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A Study on the Role of Molecular Cytogenetics for Cancer Diagnosis

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Abstract – Cytogenetic aberrations may escape detection or recognition in traditional karyotyping. The past decade has seen an explosion of methodological advances in molecular cytogenetics technology. These cytogenetics techniques add color to the black and white world of conventional banding. Fluorescence in-situ hybridization (FISH) study has emerged as an indispensable tool for both basic and clinical research, as well as diagnostics, in leukemia and cancers.

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INTRODUCTION

FISH can be used to identify chromosomal abnormalities through fluorescent labeled DNA probes that target specific DNA sequences. Subsequently, FISH-based tests such as multicolor karyotyping, comparative genomic hybridization (CGH) and array CGH have been used in emerging clinical applications as they enable resolution of complex karyotypic aberrations and whole global scanning of genomic imbalances. More recently, crossspecies array CGH analysis has also been employed in cancer gene identification. The clinical impact of FISH is pivotal, especially in the diagnosis, prognosis and treatment decisions for hematological diseases, all of which facilitate the practice of personalized medicine. This review summarizes the methodology and current utilization of these FISH techniques in unraveling chromosomal changes and highlights how the field is moving away from conventional methods towards molecular cytogenetics approaches

The rationale of classifying hematological malignancies is based on the separation of diseases with distinct clincopathologic and biologic features. Recognizing the association between specific cytogenetic abnormalities and certain morphologic and clinical features, the World Health Organization has categorized four unique subtypes of acute myelocytic leukemia according to cytogenetics. Therefore, cytogenetics study is currently considered a mandatory investigation in newly diagnosed leukemia owing to its usefulness in disease diagnosis, classification and prognostication. The vast majority of recurrent chromosomal rearrangements associated with leukemia were originally identified by cytogenetic analysis, which remains the gold standard laboratory test since it provides a global analysis for abnormality on the entire genome. Although banding techniques represent the central theme at every cytogenetics laboratory, it is sometimes difficult to karyotype the tumor cells from a patient owing to unfavorable factors such as low specimen cell yield, low mitotic index, poor quality metaphases and other technical difficulties. In addition, these techniques demand expertise such that the interpretation of variant translocations or complex karyotypic configurations may challenge even the most experienced cytogeneticist. The fluorescence in-situ hybridization (FISH) technique can be used to map loci on specific chromosomes, detect both structural chromosomal rearrangements and numerical chromosomal abnormalities, and reveal cryptic abnormalities such as small deletions. It has managed to overcome many of the drawbacks of traditional cytogenetics. FISH is routinely applied in the clinical laboratory and allows nearly unlimited and targeted visualization of genomic DNA using either metaphase spread, interphase nuclei, tissue sections, or living cells. FISH applications are particularly important for the detection of structural rearrangements such as translocations, inversions, insertions, and microdeletions, as well as for identification of marker chromosomes and characterization of chromosome breakpoints.

FISH is essentially a molecular technique which has greatly enhanced the accuracy and efficiency of cytogenetic analysis by bringing together cytogenetics and molecular biology. The impetus for many of these FISH technology innovations has been the direct result of an increased understanding of the sequence, structure and function of the human genome, which has highlighted the intricate marvel of the DNA architectural blueprint housed within our chromosomes. This review will summarize the development, current utilization and technical pitfalls of molecular cytogenetics techniques in clinical and research laboratories. Furthermore, this article highlights how, with advancements in technology, the

study of chromosomal abnormalities is moving away from conventional methodologies towards molecular cytogenetics approaches.

REVIEW OF LITERATURE

FISH versus cytology Bladder cancer is most frequently diagnosed when investigating hematuria. Urine cytology is performed on a urine sample which is centrifuged and the sediment is examined under the microscope to detect abnormal cells that may be shed into the urine by a cancer. However, many early bladder cancers may be missed by this test and hence a negative or inconclusive test does not effectively rule out bladder cancer. Increased chromosomal instability and aneuploidy are characteristic of bladder tumor progression. Using the FISH method, a mixture of CEP 3, CEP 7, CEP 17, and locus specific identifier p16 FISH probes is used to enumerate chromosomes 3, 7, and 17 and detect the 9p21 locus deletion on chromosome 9, which is a non-invasive strategy for bladder cancer screening. FISH versus immunohistochemistry Since patients with HER2 amplified breast carcinoma who receive trastuzumab (Herceptin) have improved clinical outcomes, accurate HER2 testing is essential for quality patient care. Clinical practice guidelines for HER2 testing in breast cancer have recently been published. In practice, the HER2 status can be determined by immunohistochemistry (IHC) and FISH methods. IHC detects HER2 transmembrane protein by using a labeled antibody. Studies show that non-amplified cells with negative IHC staining have less than half of the receptors compared with those with HER2 gene amplification. A category of intermediate HER2 expression status (2+) exists with staining intensity - between that of clear-cut negative (0 and 1+) and positive (3+), and these cases should undergo FISH to document the HER2 status. Currently, based on the more tedious nature and higher cost of FISH, most countries have recommended two-step testing for HER2 status, with FISH used to confirm 2+ IHC staining. FISH versus conventional cytogenetics Although translocation and PML-RARα fusion are regarded as highly specific for APL, t(15;17) (q22;q21) not associated with APL and negative for PML-RARα rearrangement has been reported. Detailed molecular analysis showed no evidence of PML-RARα rearrangement, thus confirming that the translocation breakpoints in this patient did not involve the PML and RARα genes. These observations showed that morphologic, cytogenetic and FISH/molecular features must all be considered for an accurate diagnosis of APL. These cases highlighted the importance of combined modalities. FISH versus molecular biology The advantage of FISH is for the detection of chromosomal translocations that are not amenable to polymerase chain reaction (PCR) detection due to scattering of breakpoints throughout the gene, such as CBFβ rearrangement.

