Journal of Advances in Science and Technology

Vol. VII, Issue No. XIII, May-2014, ISSN 2230-9659

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AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. VII, Issue No. XIII, May-2014, ISSN 2230-9659

A Study on the Impact of Molecular Techniques on Cytology

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Abstract – Cytology is an important discipline in the research, diagnosis and treatment of human diseases, and has traditionally involved the study of cellular morphology using a microscope. However, the recognition that genes play an essential role in growth and development has led researchers and clinicians to include the analysis of chromosomal and genetic abnormalities as part of their investigations. Genetic information can provide an in-depth understanding of the underlying causes of many human diseases, especially congenital diseases and those with a significant genetic component such as cancer.

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INTRODUCTION

As part of the natural evolution of cytological techniques, the use of cytogenetics as a clinical tool first occurred in the late 1950s, when an additional copy of chromosome 21 was shown to be associated with Down syndrome. The chromosomal profile of each cell, known as the karyotype, subsequently became a useful way of characterizing a disease. Improvements to chromosome staining techniques during the late 1960s and early 1970s made it possible to distinguish sections within a chromosome, facilitating the first classification of disease-causing genetic duplications, translocations and inversions.

Building on the work from prior developments, the structure of the DNA double helix was published, which was a key step in linking chromosomes, heritability, and morphology to a decipherable genetic code. As a patient's body is built and regulated following the guidelines laid down in his or her DNA, this information can often be used to make disease diagnosis even in the absence of any obvious symptoms. This is especially useful for identifying diseases early during their development or when external symptoms are inconsistent, ambiguous, or nonexistent.

Within a clinical laboratory, it is now common to see a fusion of cytological and cytogenetic analysis, with the relative usefulness of each depending on the disease being investigated. As high resolution molecular techniques such as microarray analysis and DNA sequencing become cheaper, faster, and easier, the reliance on these techniques is likely to grow, having a significant impact on how diseases are researched, diagnosed, and treated in the clinic.

The knowledge that chromosomes were composed of DNA also made it possible to classify a cell's karyotype with greater resolution, by facilitating the development of DNA-sequence specific chromosome banding methods such as fluorescent *in situ* hybridization (FISH). Using this technique, known DNA sequences are labeled with fluorescent molecules and used to identify specific chromosomes of interest, as well as discreet regions of a single chromosome at increased resolution. This makes it possible to visualize even smaller deletions and duplications than could be identified using traditional chromosome staining.

Although chromosome-labeling techniques such as FISH have been an important tool in clinical laboratories for the last few decades, karyotyping requires significant expertise and time, while genetic abnormalities caused by very small variations are difficult to reliably detect using karyotyping. For this reason, their use for diagnosis has been limited to a subset of diseases, particularly those where balanced translocations occur. However, the recent development and optimization of modern molecular techniques such as DNA microarrays and high-throughput DNA sequencing means that the use of cytogenetics is rapidly superseding traditional cytology as the method of choice for diagnosing a wide range of diseases. These techniques offer greater resolution than ever before, as well as fast, reproducible, and reliable analysis with a greater scope for process automation.

RESEARCH STUDY

DNA microarrays, sometimes known as DNA chips, are a large collection of short probes of DNA, usually less than 100 nucleotides in length, attached to a

solid surface such as a glass slide. The most common uses of microarrays are for genome-wide variation studies and the analysis of gene expression levels, although many different designs exist. The basic premise for any DNA array is that each probe is designed to target a specific DNA sequence in the sample being analyzed, providing information such as the presence, absence, or copy number of that sequence. This data can then be used to infer the existence of atypical point mutations, deletions, insertions and copy number variations (CNVs), or the expression level of a gene of interest.

DNA microarrays offer several advantages over *in situ* hybridization methods. First, the large density of probes now available on a single chip allows the user to screen the entire genome of an individual in a single experiment. DNA microarray processing is also well suited for analyzing many samples in parallel, increasing the speed of analysis and making it easier to robustly and reliably compare results. The resolution of DNA microarrays also supersedes that of *in situ* techniques, with some arrays even capable of distinguishing changes in a single nucleotide (single nucleotide polymorphisms, or SNPs). This is especially important: even though SNPs appear to be subtle genetic variations at first glance, they can have a large impact on disease incidence and severity.

