The Roles of the Fluorescent In Situ Hybridization (FISH) and Comparative Genomic Hybridization (CGH) Techniques in the Detection of the Breast Cancer

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Abstract

This paper aimed to understand and compare the two popular cytogenetic techniques of fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) in detecting breast cancer chromosomal abnormality. Several chromosomal anomalies play a role in the development of breast cancer, and the two above approaches play an important role in confirming fluorescence in situ hybridization in particular (FISH). However, comparative genomic hybridization has developed DNA copy number profiles for most of the publicly available breast cancer cell lines for the FISH methods rely on the fluorescent probes. Chromosomal profiles can be generated for the suspected chromosomal abnormality, copy number changes between the tumour and the DNA control can be compared, and the results can be registered. Today, modern cytogenetic tools such as fluorescence in situ hybridization (FISH) are more commonly used to detect any microdeletion that cannot be detected by conventional cytogenetic karyotypes that involve a high rate of cell division and good chromosomal morphology, which pose challenges for cytogeneticians, and a long period of testing and research. Usually, this is a problem for physicians, and there are still many drawbacks and disadvantages concerning the high benefits, such as false findings. Normal chromosome in situ hybridization requires the hybridization of a labelled DNA probe into denatured chromosomal DNA present in metaphase chromosomes in an air-dried microscope slide preparation. Metaphase spreads are used for traditional chromosome FISH (metaphase FISH). Positive and positive signs of hybridization also appear as a double spot, corresponding to the hybridized probe for both sister chromatids. A further extension of chromosome painting is comparative genomic hybridization (CGH). CCH involves simultaneous chromosome painting in two different colours using complete DNA from two similar sources as probes, which reveal variations concerning the benefit or loss of sub-chromosomal regions or even entire chromosomes.

Keywords: FISH; CGH; probe; classical cytogenetic; Chromosomal regions and hybridization.

INTRODUCTION

In terms of histology, clinical response, diffusion trends to distant locations, and patient outcomes, breast cancer is heterogeneous (Prat et al., 2011). Nevertheless, the most common cause of death from cancer worldwide is breast cancer. Rates differ about five-fold across the world, but they are growing in regions with low disease rates until recently (Key et al., 2011). Furthermore, the availability of cytogenetics and cytogenetics technologies has enhanced the detection and recognition of molecular tumour signatures and the understanding of the initiation and progression of cancer (Ribeiro et al., 2019).

The fluorescence in situ hybridization (FISH) technique that plays a leading role in diagnostic pathology has provided valuable information on genomic variations in malignant cells for its single-cell study (Das et al., 2013). FISH is a molecular tool in which both metaphase chromosomes and interphase nuclei identify numerical and structural defects. FISH is widely used to identify translocations and determine gene deletion and amplification in tumours as a prognostic and diagnostic method (Brown et al., 2007). The technique utilizes the inherent capacity to hybridize complementary strands of DNA or RNA from various sources. The theory of annealing a labelled nucleic acid probe to complementary sequences inside cells or tissue mounted (in situ) on a microscope slide is based on in situ hybridizations (Karimi et al., 2020).

Genomic changes, amplifications, and deletions may reduce the complexity of genomic data observed by comparative genomic hybridization (Przybytkowski et al., 2014). By comparing the samples with reference DNA, CGH detects changes in the copy number of individual chromosomes or chromosomal regions, such as changes in relative genome size and ploidy levels in test samples. This technique was initially designed to examine the differences between solid tumours and normal tissues in chromosomal supplements (Zhang et al., 2015). It is expected that the precise classification of these genomic changes would have an important effect.
on translational and fundamental research (Climen et al., 2007). For example, comparative genomic hybridization has been instrumental in the dissection of distinct molecular pathways to malignancy of the breast and developing a direct relationship between genotype and clinical pathology (Reis et al., 2005).

