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Overview of Cytogenetic Technologies

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세포유전학 기술에 관한 고찰

강지언

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Cytogenetic analysis plays an important role in examinations of a variety of human disorders. Over the years, cytogenetic analysis has evolved to a great extent and become a part of routine laboratory testing; the analysis provides significant diagnostic and prognostic results for human diseases. Microarray in conjunction with molecular cytogenetics and conventional chromosome analysis has transformed the outcomes of clinical cytogenetics. The advantages of microarray technologies have become obvious to the medical and laboratory community involved in genetic diagnosis, resulting in greatly improved visualization and validation capabilities. This article reviews how the field is moving away from conventional cytogenetics towards molecular approaches for the identification of pathogenic genomic imbalances and discusses practical considerations for the routine implementation of these technologies in genetic diagnosis

Key words: Conventional cytogenetics, Microarray technologies, Molecular cytogenetics

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INTRODUCTION

Cytogenetics is the study of the relationship between chromosomal aberrations and genetic diseases in human beings [1]. The cytogenetic analysis extends beyond the simple description of the chromosomal status of a genome. It allows the study of fundamental biological questions, such as the nature of inherited syndromes, genomic alterations that are involved in tumorigenesis, and three-dimensional organization of the human genome [2]. Over the years, cytogenetic techniques have been used to unravel the three-dimensional organization of the genome and epigenetic features of higher-order chromatin structure [1, 3-8]. Throughout the history, various discoveries and techniques have revolutionized the field of human cytogenetics (Table 1) [1].

Microarray that merges molecular cytogenetics with conventional chromosome analysis has transformed the outcomes of clinical cytogenetic testing. In the last few years, the robustness of these technologies has provided an accurate diagnosis of genetic alterations of congenital and acquired aberrations and appropriate clinical management in a timely and efficient manner [9]. The application of this technology in genetic diagnosis has provided distinct advantages over traditional cytogenetic analysis in detecting microscopic and submicroscopic chromosomal aberrations [10].

In this article, the diagnostic approaches deviated was

Table 1. Comparison of technical details between conventional cytogenetic and major molecular approaches

Techniques	Method	Characteristics	Application	Advantages
Conventional cytogenetics (G-banding, R-banding)	Cell culturing	Special dye generate banding pattern for each chromosome	Detection of numerical and structural chromosomal anomalies	Genome wide screening for chromosome level abnormalities
Conventional FISH	Molecular technique	Labeled DNA is used as a probe to search for target sequences in chromosome	Detect all types of balance and unbalanced defects	Interphase cytogenetics possible Simple procedures
Spectral karyotyping	Arresting cell in metaphase	Chromosome specific probes allows the painting of every chromosome	Detection of rearrangements including complex anomalies	Fast characterization of euchromatic marker chromosome content
CGH	Molecular technique	Comparative hybridization of differentially labeled total genomic tumor DNA and reference DNA	Identify and assess biomarkers Gene discovery, functional analysis	Whole genome wide screening of genomic anomalies No need for cell culture
Array-CGH	Molecular technique	Identification of DNA sequences by specific DNA binding proteins in cells	Identification of cryptic rearrangements (aneuploidy, deletions, duplications or amplifications)	High-resolution target-specific detection of gene amplification, submicroscopic information on imbalances

Abbreviations: FISH, fluorescence in situ hybridization; CGH, Comparative genomic hybridization.

discussed from conventional cytogenetics towards molecular approaches for the identification of genomic imbalances pathogenically and the practical considerations for the routine implementation of the technology in genetic diagnosis.

MAIN ISSUE

1. Classic cytogenetics analysis

Conventional banded karyotyping is recognized as the gold standard for the diagnosis and prognosis of genetic diagnosis. It has been used for scanning genome alterations that involve both gains and losses of portions of the genome, as well as rearrangements within and among the chromosomes [9]. Karyotyping analysis has been used to prove the causal association between specific chromosomal abnormalities and clinical syndromes such as congenital anomalies, developmental delay (DD), and mental retardation (MR) [11]. In tumor genetics, conventional single cell and metaphase cytogenetics are essential for disease monitoring, tumor staging, and research purposes to identify chromosomal regions harboring putative tumor suppressor genes and proto-oncogenes [12].

