FISH - A Revolutionized Cytogenetics

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Introduction

Fluorescent in-situ hybridization (FISH) is a molecular cytogenetics technique which was introduced in late 1980s. The molecular era of cytogenetics started with the procedure of in situ hybridization. With the use of fluorescent probes in the technique, the in situ hybridization is called as FISH. It is based on the same principle as the Southern blot method. It is a powerful cytogenetic technique, localizes the presence or absence of specific DNA sequences on chromosomes.

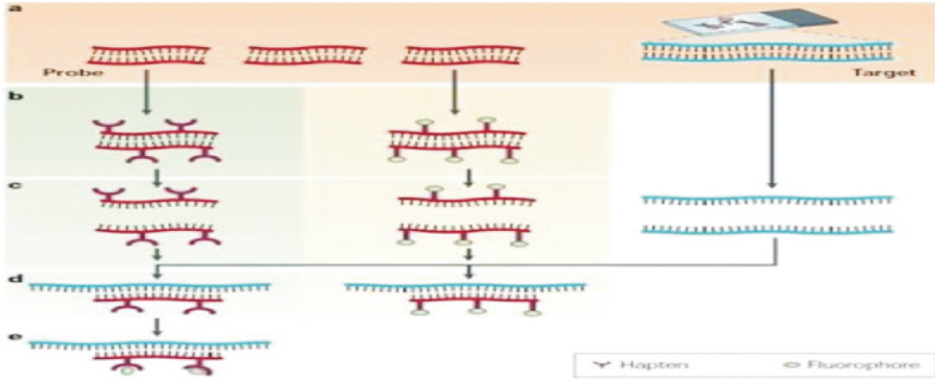
We as oral health professionals know that squamous cell carcinoma is the most common cancer of oral cavity encompassing 90% of all oral malignancies with about 3,00,000 new annual cases worldwide.[1-3] Molecular changes in oral squamous cell carcinoma (OSCC) are well documented with wide range of genetic changes. In OSCC the proliferative activity of oral mucosa is due to the malignancy which in turn is activated by the multiple mutations in the growth regulatory genes. These genetic changes are also the focus of attention in dentistry especially in oral and maxillofacial pathology.

Over the years the cytogenetics has expanded rapidly. It plays an important role in cancer diagnostics and management. Among the advanced molecular techniques, FISH has a perfect balance of high specificity, sensitivity and rapidity and used in routine clinical labs. FISH has gained it's importance in diagnosis due to it's simplicity and reliability in evaluating the key biomarkers in various tumours.[4]

Principle

FISH works by exploiting the ability of one DNA strand to hybridise specifically to another DNA strand. FISH uses the fluorescent DNA probes to target specific chromosomal locations in the nucleus resulting in the coloured signals which can be detected by the fluorescent microscopy. It localizes the presence or absence of specific DNA sequences on chromosomes.

FISH doesn't require cell culturing and uses fresh or paraffin- embedded interphase nuclei for rapid evaluation. FISH can also be performed using the bone marrow or peripheral blood smears. Once the sample fixed to the microscopic slide, the desired cells are hybridized to a nucleic acid probe which is labelled with a reporter molecule. This reporter molecule is either attached to fluorochromes that enables direct detection or a hapten that can be detected indirectly.[5-6] The second method depends on Immunohistochemistry (IHC) for probe detection which is based upon the binding of antibodies to the specific antigens. This antigen- antibody binding is demonstrated with a coloured histochemical reaction that is visible by light microscopy or fluorochromes with ultraviolet light.[7] IHC is limited by the availability of antibodies. Reporter molecules most frequently used for direct detection are FITC, Rhodamine, Texas Red, Cy2, Cy3, Cy5 and AMCA. For indirect detection the reporter molecules used are Biotin, Digoxigenin and Dinitrophenol. Fluorescent dyes are non-protein molecules that absorb light and remit it at higher wavelength.



[Figure: Macmillan publishers Ltd: Nat Rev Genet 6: 782- 792]

With the discovery of numerous disease related genes in recent years the applications of FISH has also broadened and includes more genetic diseases, solid tumours and hematologic malignancies.