CHARACTERIZATION TYPES OF BREAST CANCER

Over 100 types of cancers consist of multiple subtypes capable of arising in a single type of organ or tissue. Most cancers can be defined at a very general level based on their originating tissue location. In epithelial cells, the most common type of cancer, comprising more than 80 per cent of all cancers, occurs; these cancers are called carcinomas. Depending on whether the epithelial cells of origin are part of the protective epithelial layer (squamous cell carcinomas) or have secretory properties (adenocarcinomas), most carcinomas can break into squamous cell carcinomas or adenocarcinomas (Smail 2016 and Smail 2020). Breast cancers are a complex and heterogeneous community of diseases, and patients are treated with clinicopathological features and estrogen receptor status and HER2 guidance. However, for the individualization of treatment, these factors are not necessary. Microarray-based gene expression profiling has contributed to a paradigm change in the understanding of breast cancer. It has been shown conclusively that breast cancer is not a single disease (Weigelt et al., 2010).

Joint hormone receptor-defined subtypes of breast cancer (H.R.; estrogen receptor [E.R.] and progesterone receptor [P.R.]) and HER2 status (Howlade et al., 2014). Triple-negative (TNBC) breast cancers do not express hormone receptors or over-express HER2. As characterized by low five-year survival and high recurrence rates after adjuvant therapy, poor prognosis is associated with these conditions. Overall, TNBC has striking similarities with basal-like breast cancers (BBC), but they are found the same in various studies (de et al., 2011). Five tumour subtypes (luminal A, luminal B, HER2-enriched, basal, and claudin-low) have been identified in breast cancer gene expression studies, each of which has specific biological and prognostic features (Prat et al., 2010). Four major intrinsic molecular subtypes of breast cancer, known as luminal A, luminal B, HER2-enriched [HER2E], and basal-like, were identified in global gene expression analysis studies. These molecular entities have shown major differences in terms of occurrence, risk factors, baseline prognosis, age at diagnosis, and response to treatment. Because of its high frequency, lack of successful targeted therapies, weak baseline prognosis, and propensity to impact younger women, the basal-like subtype is of particular clinical concern (Prat et al., 2013).

A promising new diagnostic area for estimating the risk for metastatic relapse and metastatic progression in cancer patients is the Circulating Tumor Cell (CTC) study (Lianidou et al., 2011). RNA sequencing found that PDX cells developed in the mammary gland were identical to those studied in culture for global gene expression. Carboplatin was cytotoxic to WHIM30 but not WHIM2 in vitro, while both lines were cytotoxic to bortezomib, dacarbazine, and cyclophosphamide. However, these drugs have proven unsuccessful in treating in vivo both primary and metastatic WHIM2 tumours. On the other hand, carboplatin and cyclophosphamide have successfully treated WHIM30 mammary tumours and reduced brain, liver, and lung metastatic burden (Turner et al., 2018). The metastatic cascade is a series of biological processes that cause tumour cells to migrate from the primary site to a distant location and create new cancer growth. Circular tumour cells (CTCs) play a crucial role in tumour propagation. The role of CTCs in treatment failure and disease progression can be explained by their association with biological processes, including epithelial-to-mesenchymal transformation and self-seeding, defined as primary infiltration of the tumour or metastasis formed by more aggressive CTCs. CTCs are a rare and heterogeneous population of cells of proven prognostic and predictive value in some clinical contexts (Mego et al., 2010).

In addition to the typical hormone receptor-positive and hormone receptor-negative forms, studies of breast cancers using gene expression profiling have identified many major breast cancer subtypes. The luminal A and luminal B classes are the most reproducibly defined molecular subtypes among the hormone receptor-positive cancers. The major molecular subtypes identified in hormone-receptor-negative breast cancers are the HER2 and basal-like classes. Some studies have also described other molecular subtypes, such as luminal C and regular breast-like classes. Still, they are less well characterized than luminal A, luminal B, HER2, and basal types (Schnitt 2010). Several molecular-targeted therapies for breast cancer have been tested since the application of endocrine therapy for estrogen receptor (E.R.)-positive tumour types. 1. An example of effective gene-targeted therapy is genome alteration-matched treatment of breast cancer to target amplification of human epidermal growth factor receptor 2 genes (Erb-B2 receptor tyrosine kinase, ERBB2 also referred to as HER2). Gene expression-based molecular subtyping has also been broadly applied to breast cancer to aid treatment decisions (Chung et al., 2017). There are many cellular functions for BRCA1. DNA repair, cell-cycle regulation, transcriptional regulation, and chromatin remodelling have been implicated.