However, banding analysis is considered to be time-consuming and labor-intensive. Routinely, about two weeks are required to obtain the results while many potentially clinically relevant submicroscopic chromosomal abnormalities remain undetected [13]. Furthermore, it is difficult to detect microdeletions and duplications that result in significant clinical conditions such as congenital anomalies, MR, DD or intellectual disability (ID) [14]. Limited chromosome-specific banding resolution makes the characterization and correct interpretation of complex and cryptic chromosome alterations difficult to ascertain.

2. Molecular cytogenetics

To overcome the limitations of banding analysis, molecular cytogenetic techniques such as fluorescence in situ hybridization (FISH), spectral karyotyping (SKY), and comparative genomic hybridization (CGH) have emerged as successful diagnostic tools. These techniques are widely used as adjuncts to traditional cytogenetics for identifying chromosomal alterations [9]. Apparently, it is commonly employed as adjuncts to conventional methods for identifying chromosomal aberrations [15-19]. Variable molecular cytogenetic techniques have been recognized as valuable additions or even alternatives to chromosomal banding as they enhance thorough interpretation of numerical and complex chromosome aberrations by

bridging the gap between conventional banding analysis and molecular genetic studies [20-22].

FISH is based on the use of chromosome region-specific and fluorescent-labeled DNA probes. These probes are cloned pieces of genomic DNA that can detect their complementary DNA sequences and produce a fluorescent signal against background stained chromosomes that can be easily detected, thus making FISH testing ideal [23]. FISH not only allows the detection of small genomic alterations of 50 Kb to 100 Kb but also permits direct visualization of these alterations in uncultured cells [24]. FISH has been used for aneuploidy screening in prenatal specimens and certain suspected malignancies, evaluation of gene rearrangements in leukemias and lymphomas, microdeletions in contiguous gene syndromes, and rearrangements of subtelomeric regions [25]. In the past decade, FISH assays have made rapid developments in the area of detection of genomic alterations regardless of their complexity by filling in the gap between conventional chromosome karyotyping and molecular cytogenetics [26, 27]. The use of diverse, multicolor FISH assays enhances thorough characterization of numerical and complex chromosome aberrations regardless of their complexity [28]. However, the complexity of the staining pattern that can be produced with FISH is limited based on the number of FISH probes that can be distinguished. Additionally, the same optical and chromosome structure considerations can affect chromosome banding [1, 29].

CGH allows screening of the entire genome for diagnosing the aberrations and represents a variation of FISH technology with a clear advantage of revealing imbalances across the whole genome [12]. However, owing to limited resolution (5-10 Mb) of metaphase chromosomes, aberrations such as mosaicism, balanced chromosomal translocations, inversions, and changes in whole genome ploidy cannot be detected using this approach [30].

Overall, the resolution at which copy number alterations can be detected using these molecular cytogenetic techniques is only slightly higher than conventional karyotyping. Furthermore, these experiments are labor-intensive and time-consuming, especially when multiple genomic regions are interrogated [31]. For the detection of such abnormalities, a high-resolution technique is required.

3. Clinical use of microarray technology

The use of DNA targets immobilized in an array format as a substitute of the conventional metaphase chromosome spreads represents a significant advancement. It combines fluorescence techniques with microarray platform and allows comparison of DNA content in two differentially labeled genomes: a test genome and a reference genome. Consequently, the microarray platform allows the use of thousands of individual DNA sequences throughout the genome and provides precise information about locations of any identified aberrations through a single experiment [4, 32].

Over the years, these technologies have enabled the detection of genomic imbalance including deletions, duplications, insertions, amplifications, rearrangements, and base-pair changes. In addition, multigene prognostic or predictive models equivalent or superior to those of established clinical parameters have been successfully developed. Recent discoveries of genomic aberrations underlying and promoting malignant phenotype, together with an expanded repertoire of targeted agents, have provided many opportunities to conduct hypothesis-driven clinical trials [33]. Several genomic aberrations have been discovered by employing these methodologies. They are now being used as predictive genetic markers for the treatment with targeted therapeutics. Advent of microarrays in clinical cytogenetics has imparted a significant impact on our existing knowledge of the genetics of human disorders and is currently leading to an unprecedented speed of acquisition and amount of new knowledge [34-38].

