromosome (FIGURE 6B). Ratio profiles represent

tumor genome to reference genome fluorescence hybridization signal intensities and are indicative of copy number changes [76]. Average ratio profiles are based on the analysis of at least five metaphase spreads (or measurements from ten copies of each chromosome). A ratio of 1.0 indicates that no copy number changes are present in the tumor. Typically, ratios of 0.8–0.75 or less indicate loss or deletion of a whole chromosome or chromosomal subregion, whereas ratios of 1.2–1.25 or greater indicate chromosomal gains. A ratio of 2.0 or higher represents high-level gene amplification (FIGURE 6B). The end result of CGH image analysis is a karyogram of tumor-specific gains and losses along the length of each chromosome (FIGURE 6C).

Applications & limitations

CGH serves as an important global screening test for chromosomal aberrations present within a tumor genome. As only genomic tumor DNA and metaphase preparations from a normal donor are needed, the challenging task of preparing high-quality tumor metaphase spread is circumvented. Perhaps most importantly, tumor DNA extracted from archived, formalin-fixed, paraffin-embedded tissue can be used [79–83]. This allows for the retrospective identification of chromosomal aberrations and thus facilitates the correlation of cytogenetic findings with histologic/histochemical information, clinical course and prognosis [15,84]. In addition, the use of archived samples allows a larger number of specimens to be utilized for study and provides a means of evaluating tumors that are difficult to culture. Lastly, one is able to analyze small subregions of a histologically defined lesion [82,83].

Another important advantage of CGH is the requirement of very small amounts of DNA (less than 1 µg). Using modified methods for extracting and labeling DNA from formalin-fixed, paraffin-embedded tissues, one can obtain sufficient amounts of DNA required for CGH. This modest amount of material can be easily obtained from a routine tissue section. Concordance between CGH analyses performed on matched fresh and formalin-fixed material is high (95%) [79,80]. In addition, 70–90% of all archived, formalin-fixed material, including very old material and tissue obtained from autopsies, has been found suitable for analysis using CGH [80]. This demonstrates the benefit of this technique in retrospective genome analysis. However, the quality of DNA extracted from formalin-fixed samples varies greatly and may not always result in uniform hybridization to the chromosomes.

CGH has been utilized for the analysis of a wide variety of tumors [85]. Chromosomal copy number changes have been mapped in common neoplasms, including

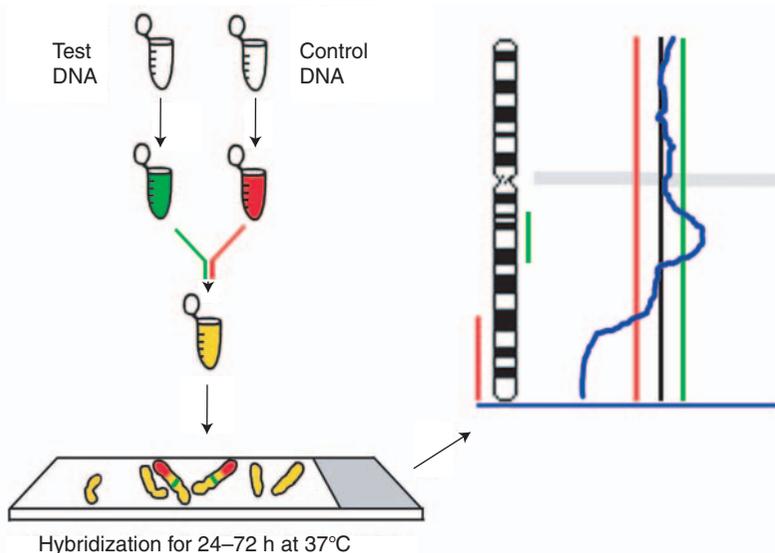


Figure 5. Schematic representation of comparative genomic hybridization experimental set-up.

Test DNA is labeled with biotin and normal diploid control DNA is labeled with digoxigenin. They are then combined (with an excess of Cot-1 DNA), denatured, briefly allowed to reanneal and hybridized to normal diploid metaphase spreads. After detection of the biotin with avidin-FITC and the digoxigenin with mouse antidigoxigenin followed by goat antimouse TRITC, the metaphases are imaged, the ratio of green to red fluorescence intensity along the length of each chromosome plotted, and the gain/loss profiles generated.

lung [86–91], breast [81,92–97], colon [98–103], brain [104–107], prostate [108–113], head and neck [114–119], hematologic [120–123], kidney [124–130], ovary [131–136], bladder [137–142] and uterine cervical cancers [143–148]. Analysis of these tumor types has led to the discovery of general patterns of chromosomal aberrations, with both the number and genomic distribution of such aberrations defining certain tumor types [84]. For example, extra copies of chromosomes 1, 8, 17, and 20 are typically seen in breast cancer, along with a loss of chromosomal arms 13q and 17p, the loci of the retinoblastoma and p53 tumor suppressor genes, respectively [81,93]. In glioblastomas, on the other hand, a gain of chromosome 7 and loss of 10 appear to be a hallmark of tumor progression [149–151].