On the contrary, functions assigned to BRCA2 were specifically limited to DNA recombination and repair processes. In the regulation of RAD51 activity, BRCA2
has a role. RAD51 is a highly conserved DNA recombinase involved in double-strand break repair and replication fork arrest (Da and Lakhani. 2010).

**CHROMOSOMAL ABNORMALITY IN BREAST CANCER**

The most common cytogenetic anomalies observed in human breast carcinoma are modifications to the long arm of chromosome 1 (Bièche and Lidereau. 1995). For four ductal breast carcinomas, the cytogenetic analysis showed a net benefit of 1 q in all tumours. The only improvement in the first tumour was that one chromosome 16 was replaced by a chromosome derivative consisting of 16p and 1q (Pandis et al., 1992). Active X chromosome duplication and Xi loss characterized almost half of the cases of sporadic basal-like cancers studied (Richardson et al., 2006). Grade I and tubular breast carcinomas have a limited number of genomic alterations with extremely recurring 16q losses, while grade III breast carcinomas also have complex genotypes with 11q, 14q, 8p, 13q loss; 17q, 8q, 5p gain; and high-level gains (amplification) on 17q12, 17q22-24, 6q22, 8q22, 11q13, and 20q13 (Simpson et al., 2005).

Amplification of chromosome band 11q13 protooncogenes (MYC, ERBB2) and DNA; TP53 mutation; and loss of heterozygosity of chromosome and chromosome arms 1, 3p, 6q, 7q, 8p, 11, 13q,16q, 17, 18q, and 22q are the main forms of genetic defects commonly found in breast tumours (Bièche et al., 1995). In breast cancer, abnormalities of chromosome 17, recognized over two decades ago to be important in tumorigenesis, frequently occur. Changes in unique chromosome 17 loci, including amplification of ERBB2, loss of P53, loss of BRCA1, and amplification or deletion of TOP2A, are considered to play an important role in breast cancer pathophysiology (Reinholt et al., 2009). A single amplicon spanning several megabases was originally thought to include 11q13 amplification. Still, more recent data identified four core regions within 11q13 that can be amplified separately or separately together in various combinations (Ormandy, 2003).

Chromosomal alterations can be tested using G-banding karyotype and multicolour Fluorescence in situ hybridization (FISH) on metaphases (Rondón et al., 2014). Fluorescence in situ hybridization (FISH) allows the number of the gene or chromosome copies in archival tissues to be measured in situ and linked to morphology and clinical outcome (Watters et al., 2003). For example, cytokinetic defects are characterized by chromosomal instability in an inherited cancer syndrome and may help explain why BRCA2-deficient tumours are also aneuploidy-deficient (Daniels et al., 2004). The innovation is known as Comparative Genomic Hybridization (CGH), sets out methods for evaluating the relative number of copies of nucleic acid sequences in or in parts of one or more subject genomes (e.g. a tumour cell) as a function of the position of certain sequences in the reference genome (e.g. a regular human genome) (Pinkel et al., 2011). Initial CGH array applications for breast cancer analysis and the mechanisms by which various types of copy number changes can occur have been described (Albertson 2003).

**HOW DOES FLUORESCENCE IN SITU HYBRIDIZATION WORKS?**

Fluorescence in situ hybridization by a fluorescently labelled probe detects nucleic acid sequences that precisely hybridize within the intact cell to its complementary target sequence (Moter and Göbel, 2000). Fluorescence in situ hybridization has been used in interphase nuclei to image complex genomic DNA sequences. Unreplicated DNA parts give singlet hybridization signals in normal diploid cells, whereas replicated loci are characterized by doublets (Selig et al., 1992). FISH on 3D preserved nuclei, compared to FISH on metaphase chromosomes and traditional interphase cytogenetics, needs special requirements concerning the consistency of the probe, fixation, and pretreatment steps of the cells to achieve the two objectives, namely the best possible preservation of the nuclear structure and at the same time, the efficient accessibility of the probe (Cremer et al., 2012). The concepts of in situ hybridizations of fluorescence are as follows: (a) DNA probe and a target sequence are the basic elements. (b) Before hybridization, the DNA probe is indirectly marked with a hapten, specifically marked with hapten by adding a fluorophore probe (right panel). (c) The labelled probe and the target DNA are denatured to yield single-stranded DNA. (d) Then they are combined, which allows complementary DNA sequences to be annealed. (e) If the probe has been indirectly labelled, the visualization of the non-fluorescent hapten using an enzymatic or immunological detection system involves an additional step. Finally, fluorescence microscopy tests the signals. Speicher and Carte adapted from (Speicher et al., 2005 and Bishop, 2010). Hapten binding is visualized using a subsequently applied fluorochrome-conjugated antibody (Waminal et al., 2018).