Aside from the discovery of specific disease genes, it has revolutionized our knowledge about the contribution of inherited factors in the development of the disease. It offers a much higher diagnostic yield for genetic testing of individuals with unexplained DD, ID, autism spectrum

disorders (ASDs), multiple congenital anomalies (MCA) than a conventional karyotyping [39]. de Vries et al. [40] studied 100 individuals with normal G-banded chromosomes and unexplained MR. All were screened by subtelomeric multiplex ligation-dependent probe amplification with normal results. Prospective studies of individuals with DD and dysmorphic features have also demonstrated that array analysis has the ability to detect any genomic imbalance including deletions, duplications, aneuploidies, and amplifications. Detection rates for chromosome alterations with microarray CGH range from 5% to 17% in individuals with normal results from prior routine cytogenetic analysis [41]. Additionally, a meta-analysis of microarray by involving 13,926 subjects with ID and/or MCA, most of whom had normal G-banded chromosomes, detected an overall diagnostic rate of 10% for pathogenic genomic abnormalities [42]. Another retrospective analysis of 36,325 patients with DD estimated that a pathogenic abnormality could be detected in ~19% of unselected DD/ID patients using genome-wide microarray assays [43]. Numerous studies on disorders of cognitive development have also revealed interesting and novel insights and opened an avenue of investigation with huge potential for the diagnosis of numerous human disorders. Microarrays testing are now recommended as a first-tier test, as they have replaced standard karyotype for postnatal disorders including DD, ASD, and MCA [44-47].

The higher abnormality detection yield and amenability to automation render array analysis also suitable for prenatal diagnosis. Both the findings of unclear significance and unexpected findings have been detected, varying from 1% to 5%, depending on the reason for referral [48]. In the study by Shaffer et al. [49], 151 prenatal cases with normal karyotype were retrospectively screened and two causative rearrangements were identified with a diagnostic yield of 1.3%. Frequencies of apparently benign alterations and findings of unclear significance were 7.9% and 0.6% respectively, after parental analysis [50]. In another study, targeted microarray CGH was applied for the evaluation of 300 prenatal samples, which led to the detection of 58 copy number variations (CNVs). Of those, 15 (5%) were clinically significant chromosome alterations, 3 (1%) were of uncertain clinical significance, and 40 (13.3%) were benign CNVs [51]. Meta-analysis of prenatal studies using microarray of various platforms also detected 3.6% additional genomic imbalances when G-banded karyotyping was normal, regardless of the indication for referral. In the case of referral indication being an abnormal ultrasound, the percentage increased to 5.2% [52].

CONCLUSION

Microarray technology, with the potential to identify most of the unbalanced microscopic and submicroscopic rearrangements, is likely to be the first approach towards cytogenetic testing. The technology is believed to replace most of the banded chromosome and FISH analyses in the clinical laboratory in the very near future [2]. When considering replacing traditional karyotype with microarray technology, it is important to consider that array testing cannot detect balanced karyotypic abnormalities, such as reciprocal translocations, that could be of clinical significance if they disrupt a critical gene. As newer genomic technologies enter the clinical realm, including exome and genome sequencing, it is imperative to remember lessons learned from microarrays. Discovery and interpretation of such factors will be one of the next big challenges for genome-wide clinical genetic testing. While current array-based technologies may be too expensive for routine applications, it is hypothesized that in the near future, with the introduction of massive whole-genome parallel sequencing, complete mapping of the genomic changes in malignant cells can be achieved [34]. A decrease in the cost of these technologies will probably occur with the use of automation and wider application.

요 약

세포 유전학적 분석은 인간에서의 다양한 종류의 질환을 연

구하고 진단하는데 매우 유용하게 사용되고 있다. 지난 수년 동안 세포 유전학적 분석을 통해 매우 의미 있는 결과를 얻을 수 있었으며, 현재 임상검사실에서 일반적인 검사로 확대되어 질병을 진단하고 결과를 평가하는데 매우 유용하게 사용 되고 있다. Microarray는 분자 세포 유전학적인 방법과 기존의 세포유전학적 방법이 융합된 검사방법으로 기존 검사 방법의 단점을 보완하여 유전 관련 질환을 진단하는데 매우 유용하게 사용되고 있다. 따라서 본 논문은 유전질환 진단에 있어 기존의 일반적인 세포유전학적 방법에서 마이크로어레이를 통한 분자세포유전학적 방법으로 어떻게 전환되어 왔는지, 유전 진단을 하는데 앞으로 이 검사방법들이 얼마나 의미 있게 사용될 것인지에 관하여 고찰하였다.

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