Molecular cytogenetics: Show me the colors

In this issue of Genetics in Medicine, an article by Jalal and Law entitled: "The Utility of M-FISH in Clinical Cytogenetics" is presented.[1] The authors examine the utilization of multicolor FISH (M-FISH) in clinical cytogenetics by studying seven cases, six of which were cytogenetically abnormal, to determine the efficacy and utility of this technology. They have shown that this multi-color technology is useful for the identification of marker chromosomes, derivative chromosomes, and in the analysis of complex karyotypes. In addition, they illustrate limitations of this technology and its inability to detect some specific abnormalities. They also compare its limitation to the similar technology of spectral karyotyping (SKY). This article follows a similar article in the inaugural issue of Genetics in Medicine (Volume 1) in which Levy and her colleagues described the utilization of comparative genomic hybridization (CGH) to study 12 abnormal derivative chromosomes (five markers, five unbalanced translocations, and two intrachromosomal duplications), highlighting the utility of this technology also to identify unknown chromosomal material.[2]

The study of chromosomes has a relatively short history, and since Tjio and Levan first identified the correct chromosome number of 46 in humans in 1956, there has been constant improvement and refinement of the technologies that are routinely used for chromosome identification. Analysis of chromosomes using solid staining progressed to identification by banding in 1971, which was followed shortly thereafter (1976) by utilization of high-resolution technology. At that point, advances in cytogenetics came to a standstill and questions concerning the overall efficacy of cytogenetics and its usefulness in the future began to be posed. However, the future applicability of cytogenetics became clearly delineated and apparent with the ground breaking experiments of Pinkel and Gray and of Ward and his colleagues who laid the groundwork in 1988 for molecular cytogenetics, with technology revolving around the utilization of fluorescence in situ hybridization.[3–5]

Today, cytogeneticists have an arsenal of technologies that can be used both clinically and from a research perspective to better understand chromosome structure and function. These techniques can be used to make chromosome identification, to study the mechanism of chromosomal aberrations, and to better understand the phenotypic effects of chromosomal abnormalities. These technologies run the gamut from using single chromosome painting probes to identify one specific chromosome to using single copy probes to look for specific deletions or duplications of material. Comparative genomic hybridization can be utilized to better analyze neoplasia and, as discussed in the article by Jalal and Law presented in this issue of the journal, multicolor FISH or SKY can be used to analyze marker chromosomes, derivative chromosomes, and complex karyotypes.[2,6–9] All of these technologies have made the field of cytogenetics a much more vibrant and fruitful endeavor, allowing us to unequivocally identify marker chromosomes that are found both prenatally and postnatally. It has allowed distinct phenotypic correlations to be made for many specific markers, specifically those derived from chromosomes 12, 15, 18, and 22. For example, a marker derived from chromosome 15 containing SNRPN will most likely have an abnormal phenotype, whereas the marker without SNRPN will more likely have a normal phenotype.[10–14] FISH can be used to determine the origin of extra unidentified material on derivative chromosomes and single copy probes can determine the extent of rearrangement.[15] Both subtle and complex rearrangements can be elucidated by a variety of methods. Using a series of YACs, we have delineated subtle deletions in several cytogenetically "balanced" translocations and have elucidated known genes that are either deleted or present in individuals with cytogenetic deletions.[16–17] One of the more common uses of FISH, and by many accounts one of the important aspects, is in the identification of microdeletion syndromes. These studies have taught us that the frequency of many abnormalities may be greater than we initially imagined. For example, the frequency of deletions of chromosome 22 may be as high as 1:3000. The National Institutes of Health initiative to create a FISH-BAC map, with markers one megabase apart on every chromosome, provides the opportunity to precisely define structural rearrangements.[18] We will be able to determine the precise amount of material on accessory marker chromosomes and to identify small deletions in apparently balanced translocations. We have already shown this phenomenon utilizing YACs, but it will be more efficacious with BACs.

With the identification of unique subtelomeric regions on each individual chromosome arm, studies can be done answering whether, and if so to what extent, subtelomeric variation is clinically important. Both cryptic rearrangements as well as cryptic subtelomeric deletions have been associated with idiopathic mental retardation.[19,20] As the technology increases, it is extremely likely that multicolor telomeric probes will be available in which all of the chromosome arms can be routinely analyzed in appropriate cases.