CGH has also proven useful in establishing a phenotype/genotype correlation in solid tumor progression [101,143]. For example, in the case of cervical carcinoma, CGH analysis of DNA gathered from normal cervical epithelium, different stages of dysplasia and invasive carcinomas revealed distinct chromosomal aberrations previously undetected with conventional cytogenetic analysis. Specifically, a 3q gain was observed only sporadically in severe dysplasias, while it was observed in nine out of ten invasive carcinomas [143]. This indicates that the 3q gain is perhaps the defining aberration required for transformation of cervical epithelium from an *in situ* to an invasive lesion, making genes in this region potential clinical markers for cervical dysplasia progression [13]. CGH has been widely applied, together with FISH and SKY, to better understand chromosomal changes that occur in mouse models for human diseases. The cytogenetic analysis of murine models and their comparison to human tumors offers the opportunity to follow rearrangements during tumor evolution and to identify aberrations that are conserved across species boundaries [152].

While CGH has no doubt proven to be an accurate means of identifying chromosomal gains and losses, it does have its limitations [134]. For instance, since copy number changes are detected relative to the average copy number in the entire tumor, one is unable to determine the relative ploidy of a given tumor. Resolution limitations also exist due to the length of the metaphase chromosomes hybridized to. While the smallest detectable deletion is estimated to be 3–5 Mb, the typical resolution for identifying copy number changes is somewhere in the range of 10–20 Mb, while high-level amplifications can only be detected when 20–40 kbp in length [153].

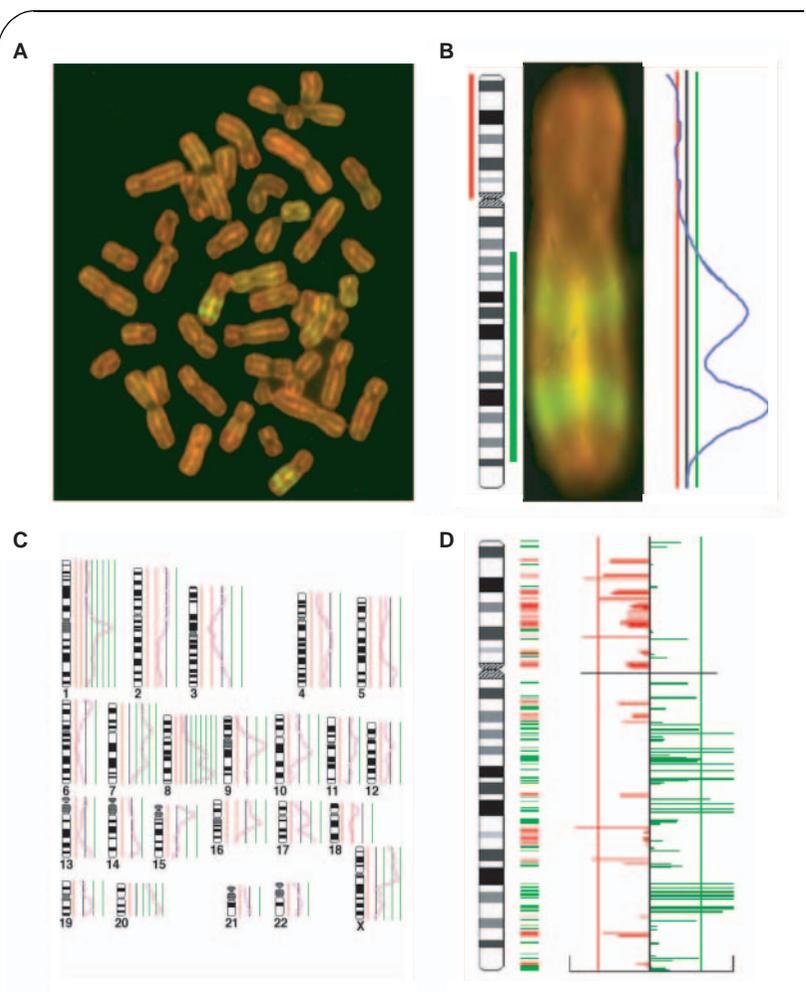


Figure 6. Fluorescent images of chromosome metaphases. (A) Fluorescence image of metaphase after CGH hybridization. (B) Close-up of chromosome 8 from metaphase in (A) showing two amplicons in the human breast cancer cell line SKBR3. Red bars to the left and green bars to the right of the chromosome ideogram illustrate those regions that are lost or gained, respectively. These determinations are based on the plotted ratio of green:red fluorescence intensity along the length of the chromosome (right). (C) CGH profiles of the entire metaphase in (A) without loss/gain bars. (D) Array CGH presentation of SKBR3 data. Red bars to the left and green bars to the right in the bar graph illustrate those regions that are lost or gained, respectively. Each spot on the array is plotted along the length of the chromosome 8 ideogram as red or green lines. CGH: Comparative genomic hybridization.

