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PRINCIPLE & TECHNIQUE OF COMPARATIVE GENOMIC HYBRIDIZATION

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ABSTRACT

Comparative genomic hybridization (CGH) is a molecular cytogenetic method for the detection and mapping of chromosomal gains and losses. These techniques are serves to provide with essential information in cases pertaining to clinical applications in oncology and medical genetics and also basic evidence on fundamental characteristics of genome structure. It has been applied primarily as a research tool in the field of cancer genetics. The technique is applicable in clinical genetics as it helps in detecting the origin of extra or missing chromosomal material and provides a wide screening for unbalanced aberrations. Tumor DNA is labelled with a green fluorochrome, and is consequently mixed with red labelled normal DNA. This is then hybridized to normal human metaphase preparations. The green to red fluorescence ratio signifies loss or gain of genetic material in the tumor at that specific locus. Thus, this review provides a comprehensive explanation of the CGH technique.

KEYWORDS: Fluorochrome; DNA microarray; Genome; Hybridization.

INTRODUCTION

Comparative genomic hybridisation (CGH) is considered to be one of the contemporary approaches that can provide ample amount of information the biological status or function of the genome.[1] These techniques can provide valuable information that is useful for clinical applications in oncology and medical genetics and also basic evidence on fundamental characteristics of genome structure.[2] It has been applied primarily as a research tool in the

field of cancer genetics. CGH may be applicable in clinical genetics because it aids in unequivocally detecting the origin of extra or missing chromosomal material and provides a wide screening for unbalanced aberrations.[3] CGH is a dual fluorescence in situ hybridisation protocol that detects global gains and losses of genomic regions by competitive DNA hybridisation.[4] The principle of the CGH technique was first described by Kallioniemi *et al.* (1992) and was technically improved later.[5]

PRINCIPLE AND METHODOLOGY

CGH was the first to effectively scan the entire genome for variations in DNA copy number. In this procedure, total genomic DNA is isolated from test and reference cell populations, differentially labeled and hybridized to metaphase chromosomes or, more recently, DNA microarrays.[6] An equal amount of tumor and normal DNA are labeled with biotin and digoxigenin respectively, by nick translation.[5] Regions of DNA exhibiting areas such as deletions, duplications, or amplifications are seen as changes in the relative ratios of red (areas of loss) and green (areas of gain). Subtle changes in color may not be discernible by the naked eye, requiring high-tech image analysis software for quantification.[7]

The relative hybridization intensity of the test and reference signals at a given location is primarily proportional to the relative copy number of those sequences in the test and reference genomes. The increase and decrease in the intensity ratio specifically indicate DNA copy number variation in the genome test cells wherein more than two genomes can be competed concurrently if characteristic labels are available. Data are typically normalized so for the genome to some standard value (1.0 on a linear scale or 0.0 on a logarithmic scale).[6]

Techniical Considerations

CGH technique involves fluorescence in situ hybridization (FISH) technique that helps in detection and mapping of chromosome imbalances in a tumor genome with the help of total genomic DNA as a probe.8 It enables us to analyse the entire genome in frozen or paraffin-embedded tissue, specifically exhibited in tumor biology.[9]

Hybridization signals

CGH generates hybridization signals that are suitably intense and specific so that copy number changes can be detected. The signal intensity on an array element is affected by number of factors including the base composition, proportion of repetitive sequence content, and amount of DNA in the array element available for hybridization.[2] Most significantly,

production variability among different arrays is precisely compensated such as the amount of DNA in array elements. When the intensities become non-linearly related to genomic abundance due to processes disturbing the test and reference genomes equally, such as saturation of array elements or re-association of double-stranded nucleic acids during hybridization; ratio accuracy is still intact. The complexities of the genomic DNA and in the array elements considerably affect signal intensities. Thus, it plays a dominant role in determining the genomic resolution of different array CGH technologies.[6]

Genome characteristics and copy number measurements

The change in ratio produced by a copy number change is influenced by several intrinsic characteristics of the specimen DNA. The high-copy repetitive sequences dispersed throughout mammalian genomes are of great importance. These can hybridize to array elements that contain copies of the repeats, such as those made from genomic and cDNA clones, overwhelming the signal due to the unique sequences.[2] A defined quantity of Cot-1 DNA is added for suppression of repetitive DNA sequences and the probe mixture then is denatured in buffered formamide/dextran sulfate for 3 min at 75°C and pre-annealed at 37°C for 20 min. The probe mixture is then hybridized to a normal lymphocyte metaphase slide denatured in 70% formamide/2× SSC/sodium phosphate buffer, covered with a cover slide, and sealed with rubber cement. Hybridization is carried out for 3 days at 37°C in a humidified chamber. For fluorescence detection, the slides are stained with avidin-fluorescein isothiocyanate and anti-digoxigenin-rhodamine, followed by counter-staining with DAPI[4],6-diamidino- 2-phenylindole). The slides then are mounted in anti-fade solution.[5]

Specimen preparation

The quality of genomic DNA preparations has an abundant effect on the resulting data. Although isolating genomic DNA from fresh and frozen specimen with numerous published protocols and commercial kits; there appears to be an unknown class of contaminants that occasionally co-purify with the DNA and produce abnormally high “noise” in the ratios. This noise is typically not random because relabeling a different aliquot of the same DNA reproduces exactly the same noisy pattern. Repurifying or, better, reisolating the DNA may help significantly. CGH array procedures usually utilise 300 ng to 3 μg of specimen DNA in the labeling reaction, employ random primer labelling (amplifying the DNA) so that several micrograms are used in the hybridization.[6]