Genetic Mutations

Cell cycle controls are deranged in most tumours. Tumour cells are triggered more readily and without restraints. DNA replication in a cell cycle is an accurate process and if there is any DNA damage repairing process starts. But if there is a failure in the DNA repair, there is genetic damage and it leads to mutations and chromosomal rearrangements, leading to activation of growth- promoting oncogenes, inactivation of tumour suppressor genes and alterations in genes that regulate apoptosis. Due to these altered genes there is unregulated cell proliferation and decreased apoptosis leading to clonal expansion. Cell cycle checkpoints results in the delay or arrest of cell cycle thus triggering apoptotic pathways. Loss of mitotic checkpoints control is a common event in human cancer cells leading to tumourigenesis. There are two cell cycle checkpoints, one is G1/S transition where arrest is mediated by p53by inducing cell cycle inhibitor p21, and other is G2/M transition which involves both p53 dependent and independent mechanism.

P53 is the most common target for genetic alterations in human tumours. Little over 50% of human tumours owns the mutations of this gene. Major activity of p53 protein is cell- cycle arrest and apoptosis initiation in response to DNA damage. P53 activation leads to upregulation of a. p21 which is a CDK inhibitor, b. GADD45 which causes DNA repair and of c. BAX which is an apoptosis gene. All these genes upregulation leads to either successful repair or apoptosis. If the cell is with mutations or loss of p53, there is DNA damage and p53 dependent genes are not activated; hence no cycle arrest and no DNA repair leads to mutant cells with further expansion and additional mutations and finally results in malignant tumour.

Probes

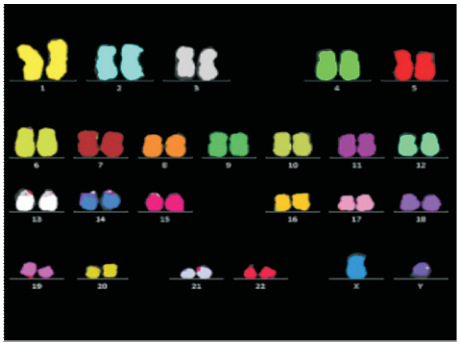
The FISH technique is dependent on probe hybridization with fluorescent tag, complementary in sequence, to a section of DNA on a target gene. The tag and probe are applied to a sample of interest under conditions that allow for the probe to attach itself to the complementary sequence in the specimen if it is present. Once the specimen has been treated, excess fluorophore is washed away and sample is visualized with fluorescent microscopy. By quantifying the amount of fluorescence, it can be determined if the type of cell the probe was designed for is present, and if so then how much of it is present in a sample.

The potential of various applications of FISH is enhanced by multicolour detection of simultaneously hybridized probes. This is useful particularly when structural chromosome aberrations involving different chromosomal regions are to be diagnosed or when several numerical aberrations should be detected in parallel.[7] One of the critical consideration in FISH is the probe choice. There are wide range of probes which can be used for hybridization.

Broadly there are three types of probes and each has different applications; locus specific probes, whole- chromosome painting probes and repetitive sequence probes.

Whole- chromosome probes are the complex DNA probes. These are derived from a single type of chromosome that are flow- sorted or microdissected and provide accurate coverage with excellent signal specificity and high fluorescent intensity.[8] These probes are used to identify the whole human chromosomes, chromosome rearrangements,

analysis of translocation events, mutagenesis analysis, radiation and sensitivity testing and identification of human chromosomes on hybrid cells. These probes are also called as chromosome painting probes. Chromosome 'painting' is a technique which involves hybridization of fluorescently labelled chromosome-specific, composite probe pools to cytological preparations, and identify the structural as well as numerical chromosomal aberrations in human pathology with high specificity and sensitivity.[9] Chromosome painting allows the individual chromosome visualization in metaphase or interphase cells. This technique may also be used to identify cross- species homology by labelling probes from one species for hybridization with chromosomes from another species. These probes are actually the collection of smaller probes. Each of these probes binds to a different sequence along the length of a given chromosome and each of these probes are with different fluorescent dyes so each chromosome is labelled to its own unique colour resulting in full colour map of chromosome known as spectral karyotype.



