Review Article

Application of Fluorescence in situ Hybridization (FISH) Technique in Medical Science

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Abstract:

Fluorescence in situ Hybridization (FISH) is a powerful tool used in the detection of chromosomal abnormalities. Fluorescence in situ Hybridization (FISH) is a pivotal cytogenetic technique that has provided significant advances in both the research and diagnosis of hematological malignancies and solid tumors. From medical perspective, Fluorescence in situ Hybridization (FISH) can be applied to detect genetic abnormalities such as characteristic gene fusions, aneuploidy, and loss of a chromosomal region or a whole chromosome or to monitor the progression of an aberration serving as a technique that can help in both the diagnosis of a genetic disease and suggesting prognostic outcomes. Fluorescence in situ Hybridization (FISH) is based on denaturation of target DNA/RNA and probe DNA/RNA, followed by hybridization. Fluorescent probes bind to only that region of the chromosome which shows a high degree of sequence complementarity. Microscopy and imaging is done to identify the chromosomal/gene rearrangements. Fluorescence in situ Hybridization (FISH) can also be applied to such research applications as gene mapping or the identification of novel oncogenes or genetic aberrations that contribute towards various cancers. The aim of this review article is to highlight the concept of Fluorescence in situ Hybridization (FISH), its application, and its advantages in medical science.

Keyword: *FISH, Genetic anomalies, Cytogenetic, Medical Science, probe DNA/RNA.*

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Introduction:

Medical Science has seen some tremendous modifications in cytogenetic techniques over the past few decades. The introduction of Fluorescence in situ Hybridization (FISH) has been one of those major changes that have impacted the diagnosis, treatment and research of haematological malignancies and genetic disorders[1]. Chromosome banding techniques revolutionized cytogenetic analysis and have been pivotal in the understanding of genetic changes in both constitutional and acquired diseases[1,2].

After being introduced in the late 1980s, the Fluorescence in situ Hybridization (FISH) technique has rapidly increased the qualitative advantages of cytogenetic analyses, such as it can readily detect trisomies and translocations in metaphase spreads and interphase nuclei using entire chromosome-specific DNA libraries, was heralded as a further revolution in cytogenetic analysis[3].

Furthermore, it is economical in terms of the time it takes. By Fluorescence in situ Hybridization (FISH) technique specific cytogenetic abnormality along with a copy of aberrations numbers, can be measured and outlined properly. Ultimate the treatment has become easier and life expectancy of people has been increased[4].

All these unique features of high sensitivity and specificity of Fluorescence in situ Hybridization (FISH) and the speed with which the assays can be performed have made Fluorescence in situ Hybridization (FISH) a powerful technique with numerous applications, and it has gained general acceptance as a clinical laboratory tool[1,5].

Mechanism of Fluorescence in situ Hybridization (FISH) procedure:

The Fluorescence in situ Hybridization (FISH) technique tailor binding or annealing specific

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nucleic acid probes to their counterpart DNA or RNA sequences labeled with fluorescent and consequent visualization of hybridization signals of those specific probes is detected when any nucleic abnormality is present[7]. The DNA or RNA sequences, at first, from the tissue of interest are being denatured to get single strand, and that tissue of interest can either be formalin-fixed, paraffin-embedded section or fresh-frozen tissue. [8] Then, the selection of a FISH probe which is a pivotal phase of this technique as it solely detects the targeted chromosomal anomalies by the assigned probes. The choices, usage as well as the execution of FISH probes depend upon the diseases' anomalies or anomalies under field of interest[9].

The fluorescent labeling of the probe after its selection as probe for the interested investigation can either be done directly or indirectly. The flulochrome(s), in direct fluorescence labeling, is directly attached to the specific probe DNA; whereas in indirect labeling, a hapten is included in the probe DNA. The flurochrome(s) in direct fluorescence labeling is identified by the fluorescence microscope; on the other hand, hapten, through an immunohistochemical process, is identified by a fluorophore-tagged antibody executed in opposition of that hapten. Next, the hybridization process in which the DNA or RNA sequences of interest and the fluorescence labeled probe brought together; and at that time, the sequences of interest are annealed by the fluorescent labeled probe[10]. The slides are then examined after post hybridization washing process which takes away the excessive unbound probe.

