Medical Implementation of Microarray Technology

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ABSTRACT

Microarray technology represents a critical new advance in molecular cytogenetics. The development of this approach has provided fundamental insights into the molecular pathogenesis in clinical cytogenetics and has provided a clue to many unidentified or unexplained diseases. The approach allows a comprehensive investigation of thousands and millions of genomic loci simultaneously and enables the efficient detection of copy number alterations. The application of this technology has shown tremendous fluidity and complexity of the human genome, and has provided accurate diagnosis and appropriate clinical management in a timely and efficient manner for identifying genomic alterations. The clinical impact of the genomic alterations identified by microarrays is evolving into a diagnostic tool to identify high-risk patients better and predict patient outcomes from their genomic profiles. The transformation of conventional cytogenetics into an automated discipline will improve diagnostic yield significantly, leading to accurate diagnosis and genetic counseling. This article reviews cytogenetic technologies used to identify human chromosome alterations and highlights the potential utility of present and future genome microarray technology in the diagnosis.

INTRODUCTION

Cytogenetic analysis has provided fundamental insights into the molecular pathogenesis of a variety of human disorders [1]. In the last decade, cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in tumorigenesis and the three-dimensional organization of the human genome (Figure 1) [2]. However, these conventional investigations have technical limitations result in the underestimation of the degree of chromosomal alterations and limited by its capacity to detect only screen DNA targets rather than the whole genome.

Microarray technologies circumvented these limitations of conventional cytogenetic analysis. It simultaneously evaluates regions across the entire genome for patients with suspected genome imbalance with higher resolution and excellent throughput. The multiplex format of the test permits simultaneous evaluation of multiple disease specific loci and subtelomeric regions, resulting in a more efficient
consideration of possible diagnoses and cost savings over ordering testing of each locus individually [3]. Additionally, his technologies are revealing the tremendous fluidity and complexity of the human genome and transforming the field of cytogenetics [4]. In this article, we review the overview of methods of cytogenetic and highlight a potential utility of array based technologies in the identification of cytogenetic abnormalities and the implications for clinical applications (Figure 2).

### MAIN ISSUE

1. **Conventional cytogenetics**

   The invention of chromosome banding techniques in 1970 led to the discovery of numerous structural chromosome aberrations and their association with human diseases [5]. Since the development of karyotyping techniques, microscopic analysis has been used as the gold standard for the diagnosis and prognosis of genetic disorders. It has been used for scanning the genome for aberrations that involve both gains and losses of portions of the genome, as well as rearrangements within and among chromosomes [6].

   This enabled to prove the causal association between specific chromosomal abnormalities and genetic disorder [7].

   However, karyotyping investigation is not sufficient for the identification of the origin of extra material on chromosome or correctly assigns other structural chromosomal alterations. Furthermore, these techniques require a high rate of cell division and good chromosomal morphology, which represent challenges for the cytogeneticists, and a long period for assaying and analyzing, which usually is a challenge for physicians [8].

2. **Fluorescence in situ hybridization**

   The molecular cytogenetic methods including fluorescence in situ hybridization (FISH), multiplex-FISH, spectral karyotyping (SKY) and comparative genomic hybridization (CGH) have provided valuable diagnostic and prognostic information for the detection of genomic defects in genetic diseases [9].

   The use of these techniques enhances the thorough interpretation of numerical and complex chromosome alterations, bridging the gap between conventional banding analysis and molecular genetics studies [5].

   The introduction of FISH circumvented the
limitations of traditional cytogenetics and became an integral part of a comprehensive clinical cytogenetic evaluation. FISH is based on the use of chromosome region-specific, fluorescent-labeled DNA probes. The technology not only allows the detection of small genomic alterations of 50 Kb to 100 Kb, but also permits the direct visualization of these alterations in uncultured cells [4]. FISH allows the detection of genomic imbalances with great accuracy, it can only probe specific sequences that are known and suspected to be associated with known syndromes. In most diagnostic laboratories, karyotyping analysis, in conjunction with targeted FISH, is routinely performed to detect recurrent alterations with prognostic implications.