The choice of the probe is one of the most significant factors in FISH research. It is possible to use a large variety of probes, from whole genomes to tiny cloned probes (1–10 kb). There are three types of probes in general, each with several applications: full-chromosome painting probes, repetitive sequence probes, and locus-specific probes (Bishop, 2010). Well-prepared chromosome distribution and an effectively labeled probe are prerequisites for a good FISH experiment. Nick-translation, random priming, and PCR provide multiple enzymatic methods for labelling probes. The most common use of nick-translation is
In conducting efficient 3D FISH experiments, there are two major considerations.

to maintain nuclear morphology as much as possible while keeping DNA sufficiently accessible for probe hybridization, the option of cellular treatments, including fixation, pre-and post-hybridization steps (Bolland et al., 2013).

Figure 1. The FISH spot detection algorithm (Les et al., 2014).

Figure 2. Fluorescence in situ hybridization (FISH) applications in genetic diagnostics on FFPE content in solid tumors: A-1p19q probe: a-1 deletion of 1p32 locus, a-2 natural signal pattern (cell on the left) and 19q13 locus deletion (cell on the right), a-3 normal signal pattern (Abbott Molecular), B-dual fusion probe: COL1A1 and PDGFB loci fusion and normal signal pattern (ZytoVision), C-break apart probe: c-1 ALK gene rearrangement, c-2 normal signal pattern (Abbott Molecular), D-break apart probe: EWSR1 locus rearrangement (Abbott Molecular), F-break apart probe: F1 rearrangement (Empire Genomics), G-locus specific probe: g-1 HER2 locus amplification, g-2 normal signal pattern (Abbott Molecular), H-break apart probe: normal SS18 locus signal pattern, green colour—PDGFB gene locus, yellow colour—the fusion of COL1A1-PDGFB and PDGFB-COL1A1 (Chranowska et al., 2020).

Figure 3. Detection of di-centric, tri-centric, and tetra-centric ring chromosome 18 using a centromeric probe D18Z2 for chromosome 18. The left panel shows regular chromosome 18, the top dicentric ring 18 and the bottom tetracentric ring 18. The right panel shows dicentric ring 18 and FISH insets pericentric/tetracentric ring 18. (b) Identification of a 2q32/16p13.3 translocation derivative of chromosome 16 by complete chromosome painting probes for chromosome 2 (WCP2) and 16 (WCP16). (C) Identification by dual-colour double fusion probes of ABL1/BCR gene fusions in interphase and metaphase cells (thin arrows point to the normal signal and thick arrows point to the abnormal fusion signals). (d) Diagnostic use of the ETV6 and RUNX1 probes for the identification of two cryptic t(12;21) (p13;q22) fusion signals, loss of ETV6 signal and gain of three additional RUNX1 signals (thin arrows point to the fusion signals and thick arrows to extra RUNX1 signals). All images are from Yale clinical cytogenetics laboratory (Cui et al., 2016).
FISH

Figure 4. Using whole-chromosome-painting probes, Fluorescence in situ hybridization (FISH). To rule out the presence of an additional copy of chromosome 19 (i.e. trisomy 19) in this metaphase distribution, derived from a glial cell taken from a mouse's brain, chromosome painting probes were used. Chromosome 19 is green in colour (McNeil and Ried 2000).

**IMPORTANT COMPARATIVE GENOMIC HYBRIDIZATION IN EARLY DETECTION OF BREAST CANCER**

Comparative genomic hybridization (CGH) has emerged to promote the aggregation of high-resolution data of cancer-associated genomic imbalances as a high-throughput genomic technology. High-resolution genomic microarray CGH (Li et al., 2020). The rapidly growing CGH publication database already contains around 1500 tumours and is beginning to reveal genetic anomalies characteristic of certain forms of tumour or stages of tumour progression (Forozan et al., 1997). Comparative genomic hybridization has created DNA copy number profiles for most of the publicly available breast cancer cell lines (Forozan et al., 2000). High-resolution CGH analysis of breast cancer shows non-random associations between particular amplicons in many regions where DNA copy number is frequently obtained or lost. That specific genetic modification is preserved in breast cancer cell lines despite repeated passage through tissue culture (Climent et al., 2007).