Another limitation of CGH is its inability to detect chromosome rearrangements that are balanced (i.e., do not result in increased/decreased genomic content), such as inversions and balanced translocations. In addition, it does not provide information regarding the nature of chromosomal segments involved in copy number alterations. Lastly, CGH averages out alterations in genomic content over the entire tumor population. Gains or losses are therefore only detected when they are present in greater than 60% of the cells from which the DNA was extracted. Thus, information pertaining to clonal heterogeneity or genetic diversity is lost using this technique and tumor-specific aberrations can be obscured if the surgically obtained specimen contains a

large population of normal surrounding tissue or infiltrating cells. For this reason, some studies have resorted to laser capture microdissection to isolate morphologically aberrant tumor cells from paraffin-embedded histology sections [112,127,154–159]. There exist a few databases worldwide that contain the CGH profiles for numerous tumor specimens [202–205].

Some of the limitations of conventional CGH have been overcome with the recent development of matrix or array CGH [160,161]. Using this technique, one can hybridize total genomic DNA to a chip spotted with BAC clones, complementary DNA or oligonucleotide sequences. As with conventional CGH, a reference and tumor sample are labeled separately and hybridized to the same sequences on the slide. A laser scanner is used to acquire fluorescence hybridization signals. The analysis software measures intensities for each hybridization spot and ratio values between the normal reference channel and the tumor channel are calculated. The ratio values for each gene are then plotted onto chromosome ideograms based on their mapping position, which results in a high-resolution mapping of specific genomic imbalances (FIGURE 6D). The resolution that can be achieved with this type of analysis depends on the amount of the genome represented by the spotted DNA, the length of the spotted sequence (i.e., oligos vs. BAC clones) and the size of the moving average used to reduce the amount of experimental noise. The recent development of single nucleotide polymorphism (SNP) arrays has greatly facilitated deletion detection (e.g., Affymetrix GeneChip[®] Mapping 10K Array). However, the resolution of SNP arrays is currently limited to approximately 10,000 SNPs. One would expect that only a subset of these loci would be informative (heterozygous). In time, SNP arrays will reach sufficient resolution and other whole-genome scanning technologies, such as the representational oligonucleotide microarray analysis technique, will become more widely available.

Another method, tiling path array CGH, spans the human physical map with over 30,000 BACs and provides the most comprehensive method for examination of tumor genomes for regional copy number alterations [162].

The use of FISH along with its various applications such as CGH and SKY, have greatly enhanced the ability of conventional cytogenetics to detect both numerical and structural chromosome aberrations in human and mouse. These advancements have had an impact on the identification of tumorigenic events in human samples and the ability to make useful correlations with their respective mouse models.

Expert opinion

FISH has proven useful in clinical cytogenetics to identify marker chromosomes, detect microdeletion syndromes and in the prenatal diagnosis of aneuploidies. A variety of probes are commercially available for determining the copy number of oncogenes and tumor suppressor genes. The detection of genetic aberrations in solid tumors and hematologic malignancies with FISH is a powerful method for more specific diagnosis of some cancers and contributes to the differential diagnosis of disease. As these technologies move from the research bench to the clinical bedside, they will enhance the stratification of cancer patients so that their treatments can be more specifically tailored. A prime example of this is the fact that breast cancer patients with *Her2/neu* gene amplification are treated specifically with the monoclonal antibody trastuzumab (Herceptin[®], Genentech, Inc.). The antibody targets cells overexpressing *Her2/neu* and interferes with the protein product of this amplified gene, thereby abrogating its effect and controlling tumor cell growth. More progress is also being made in the area of hematologic malignancies in terms of designing drugs to specifically interfere with the protein products of fusion genes.

Five-year view

Comparative cytogenetics between human tumors and their respective mouse models will enable a further narrowing of the regions of importance for tumorigenesis and will hopefully lead to the identification of specific genes whose alteration is involved in tumor development. This type of analysis will benefit from the increasing number of CGH array platforms available that result in a continuously increasing resolution of mapped loci. Comparison between array CGH and expression data obtained on the same platforms will provide a tool to better investigate the mechanisms by which alterations in chromosome copy number are responsible for changes in gene expression.

One of the main applications to clinical investigation of molecular cytogenetics is the identification of specific chromosome aberrations that could serve as markers for the detection of early transformation events. For example, one of the future goals will be the use of interphase FISH in cytologic specimens to detect cytogenetic abnormalities in otherwise morphological normal tissue. This will allow the identification of those abnormal cells that will become tumorigenic [206].

Key issues

- Many tumor cells are characterized by complex chromosome rearrangements resulting in the gain or loss of specific chromosomal regions.
- The identification of specific imbalances has made it possible to clone the specific genes responsible for malignancy in some cases.
- The molecular cytogenetic techniques described herein allow a comprehensive and detailed analysis of complex chromosome rearrangements and changes in gene copy number.

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