FISH procedures are extremely useful in identifying specific suspected chromosome abnormalities and are highly specific for regions that are suspected to be involved on the basis of clinical findings [10]. It offers numerous possibilities for identifying genomic imbalances with great accuracy and has become an important complementing application in genetic diagnostics [5]. However, the complexity of the staining pattern that can be produced with FISH is limited by the number of FISH probes that can be distinguished, and the same optical and chromosome structure considerations that affect chromosome banding [11].

3. Comparative genomic hybridization

CGH represents a variation on FISH technology with the clear advantage of revealing imbalances across the whole genome without involving cell culture [5]. In CGH, a DNA sample is extracted with a known, typically normal karyotype and compared to the DNA sample obtained from an individual with an unknown karyotype or a known abnormal karyotype [12]. It has altered our view of cancer biology, revealing that tumors of the same type have similar patterns of
regional gain and loss, and that the frequency of copy number changes increases with tumor progression [13]. In many studies, CGH has been helpful cytogenetics tool for identification of oncogenes and tumor suppressor genes involved in the initiation and progression of solid tumors, guiding the more detailed genetic analysis to specific chromosomal regions. Apart from these oncological applications, this approach can also be used to study chromosomal aberrations in fetal and neonatal genomes [14].

However, CGH has many of the same limitations found in conventional cytogenetics. A main disadvantage of this technique is its inability to detect mosaicism, inversions, balanced chromosomal translocations, and whole-genome ploidy alterations. Furthermore, CGH provides no information about the structural arrangements of chromosome segments that are involved in gains and losses [2].

For the detection of these types of alterations, other techniques, such as SKY and M-FISH, can be used. These techniques are similarly based on combinatorial probe-labeling schemes with five spectrally discrete fluorochromes that uniquely paint all 24 human chromosomes. Thus, both multicolor karyotyping techniques are specially tailored to identify and characterize the complex chromosomal rearrangements found in genetic diseases. Nevertheless, these methodological approaches are unable to detect intra-chromosome rearrangements, such as inversions and deletions, since these specific abnormalities maintain the correct color for abnormal chromosomes.

Overall, the resolution at which copy number changes can be detected using these molecular cytogenetic techniques are only slightly higher as compared to conventional karyotyping and all experiments are labor intensive and time consuming [7]. For the investigation of such rearrangements, a high-resolution technique and excellent throughput is required.

4. Clinical application of whole genome technology

Microarray technology represents the technical convergence of molecular genetics and cytogenetics and is rapidly revolutionizing conventional cytogenetics [5]. The use of this approach gives rapid results and multiplex detection of both numerical and unbalanced structural abnormalities with much higher resolution and wider coverage than other molecular cytogenetic techniques. Well-designed arrays for clinical use allow for straightforward interpretation and are likely to provide diagnoses in a substantial number of currently undiagnosed cases of human genetic disorders [15]. In last decades, this technology is poised to revolutionize modern cytogenetic diagnostics and to lead to a new understanding of the genome and its relationship to health and disease [16].

The main advantage of the application of microarray is able to uncover additional genetic alterations that are not detectable with conventional cytogenetic analysis. Many studies aimed at assessing the diagnostic capabilities of this approach in screening for hidden chromosome aberrations in patients with an apparently normal karyotype and in further characterization of chromosome abnormalities detected by routine banding analysis [10]. The advent of microarray technologies has led to the detection of large numbers of copy number variant (CNV) in patients with developmental delay (DD) and/or multiple congenital anomalies (MCAs) as well as in healthy individuals [10]. For the last years, CNV studies in many genetic disorders have revealed interesting and important new insights and have opened an avenue of investigation that holds great promise for many human diseases [17-20].

5. Postnatal diagnosis by microarray

In postnatal diagnosis of patients with mental retardation (MR), autism spectrum disorder (ASD) or unexplained DD, microarray has led to uncover additional genetic alterations that are not detectable
with conventional cytogenetics. The prevalence of MR, ASD, and DD are reported to be 1∼3%, 0.67%, and 3.7%, respectively [21].

With conventional methods, the diagnostic yield is about 3∼4%; with subtelomere FISH, the yield is 5∼7% [22]. The positive yield for clinically relevant CNVs with microarray cytogenetics is 15∼20% [23]. In the study of Dave et al [24] the additional application of microarray increased the identification of genomic imbalances by approximately 8% in the population of DD with a normal conventional banding results.