In the tissue of interest with a homologue DNA sequence, the each distinct fluorescent dot detects the single facsimile of the chromosomal locus. The two dots detect diploid nuclei, and the number of more than two dots will be detected in case of duplication in the location of interest. However, any decrease in number of dots or no dots might be resulted in case of loss in the location of the interest. In experiments, a multicolor Fluorescence in situ Hybridization (FISH) detection process could have several probes e.g. different probes labeled with different colors like red, green, yellow, and aqua that allows simultaneous cross-examination of multiple cytogenetic signatures. Overlapping wavelength spectrums of the currently available fluorochromes limit the maximum number of probes in a single experiment to four [11].

Different probes labeled with same fluorochrome can be distinguished by the proportion of those used fluorochromes; and these ratio labeling techniques permit in a single visualization to image the all 46 human chromosomes where each having different distinct color. The technologies used in these purposes have wide range of usage e.g. MULTIPLEX FISH (M-FISH)[12], SPECTRAL KARYOTYPING[13] and COMBINED BINARY RATIO LABELLING (COBRA)[14].

To obtain a significant result, sufficient number of cells must be measured due to the sophisticated inconsistency of Fluorescence in situ Hybridization (FISH) signals. Fluorescence in situ Hybridization (FISH) results depend upon the process and methodology can be presented in two distinct ways:

- In microscopic and manual counting of labeled cells Fluorescence in situ Hybridization (FISH) evaluating process, the result is present as cells per unit (liter of liquid or gram of solid[3,19].
- In advanced microscopy techniques and digital imaging FISH evaluating process, the result is present as on a relative volume or area basis that can also be transformed to cells per unit of liquid or solid[3,19].

Fluorescence in situ Hybridization (FISH) technique results are usually reported as a percentage of nuclei containing more than two copies of a particular locus of interest or as a percentage of cells showing a loss or gain of a particular chromosomal region[15].

Use of Fluorescence in situ Hybridization (FISH) in Medical Science:

Fluorescence in situ Hybridization (FISH) signals in S phase nuclei enumerate the ordered process of human genome replication timing throughout the S phase of cell cycle correlates

with nuclear position, chromatin structure and gene activity that consequently envisaged the aberrations of chromosomal abnormalities leads to the congenital disorders or diseases occurred in patients ranging from neonatal to adult age[16, 17].

The child development disability regarding congenital disorders i.e. Turner syndrome, Klinefelter syndrome, Down syndrome as well as other congenital diseases including different types of cancers, and dermatological aberrations can be understood and determined more efficiently by Fluorescence in situ Hybridization (FISH) than the conventional cytogenetic (CC)[18.19].

To detect chromosomal translocation which play vital role detecting and selecting medical approaches or actions in various leukemia, the Fluorescence in situ Hybridization (FISH) technique considered as effective standard in this regard[20].

Fluorescence in situ Hybridization (FISH) test, in therapeutic implication, smoothen the way for different diagnosis in chronic lymphocytic/ myeloid leukemia (CLL/CML) which remains sluggish in some patients but can be vigorous in others where common most recurrent chromosomal aberrations are trisomy 12, del(13q), del(11q), del(17q) and del(6q);and other types of B-cell lymphoma or leukemia. In addition, this technique is also an effectual procedure to identify the cyclin D1 arrangement, usually in various reasons when the immunohistochemistry is not contributing [21,22].