Recent results released and discussed at scientific meetings have suggested higher rates of implantation and pregnancy after microarray testing, resulting in changes expected for quite some time. By using markers spanning much of the genome, it is not only possible to detect aneuploidy in single cells but also translocations. The validation results indicate that the CGH array in single cells has a resolution of 6 Mb. Therefore, most translocations can be tested as this is also the limit of karyotyping. Translocations of smaller exchanged fragments may also classify the translocation, as three out of the four fragments are above 6 Mb fragment (Munné, 2012). Over the past decade, genomic microarray technology has greatly matured. The technique offers a locus-by-locus measure of the variance of DNA copy-number (CNV) and represents another way to improve mapping resolution. Postnatal chromosomal array techniques have far higher diagnostic results (15-20 per cent) than G-banded karyotyping does for genetic testing of people with unexplained developmental delay, intellectual impairment, autism, or other congenital abnormalities. As the first-tier cytogenetic diagnostic test for people with these diseases, the International Standards for Cytogenomic Array Consortium recommends it (Lee et al., 2012). The genotyping of metastatic samples, primarily focused on array-based comparative genomic hybridization (aCGH) and next-generation sequencing, is the growth of targeted therapies and the emergence of personalized medicine (NGS). Another solution to resolving aCGH's drawbacks, such as the repeat-rich regions, is next-generation sequencing. The genomic analysis should be paired with expression analysis to elucidate individual genes related to breast cancer development and progression. Identification of new molecular targets for breast cancer eradication will contribute to the elucidation of the functions of the affected genes (Ueno et al., 2012).

Figure 5. The photomicrograph of Fluorescence shows the effects of comparative genome hybridization of invasive ductal carcinoma tissue. (a) Tumour tissue extracted DNA was labelled green, and (b) natural reference DNA was labelled red. (c) The chromosome of the regular metaphase was counterstained blue with DAPI. (d) Tumour and normal DNA have been hybridized to the normal chromosome of the metaphase. There is a
predominantly green colour in chromosomal regions, which were over-represented in the tumour, while regions with deletions in the tumour show a predominantly red colour. The overlap reflects the ratio of changes in the copy number between the tumour and the DNA control (Zhang et al., 2015).

**COMPARATIVE CLASSICAL CYTOGENETIC TOOLS WITH FLUORESCENCE IN SITU HYBRIDIZATION**

Hybridization of Fluorescence in situ (FISH) enables cytogenetic analyses of primary tumors without culture (Thompson and Gray 1993). In the identification and evaluation of human malignancies, one of the greatest impacts has been in the non-dividing interphase nucleus, chromosome translocations, deletions, amplification of particular genes, and chromosome number changes identified using probes ranging from whole chromosome 'paints' to individual gene-specific probes. Progress in FISH technology has also benefited from gene mapping (Price 1993). In comparison to the methods mentioned previously separate denaturation or proteinase K digestion changes are not needed for each sample. This technique allows retrospective studies of large series of tumors and is also useful for the routine diagnostic use of formalin-fixed material to apply FISH (Hyytinen et al., 1994). The FISH technique also helped us determine the degree of amplification and the size of the intrachromosomal amplified regions at metaphase and interphase (Bar et al., 1992).

Fluorescence in situ hybridization (FISH) is used in genetic toxicology for the study of chromosome damage with improved efficiency and precision to distinguish certain forms of chromosome aberrations, in addition to classical cytogenetic methods for scoring chromosomal aberrations (Hovhannisyan, 2010). The study shows the utility of a combination of classical karyotyping and FISH to determine the chromosome origin of double-minute chromosome amplified DNA sequences in cancer cells (Giollan et al., 1996). FISH identified Cells with clonal chromosomal defects with rates of aneuploidy ranging from 6% to 92% (median 59%). In addition, there was a gain of centromeric signals for chromosome 11, most likely corresponding to hyperdiploid; aberrations of chromosome 17 in specimens from 26 patients (87%) were hyperploid as well; however, four cases (13%) showed loss of chromosome 17 centromeres (Fieg1 et al., 1995). Modern high-throughput techniques affect research to classify new genomic regions associated with tumours (Liehr et al., 2015).