Interphase analyses have become much more routinely utilized for the rapid prenatal detection of aneuploidy or for the detection of a Bcr-Abl fusion in chronic myelogenous leukemia. A large number of prenatal laboratories are currently doing prenatal interphase analysis, to a limited degree, to rule out aneuploidy of chromosomes 13, 18, 21, X, and Y.[21] Its applications in cancer cytogenetics have vastly multiplied, in which probes for several different site-specific translocations have been developed. These probes, such as those developed for detecting the Bcr-abl rearrangement in CML, can not only be

effectively used for studying interphase cells, but have become extremely important for monitoring the effectiveness of treatments.[22] The utilization of molecular cytogenetics has vastly expanded not only in the clinical realm but also in the research area. Here, this technology has become much more routinely used to better understand meiosis, cell, and nucleus architecture, and in the identification of mouse chromosomes, especially in the creation of embryonic stem cells.

The newest FISH technologies are the multi-color karyotyping techniques as described by Jalal and Law in this issue.[1] Three major different types of multi-color FISH are available: M-FISH, SKY, and Rx-FISH. M-FISH was first described by Spiecher et al. in 1996.[23] This technique is based on a combinatorial labeling approach in which six different fluorochromes are utilized in combination, yielding a possible 63 combinations (2^n-1). Using these fluorochromes with optical filters between 350 and 770 nm, they visualized 27 combinatorially labeled probes simultaneously. These were analyzed using sophisticated software allowing each individual chromosome to be pseudocolored. In the same year, Schrock et al.[24] reported multi-color karyotyping that was interferometer-based spectral imaging, in contrast to the fluorochrome based system described above. They used an interferometer to generate a fluorochrome-specific optical path difference that provides spectral information. In conjunction with a CCD camera, the fluorescence emission spectrum can be recovered simultaneously at all points. Muller et al., in 1997, proposed using cross-species multi-color banding (Rx-FISH),[25,26] utilizing probes from flow-sorted gibbon chromosomes. Combinatorial labeling was used and a unique pattern of karyotypic banding involving different colors on each chromosome was generated. Other offshoots of this technology include a multi-color chromosome bar code and high resolution multi-color banding, both of which allow the differentiation of the chromosome at specific regions.[27,28] As described above, all of these methods can be utilized for a variety of studies. This includes clinical cytogenetics (e.g., the determination of markers and de novo duplications), cytogenetics of neoplasia, radiation biology, cellular architecture, and comparative cytogenetics.

What is truly remarkable is not the advancement of molecular cytogenetic technology but its acceptance and absorbance into the clinical cytogenetics laboratories. These techniques have become much more routinely utilized to expand each laboratory's capability to make proper diagnoses. The vast majority of laboratories in the United States currently have some type of computerized FISH analysis system. More than 70 laboratories already have a spectral karyotyping system. The big question for clinical cytogenetics does not involve how the new technology should be used but what technology is necessary to use. It must also be decided when to use it, how to make it cost effective, and how to have the proper labor effectiveness when performing the technique. As in all fields, technology does not come cheaply. It becomes incumbent upon every laboratory to be able to integrate the technology and maximize its utilization, while still running a well-organized and fiscally responsive laboratory.

In all respects, the future continues to remain bright for molecular cytogenetics. Techniques continue to be tested and to ultimately find their proper place in the clinical laboratory. As work continues, we will see the future development of multi-color telomere probes, a 1 Mb BAC map for all chromosomes, and comparative genomic hybridization with an array technology that might allow for the rapid detection of both deletions and duplications within the genome. These developments should ultimately provide the opportunity to clearly delineate all abnormalities on the molecular level. This will provide detailed phenotype-karyotype for detecting abnormalities, allowing both prenatal and postnatal prognosis of these chromosomal aberrations. All of these technologies are continually being tested and absorbed within the clinical laboratories, which ultimately must determine the best way to diagnose patients and to determine how this technology can best be successful.

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