Fluorescence in situ Hybridization (FISH) is also a good tool to identify as well as to measure other hematopoietic malignancies i.e. multiple myeloma (MM), and myelodysplastic syndrome (MDS).The early stage of tumor development in MM, there has been the likeliness to miss the detection of low proliferation magnitude of tumor cells during primary translocation by CC, whereas Fluorescence in situ Hybridization (FISH) is comparatively more effectual due to its capability to analyze interphase nuclei and a little of genetic anomalies.[23] In MDS, metaphase chromosomal banding considered as the objective standard for clonal banding detection not possible by CC but can be done by FISH, because of its identifying competence in minor abnormal clones as well as cryptic chromosomal anomalies that in consequence helps the physician, therapeutics as well biomarkers to take proper judgments in this regard[24].

The most common type of chromosome relocation to categorize the subtypes of prostate cancer in respond to specific therapies, fusion of TMPRSS2 with oncogene ERG, was effectively functioned with a dual color ERG- break FISH which can detect ERG gene not indicating the 5'-partner to what the ERG is fused to used.After that, a tri-color Fluorescence in situ Hybridization (FISH) assay combining the red/ green spaced out and an orange color fusion probes for TMPRSS2, and 3' region of the ERG respectively and recently, a four color FISH assay can detect either TMPRSS2 or ERG rearrangements regardless of partner gene has been developed.[25, 26] Human epidermal growth factor receptor factor-2 (HER-2), also called c-erbB-2 located at chromosome 17q12-21.32, and programmed in a trans-membrane protein of 185 kDa, escalation results breast cancer; so far, Fluorescence in situ Hybridization (FISH) application is most thriving in this regard to choose a targeted therapy[27].

Melanoma, skin malignancies related to melanin producing heterogenetic group, was most accurately detected by FISH probes in 2009 melanoma. Each set consists of four probes targeted 6p25 (RREB1), 6q23 (MYB), 11q13 (CCND1), and centromere 6 (CEP6); thus detected best sensitivity and specificity, 86.7 and 95.4%, respectively. The optimal discriminatory algorithms for defining positive Fluorescence in situ Hybridization (FISH) results based on these four probes were also established[28]. These four probes were also established in these circumstances and are now commercially available and have been tested in a number of subsequent studies to aid in the diagnosis of ambiguous melanocytic lesions.

In detecting and measuring the tumors with comprehensive metastatic potential, the Fluorescence in situ Hybridization (FISH) technique has the potentiality to play a future

role e.g. monosomy 3 analysis by FISH to confirm the metastatic uvealmelanom, but the clinical usability in these issues yet to be finalized. Patients having myeloid malignancies and neutrophilic dermatoses, the Fish has been utilized to appraise bone marrow as well as skin infiltrates in recent times[29].

In near future, the three dimensional Fluorescence in situ Hybridization (FISH) (3D FISH) will play a vital role in detecting measuring, enumerating the chromosomal anomalies within the tissue in its natural context; and annexing the compatible relationship with the cutting edge multi-color three dimensional imaging technology enable the Fluorescence in situ Hybridization (FISH) technique much more pragmatic to study the higher order chromatin structure as well as to visualize all the 46 chromosomes of the human being which in consequent will pave way for minute level accuracy in assessing the disorders or diseases regarding cytogenetic[30, 33].

Moreover, the four dimensional Fluorescence in situ Hybridization (FISH) (4D FISH) that is in vivo or in living cells fluorescence labeling probe technique will enable the physicians, researcher as well as biomarkers to take in time decisions appropriately though it is at present has limitation to apply[31, 32].

Conclusion:

In comparing a regular application of clinical diagnosis, the Fluorescence in situ Hybridization (FISH) is much more specific and simple as well as reliable than the other molecular profiling techniques e.g. CGH, SNP array analysis. Moreover, recent studies have suggested that FISH can be used as a supplementary diagnostic tool in pigmented lesions, but it must not be used as a stand-alone test, and it cannot replace traditional histopathology analysis. One must correlate clinical, pathologic, and molecular information. "FISHing" in cytogenetic continues to evolve, and we look forward to future studies to further delineate its roles in various congenital diseases. At present, Fluorescence in situ Hybridization (FISH) become a handy tool for identifying specific genomic anomalies, essential information for the diagnosis of diseases, risk evaluation and presage.

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