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Comparative genomic hybridization (CGH) in molecular diagnostics

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Abstract

Comparative genomic hybridization (CGH) is a powerful molecular cytogenetic approach for identifying chromosomal abnormalities. CGH allows researchers to scan whole genomes for changes in DNA copy numbers. Starting in 2004, the array CGH became an irreplaceable method for the detection of gene mutations in people with congenital and developmental abnormalities, such as intellectual disability, dysmorphic characteristics, developmental delay, or several congenital deformities without an obvious syndrome pattern. This review focuses on the evolution of array CGH technology and its use in molecular diagnostics and its advantages over older cytogenetic tools. This review further highlights special arrays developed in the past decade which detect small intragenic copy number changes as well as large DNA segments for the region of heterozygosity.

Keywords: *comparative genomic hybridization, genome, DNA, chromosome, aCHG, cDNA, FISH*

1. Comparative genome hybridization (CGH) method

Thanks to the development of Comparative genome hybridization (CGH) method, developed in the early 1990s, for the first time the whole genome could be scanned for variations in DNA copy number [1]. Comparative genomic hybridization or CGH is a cutting-edge molecular cytogenetic method for identifying chromosomal abnormalities. After co-hybridizing DNA from a research sample and control DNA to normal metaphase spreads, probes are recognized using different fluorochromes [2]. In CGH, a fluorescence microscopy is used to detect the chromosomal regions of copy number changes in DNA sequences between the two complements by competitively hybridizing normal human metaphase chromosomes with two differently labeled genomic DNAs [3]. In order to create metaphase chromosomal spreads on a glass plate, a patient's genome and a reference genome were first co-hybridized. For hybridization to targets placed on glass slides, such as BAC or PAC (bacterial or P1 artificial chromosome) clones, array CGH was designed to replace the metaphase spread [4]. In order to find deletions or duplications linked to recognized genetic illnesses, the initial BAC arrays had "targeted" formats that contained exclusive groups of clones from particular genomic areas. The "whole-genome" arrays, which included clones chosen at regular intervals throughout the whole genome, came after these focused arrays [5]. Understanding genetic alterations in leukemia and lymphomas has been greatly improved by CGH, leading to the discovery of several genes that play a pathogenic role in each type of neoplasia [6].

2. Array CGH

The Human Genome Project's completion and the adoption of new technologies into clinical practice, such as array comparative genome hybridization (aCGH), have altered the diagnostic workup and aided in the identification of the molecular underpinnings of numerous genetic diseases over the past 15 years. Array CGH was initially created as a research tool to examine genomic imbalances in cancer, but it has developed into a crucial and frequent diagnostic tool that has replaced older cytogenetic approaches and is now recognized as a

first clinical diagnostic test for people with developmental disabilities or congenital defects [7]. In array CGH, an array comprising the DNA targets is co-hybridized with equal quantities of labeled genomic DNA from a test and a reference sample. As controls, some laboratories utilize pooled male and female DNA, while others employ individual male and female controls. In addition, different laboratories utilize same-sex or opposite-sex controls. [8] The main advantage of array CGH is the ability to simultaneously detect amplifications, deletions, duplications of any locus represented on the array [9]. The identification of prospective candidate genes is possible using array CGH. Some of the chromosomal abnormalities that have been noted in some research are probably visible under a microscope and can be found using karyotyping and FISH analysis. Later, it is shown that CGH technology has certain advantages over these traditional procedures, including a more accurate breakpoint mapping and a shorter turnaround time [10].
Numerous systems, all of which are based on the same idea of finding copy number discrepancies between two samples, are used to facilitate array CGH research. These methods differ in terms of the size of the discovered genomic fragments and the genome coverage [11].

Array CGH has been extremely helpful for people with congenital and developmental abnormalities, such as intellectual disability, dysmorphic characteristics, developmental delay, or several congenital deformities without an obvious syndromic pattern [12]. Therefore, copy number anomalies in the genes causing the clinical symptoms of individuals with hereditary illnesses can be found using high-resolution whole genome array comparative genomic hybridization (aCGH) testing. The use of a CGH can find novel candidate genes for certain illnesses in addition to anomalies in recognized disease-causing genes. There are several instances of aCGH being successfully used in disease gene discovery, which include the finding of loci for congenital diaphragmatic hernia, the role of the TCF2 gene in the genesis of multicystic dysplastic kidneys, and has also helped identify genes linked to congenital eye defects [13]. cDNA microarrays, which were first utilized in gene expression profiling, were used to pioneer the genome-wide method to array CGH. High-level amplifications and deletions may be directly connected to expression changes utilizing the same platform, which is a benefit of this method. However, the cDNA targets only cover exonic portions of the genome, rendering changes to promoter regions and other protein binding sites undetectable [14].
The gold standard for finding chromosomal abnormalities has been cytogenetic examination of metaphase chromosomes for decades. Routine karyotyping is insufficiently sensitive to identify minor chromosomal rearrangements. The CGH has a major influence on the diagnosis of people with congenital abnormalities such as intellectual impairment, developmental delay or dysmorphic features. [15] Regional heterozygotes with a neutral copy number can be detected using SNP-genotyping sequences. Genomic redistributions can be beneficial or harmful, and many of them are mediated by the structures' fundamental genome. Copy number variation (CNVs) are found in the general population, and the majority of them are thought to have arisen as a result of evolutionary processes. Our ability to evaluate the rise in the number of CNVs and separate those that cause disease from those that do not is currently restricted due to the lack of a large cohort study and good population data. Based on internal databases and ACMG data, guidelines for interpreting these CNVs have been devised [16].
Furthermore, new CGH series procedures have been created since the commencement of CGH, allowing for the resolution of higher levels of particular enlargements and deletions of the number of copies [17]. This approach, however, necessitates a reasonably clean tumor population, with any low-level abnormalities in the number of copies present in around one-third of the tumors to be found [18]. As a result, the method is appropriate for treating huge, enormous melanocyte tumors. Small melanoma that develops in a nevus or neoplasm with a severe inflammatory response has a lower chance of producing meaningful effects in thin lesions. Several FISH assays have recently been developed to help in the detection of melanocyte neoplasms, using data acquired from CGH to determine optimal probe targets [19].
Traditional metaphase CGH is based on the same premise as array CGH. Whole genomic DNA from a control (or reference) and genomic DNA from a patient (or test) are differently tagged with two distinct fluorophores and employed as probes that compete for cohybridization onto nucleic acid targets in both procedures. The objective of standard metaphase CGH is a metaphase spread reference. Single-copy alterations may be detected effectively and reliably using CGH arrays that employ large-insert genomic clones (such as BACs and P1 artificial chromosomes) [20]. The use of a range of CGH in research has accelerated gene discovery in human genetics, deepened understanding of genomic changes, and improved the study of important concepts such as DNA methylation, replication time, gene silencing and many other mechanisms related to DNA structure and function. The CGH array poses a unique set of problems in the clinical setting [21]. Diagnostic