RESEARCH STUDY

FISH probes are much larger and hence there is better coverage of non-clustered potential breakpoints than in PCR analysis. Variant transcripts generated from classic translocations may also cause diagnostic dilemmas. We encountered a rare case of p230 CML in which the e19a2 BCR-ABL fusion transcript level was underestimated by a commercial real-time quantitative PCR (RQ-PCR) reagent kit. In our patient the RQPCR result was disproportionately lower than that obtained by FISH. Since a commercial RQPCR system targets the M-bcr breakpoint catering for the classic b2a2 and b3a2 transcripts, the presence of a variant e19a2 transcript may produce an underestimated or even falsely negative result, as the primers are spaced widely apart, thus lowering the PCR efficiency. Multicolor FISH Multicolor FISH is based on the simultaneous hybridization of 24 chromosome-specific composite probes. Multicolor FISH is suitable for identification of cryptic chromosomal aberrations, such as the translocation of telomeric ends, which is difficult to detect using conventional cytogenetics alone, and the identification of unidentified (marker) chromosomes, and unbalanced chromosomal translocations that remain elusive after conventional cytogenetics analysis. These chromosome-painting probes are generated from flow-sorted human chromosomes. Chromosome-specific unique colors are produced by labeling each chromosome library either with a single fluorochrome or with specific combinations of multiple fluorochromes (combinatorial labeling). Two multicolor fluorescence technologies have been introduced, multiplex FISH (M-FISH) and spectral karyotyping (SKY).) The difference between the two techniques is in the image acquisition process; they employ different methods for detecting and discriminating the different combinations of fluorochromes after in-situ hybridization. In SKY, image acquisition is based on a spectral imaging system using an interferometer and a charge-coupled device camera.

CONCLUSION

In the past decades, innovative technical advances in the field of cytogenetics have greatly enhanced the detection of chromosomal alterations and have facilitated the research and diagnostic potential of cytogenetics studies in constitutional and acquired diseases. FISH plays a central role in combination with karyotyping to rapidly detect and verify specific chromosomal aberrations. The field of molecular cytogenetics has expanded beyond the use of FISH to other techniques that are based on the principle of DNA hybridization. The considerable gap in resolution conventional cytogenetics techniques (5-10 Mb pairs) and molecular biology techniques (base pairs) has been bridged to a large extent by FISH, which allows the assessment of genetic changes on chromosome preparations. Some noteworthy innovations that have altered the landscape of clinical and research investigations include the use of various targeted FISH techniques, multicolor FISH to identify

chromosomal alterations unresolved by karyotyping, and chromosomal CGH which offers genome wide-screening by determining DNA content differences and characterizing chromosomal imbalances even when fresh specimens and chromosome preparations are unavailable. More recently, the development of array CGH, which allows the detection of much smaller genomic imbalances, involves the use of an ordered set of defined nucleic acid sequences derived from various sources, immobilized on glass slides. Currently, the array CGH approach is poised to revolutionize modern cytogenetic diagnostics and provide clinicians with a powerful tool in their diagnostic armamentarium. The cross-species array CGH studies described testify to the notion that genetically tractable mouse models represent an invaluable tool not only to identify new cancer-causing genes but also to assess the context-dependent vulnerability of tumors to multi-target intervention strategies. However, all gains and losses identified on the array CGH should be validated by FISH or molecular confirmation analysis. Taken together, the goal of the molecular cytogenetics laboratory is to identify the type of techniques that are most useful and informative for a particular study, prepare quality experimental materials, and perform a thorough analysis to arrive at an interpretation that is useful for research and diagnostic purposes.

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