Technical improvements

CGH has been technically improved by the microdissection of specific areas of archival tumor samples using micromanipulator- directed fine needle or laser technology for obtaining small but specific tumor DNA samples. The samples are also amplified by polymerase chain reaction (PCR) for CGH use. This technique permits a comparative assessment of various areas within the same tumor and even of single cells and also of intratumoral heterogeneity. In recent times, substantial development of the specificity of the obtained data has been achieved by the introduction of array techniques (a step from analysis on crude chromosomal segments to the level of specific DNA sequence).[5]

Data analysis

The major aberrations in a genome are frequently evident by inspection however, many approaches are present to improve interpretation, the simplest one being applying thresholds. If the ratio profile has only a few well-spaced ratio levels, thresholds can be chosen by examining the distribution of all measured ratios. Several tumors, due to their non-diploid genomes and/or heterogeneity, consist of closely spaced ratio levels partially overlapping due to measurement noise. Hence, this approach is not capable of distinguishing them.[2] More urbane analytical approaches indulge in the fact that the copy number changes involve chromosome segments, so ratios at adjoining sets of loci should be identical, except for occasional abrupt steps to a new level.[6]

Digital image analysis

The digital image analysis system was developed on the Magiscan system specifically for CGH and was used primarily as described by Lundsteen *et al.* (1995). In brief, ~30 metaphases were examined before selecting 5-10 high-intensity uniform hybridizations for image analysis. Green, red, and blue fluorescence images were captured from each metaphase and were analyzed as separate grey-scale images.[3]

The hybridization is analyzed with the help of a digital image analysis system (microscope based) equipped with a cooled CCD camera and a filter system. Excitation of each fluorochrome is accomplished by using these filters in a computer-controlled filter wheel. Three fluorochromes (DAPI, Spectrum Green, and Spectrum Red) images are properly recorded and processed and a software for pseudocolor display is used. The color changes along the metaphase chromosomes are visualized by three color images.[8] Green (test DNA)

and red (reference DNA) fluorescence intensities are determined for each chromosome from p-telomere to q-telomere by integrating intensities at 1-pixel intervals along the chromosome medial axis. After background correction and normalization of the green to red ratio for each entire metaphase to 1.0, green to red fluorescence intensity-ratio profiles are calculated for all chromosomes. Data from all analyzed metaphases from each hybridization, are combined and an average ratio profile for each chromosome is determined.[3]

Interpretation of CGH images

A total of ten metaphases are analyzed for the chromosomal locations of DNA sequence gains and losses. These regions are decided by using green-to-red fluorescence intensity ratio profiles. The decision limits of the green-to-red ratios are noted for the decrease in the DNA copy number < 0.75, and for the increase in the DNA copy number >1.25, respectively.[8]

Advantages

- Unambiguous localization of extra or missing chromosomal material
- Genome-wide screening for such unbalanced aberrations,
- It depends on DNA isolation and not on than preparation of metaphase spreads.[3]

Disadvantages

- Minimal size of a chromosomal segment (alteration can be reliably shown)
- Minimal portion of a cell population carrying the respective change to be detected
- Cost of the required image analysis equipment
- Inability of CGH to evaluate some chromosomal regions
- Difficulties in detecting mosaicism
- Relatively insensitive (only chromosomal changes larger than 5 megabases can be detected)[3,5,7]

Applications

- Helps to identify relatively small chromosome translocation that cannot be distinguished by traditional Giemsa staining of metaphase spreads (karyotyping).
- Helps to identify novel gene amplification or loss in tumors.
- Chromosomal identification of marker chromosomes and extra bands on chromosomes can be done.
- Detection of cryptic translocations in families with extensive histories of mental retardation.

- Enables to map aberrations with high resolution (particularly helpful when large numbers of loci need to be analysed).
- Knowledge of copy number aberrations can have immediate clinical use in diagnosis, and in some cases provide useful prognostic information.[2,4,6,7]

CONCLUSON

Array-based CGH has proven to be a standard tool for research and diagnostics of cancer and genetic disease. This technology enables the identification of chromosomal regions of gains and losses in cancers and genetic diseases. It is one of the advanced approaches that helps to provide comprehensive information about aspects of biological status or function and also for important clinical applications.

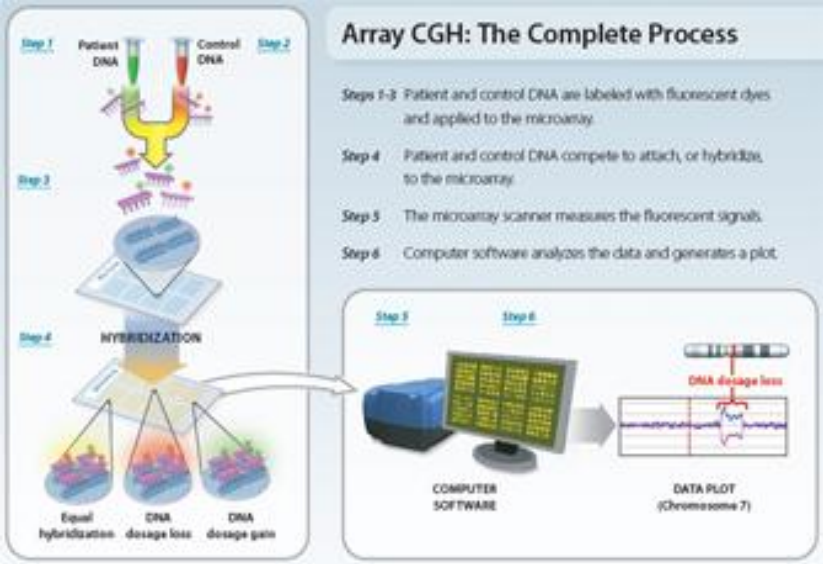

Figure 1: Microarray based CGH process.[10]

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