applications have a cautious stance in contrast to the more experimental field of research. It's important to approach CGH arrays from a different perspective. Because each clinical sample should not be viewed as a research project, diagnostic arrays should be constructed in a way that maximizes diagnostic capabilities while minimizing false positive results to give clinicians the diagnoses and information they need to manage the clinical care of people with identified chromosome abnormalitie [22]. Understanding the core mutational processes more thoroughly by array CGH may help to better understand the pathophysiology of disorders linked to genomic instability [23].

CGH was one of the earliest approaches for analyzing tumor genomes for genetic abnormalities, and it has since grown in popularity as a tool for a complete molecular cytogenetic examination of solid tumors. The approach uses normal chromosomal metaphase spreads to hybridize differently tagged tumor and reference DNA [24]. On the chromosomal DNA, both genomes fight for complementary binding sites. More DNA will hybridize to the matching band or chromosomal arm if tumor DNA is amplified, whereas deletions enable normal DNA to compete for binding [25].

The employment of unique fluorochrome markers assists in the detection of each genome. The intensity of fluorescence signals along individual chromosomes are compared to determine DNA imbalances. CGH has many of the advantages of a DNA-based technology when it comes to tumor analysis. Most crucially, it may be used with paraffin-embedded and formalin-fixed archival tumor tissues [26].Furthermore, enhanced amplification procedures are available, allowing the technique to be used on tiny tumor biopsies and minute tissues after microdissection. Because of variations in the binding kinetics of tumor and normal DNA in some particular chromosomal locations, artifactual hybridization may arise. In order to avoid these possible difficulties, only high-quality hybridizations with a strong and distinct fluorescence signal should be evaluated [27]. The use of normal chromosomes also results in low resolution in genetic imbalance assessments, which are confined to chromosomal subregions. Karyotyping is a time-consuming procedure that has yet to be automated, which is the fundamental reason for CGH's lengthy duration. CGH has influenced tumor microarray analysis, which uses competitive hybridization of differently labeled RNA/cDNA and the computation of ratio values to quantify gene expression [28]. The CGH patterns of similar cancers showed a high degree of concordance in studies on primary and metastatic tumors, which might be beneficial in establishing a clonal link. Lung cancer, on the other hand, has a high level of chromosomal instability, which is reflected in its morphological variabilit [29]. In a research comparing primary and metastatic SCC (squamous cell carcinoma) the later tumor group had more chromosomal changes, which is consistent with the tumor genetics paradigm, which states that tumor growth and metastasis formation are defined by an accumulation of genetic flaws [30].

3. Conclusion

CGH, which was invented in the early 1990s, allowed researchers to examine the entire genome as well as the tumor in order to increase or reduce the number of copies in a single experiment. One of the most notable benefits of CGH is its ability to do such a comprehensive genomic analysis in a single experiment. Since the beginning of CGH, series processes have been developed that allow for better resolution of specific enlargements and deletions of the number of copies. This method, on the other hand, requires a relatively clean tumor population, with any low-level anomalies in the number of copies present in about one-third of the tumors to be discovered. Array comparative genome hybridization (aCGH) has changed the diagnostic process and helped to uncover molecular causes of a variety of hereditary illnesses. Array CGH was first developed as a research tool to study genomic imbalances in cancer, but it has transformed into a vital and frequent diagnostic tool that has replaced older cytogenetic. CGH follows the same logic as array CGH. In both processes, whole genomic DNA from a control and genomic DNA from a test are tagged differently with two separate fluorophores and used as probes that compete for cohybridization onto nucleic acid targets. CGH was one of the first methods for examining tumor genomes for genetic abnormalities, and it has subsequently gained prominence as a tool for a comprehensive molecular cytogenetic analysis of solid tumors**.**

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