In correlating karyotype abnormalities with diagnosis, prognosis, and response to therapy in haematological neoplasias, classical cytogenetics described by chromosomal banding techniques has been effective. Such approaches, however, require a high cell division rate and good chromosomal morphology, which pose challenges for cytogeneticists, and a long period of testing and study, which is typically a challenge for doctors (Varella, 2003). Also, FISH is considered safer and has the added benefit of using several fluorochromes to differentiate between different targets simultaneously (Bartlett, 2004). These approaches range from DNA fluorescence in situ hybridization (FISH)-based kilobase-level resolution imaging approaches of individual cells to genome-wide sequencing strategies that collect nucleotide-level data from different sample types. In conjunction with the combinatorial use of multiple approaches, technical developments have led to new rearrangement groups and mechanistic insights into processes that drive structural changes in the human genome (Hu and Ly 2020). FISH and traditional cytogenetic experiments often offer a false negative outcome (Alayed et al., 2013).

**Figure 6.** Schematic diagram of karyotyping (Qaisar and Bhat, 2017).
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Figure 7. The concepts of in situ hybridization of Fluorescence. (a) A DNA probe and a target sequence are the basic elements. (b) Before hybridization, the DNA probe is indirectly marked with hapten (left panel) or specifically marked with hapten (left panel) by adding a fluorophore probe (right panel). (c) The labelled probe and the target DNA are denatured to yield single-stranded DNA. (d) They are then mixed, which enables complementary DNA sequences to be annealed. (e) If the probe has been indirectly labelled, the visualization of the non-fluorescent hapten using an enzymatic or immunological detection system involves an additional step. Finally, Fluorescence microscopy tests the signals (Shakoori, 2017).

LIMITATION OF THE FLUORESCENCE IN SITU HYBRIDIZATION

While direct preparations may be carried out, cell culture is usually needed (1-10 days), complex karyotypes with suboptimal morphology may be encountered, submicroscopic or cryptic rearrangements can result in a false-negative result, normal karyotypes may be observed after therapy-induced tumour necrosis or overgrowth of normal stromal cell support low cell density and the release of cells from the bone matrix are also issues with bone tumours (Bridge. 2008). There is still a lack of a mechanistic approach to the whole FISH system, and the key limiting steps for hybridization remain uncertain (Lima et al., 2020). The rate-limiting phase was still the slow reaction rate of the reagents used in the probe solution. In particular, hybridization was slowed by using formamide, which acts as a double-helix destabilizing agent (Nguyen et al., 2018). However, a fluorescence microscope is required for FISH, and the signals are labile and easily fade over time (Kim et al., 2011). In recent years, the combination of microfluidic techniques and FISH have tackled weaknesses in the consumption of probes and hybridization times, making the experimental process more sustainable and adaptable to high-throughput innovations (Huber et al., 2018). As cytogenetic defects have been found in samples that appear normal by morphological and conventional cytogenetic examination, the FISH analysis provides enhanced sensitivity in many cases. The combination of cytogenetic, FISH and molecular studies offers a powerful method for diagnosing and sub classifying malignant diseases into clinically and biologically important subgroups, selecting effective therapies, and monitoring the effectiveness of therapeutic regimens (Gozzetti and Le, 2000). It is also recommended that FISH and CGH findings be re-evaluated by one another.
to resolve these technological artefacts. However, CGH is of potential benefit in characterizing chromosomal alterations and could help produce tumour-specific sets of FISH probes within a few days to obtain genetic information of prognostic value (Jacobsen et al., 2000).

CONCLUSIONS

From this review article conducted the following points as follows:

1. Fluorescence in situ hybridization (FISH) more accurate than classical cytogenetic such as karyotyping

2. Comparative genomic hybridization (CGH) is very important for the create a wide range of chromosomal profile

3. Both Fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) have many limitations and disadvantages

4. Probes is the key success for both Fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH)

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