Furthermore, this approach aimed at assessing the diagnostic capabilities of microarray in identifying for hidden chromosome alterations in MR with an apparently normal karyotype. Multiple studies of hundreds of patients with idiopathic MR had normal banding results using array platforms have shown that the diagnostic yield of rearrangements increased up to 15∼20% after exclusion of inherited anomalies [25-30].

The application of well-designed arrays for postnatal diagnosis allow for straight forward interpretation and are likely to provide genetic information in a substantial number of currently undetectable by other genetic technologies targeting specific genomic regions. From all these considerations, it is clear that a high-resolution array platform covering the whole genome would provide much more informative results than one containing only low coverage limited to postnatal disease-associated regions [31-34].

6. Prenatal diagnosis by microarray

The higher abnormality detection yield and its amenability to automation render microarray also suitable for prenatal diagnosis [25]. When the referral indication was abnormal ultrasound, this percentage increased to 5.2%. In the array CGH study diagnosed two de novo unbalanced karyotype and four additional abnormalities that could not be characterized with conventional cytogenetic methods in classic microdeletion syndromes and subtelomeric rearrangements in 39 fetuses with multiple congenital abnormalities after termination of pregnancy [35]. The eight cases of microdeletions in the Yq11.23 chromosomal regions were detected by array CGH that were not found by conventional karyotyping in prenatal genetic diagnoses.

This new approach has given the clinician a greater appreciation of variability in the clinical presentation of many well-described conditions and allowed for the discovery of unsuspected imbalances in prenatal diagnosis. Given the potential described in this article, we anticipate the microarray to be the initial prenatal diagnostic approach for the identification of chromosomal alterations [9]. Although, clinical application of this approach as a universal routine test for prenatal diagnosis is premature, further investigation will allow an evaluation between the overall diagnostic yield of microarrays over routine prenatal testing with banding karyotype, as well as cost effectiveness issues [36-38].

CONCLUSION

Contemporary cytogenetics has quickly integrated the microarray technologies into clinical use and this approach have revolutionized this field and extended its applications to many areas of medicine other than medical genetics. These dramatic changes require the design of better training programs to prepare the trainees in cytogenetics to face the future challenges of this exciting discipline [16].

The application and interpretation of genomic arrays in clinical diagnosis would be desirable and capable of decreasing the risk of unexpected findings and would play a major discovery role to reveal the cryptic and/or complex nature of chromosome arrangements [39, 40]. The development of next-generation sequencing technologies that can quickly sequence an entire cancer genome will certainly lead to even more advances in our understanding of cancer genesis and our ability to diagnose, prevent and treat.
요 약

마이크로레이어 전단 기법의 발달은 세포유전학적 관점에서, 다양한 종류의 유전학적 질병과 관련하여 새로운 정보를 제공하고, 질병에 대한 기본적인 통찰력을 제공하는데 매우 중요한 역할을 제공하고 있다. 그동안 많은 연구들에서, 마이크로레이어 기술을 활용한 인간 건강의 유동성과 다양성을 입증해 주었으며, 질병의 예방을 실험하여 질병을 예방하게 됨으로써, 보다 효율적인 방법으로 질병의 진단 및 치료도에 도움이 될 수 있는 새로운 혁명을 관리 방법을 효율적으로 제공해 왔다. 앞으로 다양한 종류의 유전학적 질병과 관련하여 새로운 정보를 제공하여 질병의 진단 및 치료에 기존 세포유전학적 방법을 자동화된 마이크로레이어 방법으로 전환한다면, 보다 효율적인 방법으로 질병을 전단하고, 정확성을 향상시키며, 유전자 배열의 억제와 표준화한 특성을 발휘하는 데 매우 중요한 역할을 할 것으로 생각된다. 또한 이 분석 기법을 활용하여 건강과 인간의 건강, 질병과의 관계를 분석하여 다양한 정보를 미리 제공하여 질병을 예방하고, 질병의 진단 및 치료에도 도움이 될 수 있는 새로운 혁명을 일으킬 수 있을 것으로 기대된다.

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