[Spectral karyotyping of human chromosomes]

Repetitive sequence probes hybridize to specific chromosomal regions or structures that contain short sequences that are present in many thousands of copies.[5,8] These probes permits the highly specific chromosome enumeration and aneuploidy detection with hybridization times as short as 30 minutes.

This third types of probes are usually genomic clones which vary in size depending on the nature of cloning vector, from plasmids (1- 10 kb) to the larger vectors (80kb- 1Mb). These probes are limited to the genes or chromosomal regions commonly altered in genetic diseases or during carcinogenesis. Locus specific probes bind to a particular region of a chromosome and useful when scientists have isolated a small portion of a gene and want to determine on which chromosome this gene is located.

Advantages of FISH in Cytogenetics

The capabilities of cytogenetics have greatly expanded after introduction of FISH. This is highly sensitive, specific and have rapid turnover along with high efficiency. Processing of FISH material can be done in 4- 24 hours, and

analysis of 1000- 2000 cells can be done in 15- 45 minutes. Thus enabling the information on the cytogenetic pattern of tumour cells to be achieved within a sufficient frame of time for use in treatment strategies.[3,10]

Another advantage is the possibility to study chromosomal aberrations in non- dividing cells, unlike other cytogenetic tests which is useful for visualization of chromosomal aberrations directly in cytological preparations and tissue sections.

Cytogenetic data can be obtained from poor samples, that contain too few cells.

Limitations of FISH

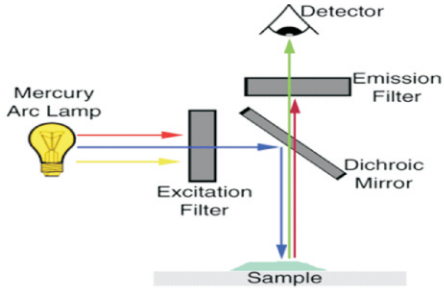
Most notable limitation of FISH is that the detection of abnormalities is limited for only those which have their probes available. It can assess only one or few abnormalities simultaneously. FISH is not a good screening tool for cytogenetically heterogenous diseases like AML or ALL. Cytogenetic data can be obtained only for target chromosome.

FISH is highly sensitive for trisomy but less sensitive for detecting chromosomal aberrations which involves chromosome loss or deletion. The processing of bone marrow and peripheral blood cells from patients with hematologic malignant diseases is relatively simple but preparation of paraffin- embedded tissues or frozen sections from solid tumours or lymph node biopsies is substantially more difficult.

Another limitation is that the technique needs fluorescence microscopy and an image analysis system.

Fluorescent microscopy involves the use of fluorescent microscope which is one of the light microscope but uses fluorescence to generate an image which is a 3D- image and study specimens which can be made to fluorescence. This microscope uses a much high intensity light to illuminate the sample. This light excites the fluorescence species in the sample for a longer wavelength. The image produced is based on the second light source or the emission wavelength of fluorescent species rather than from the light originally used to illuminate and excite the sample.

The advantage of fluorescent microscopy is that sensitivity is high enough to detect as few molecules per cubic millimetre. Also different molecules can be stained with different biomolecules allowing multiple types of molecules to be tracked simultaneously.



[Fluorescent Microscopy]

While using fluorescent microscopy the object under study is marked with a molecule called fluorophore (a dye). When fluorescent light is activated the light source for illumination is separated from the fluorescent molecule which is much weaker and this is done by an emission filter.

Applications of FISH

This cytogenetic technique is used in detection of numerical and structural chromosomal abnormalities, detection of early relapse or minimal residual disease and detection of gene deletions and gene amplifications. This technique identifies the lineage of neoplastic cells, marker chromosomes and origin of bone marrow cells following the stem cell transplantation.

FISH can also monitor the effects of therapy.

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