One technique utilizing microarrays that is becoming prevalent in clinical laboratories is array Comparative Genomic Hybridization (CGH). This involves comparing the genome of a patient with a normal reference sample in order to ascertain the location and nature of any genomic variations. Using this approach, the genes affected can be easily identified and the information used to more accurately diagnose and treat the disease. Although aCGH can be used to investigate the genetic basis of a congenital disorder in a patient at any age, there is a growing interest in analyzing the genome of embryos very early in development, so that genetic diseases can be detected early during pregnancy. In a similar way, CGH may offer the ability to improve the success of *in vitro* fertilization (IVF). Known as Pre-implantation Genetic Screening (PGS) or Pre-implantation Genetic Diagnosis (PGD), the technique involves the screening of IVF embryos for an euploidies before they are transferred for implantation into the uterus. This may improve IVF success rates.

Although now well accepted for use in congenital screening, the use of microarrays in cancer research and diagnosis has been adopted more slowly. This may be due to the genetic and morphological complexity and diversity of cancer development. However, traditional karyotyping and morphological analysis can lack the resolution and molecular insight required to precisely define a cancerous growth. Analysis using microarrays could significantly increase the accuracy of cancer diagnosis by providing highly accurate, genome-wide data.

Cancers are often genetically complex and can be triggered, maintained, or encouraged to metastasize by a wide range of spontaneous genetic changes in somatic cells, including SNPs, small insertions and deletions as well as larger CNVs. However, germ line mutations in tumor suppressor genes in either parent can also predispose individuals to the risk of cancer, creating a functional loss of hetero-zygosity (LOH). Humans have two copies of each gene, one from each parent, which can offer a form of genetic redundancy. Should one of these copies be inactivated by a random mutation, one functional copy remains. However, if a second mutation were to occur in the functional version of the gene, cancer could well develop. Similarly, there is growing evidence that a number of cancer-related genes are haplo-insufficient, so that even the loss of one functional copy of the gene is enough to favor the development of cancer (see reference [5] for review). Therefore, when assessing the genetic background of an individual, it is important to assess not only the *de novo* somatic mutations that have occurred within a given cell, but also the heterozygous, potentially "silent" mutations residing throughout the genome.

In order to more accurately understand the genetic basis behind a cancerous group of cells, microarrays can be used to screen the entire genome of the cancer using CGH. To screen for such a wide range of potential genetic variations, it is desirable to be able to detect both CNVs and SNPs using a single array. In order to achieve this, it is necessary to construct customized arrays optimized to identify aberrations known to be important predictors of disease progression and patient prognosis for a given subset of cancers. For example, arrays specifically designed to reliably detect both CNVs and SNPs for a range of hematological cancers such as Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma (MM), Myeloproliferative Neoplasms (MPN), and Myelodysplastic Syndromes (MDS) have recently been developed by Oxford Gene Technology (OGT). OGT is also a member of the Cancer Cytogenomics Microarray Consortium (CCMC), a group of more than 190 cytogenetics laboratories and microarray vendors that are working to establish standards for the design and implementation of cancer microarrays. In the future, such arrays are likely to become an important tool for improving cancer research, as well as for diagnosing and treating the disease.

THE IMPACT OF DNA SEQUENCING

While microarrays have revolutionized clinical cytology over the last decade, the next evolution of cytogenetic analysis is already underway, with affordable personal genome sequencing likely to have a big impact on clinical research and diagnosis. This technique offers the highest possible genetic resolution (down to a single nucleotide) across the entire genome. However, the clinical use of sequencing is still in its infancy, as there are still challenges to overcome before it will become the

molecular tool of choice. For example, DNA sequencing produces large datasets that require complex algorithms to assemble sequence reads into a complete genomic map. Working with such a large amount of complex data requires significant bioinformatics expertise and, even after the genome has been assembled, pulling out the biologically relevant findings from a study can still be very challenging. Because of this, current opinion suggests that in the immediate future CGH will probably continue to provide the primary solution for genome screening, while sequencing will be used to probe specific areas of the genome in detail once potential problem areas have been identified.

In the future, clinical research and diagnosis will be influenced by an increase in the technical sophistication of next generation techniques. For example, the increasing utilization of process automation will have an impact on the speed, ease, and cost of carrying out clinical genetic testing. This will help to maximize accuracy and reproducibility, while facilitating the easy implementation of high-throughput approaches. It is also likely that clinical laboratories will increasingly turn to outsourced services as a means of carrying out sophisticated genetic testing for research and diagnosis. External providers can offer a fast, cost-effective, and high-quality service by dedicating their time, expertise, and resources to specific tests. This simply cannot be achieved by in-house clinical laboratories, as they often need to be flexible enough to provide a wide range of testing options, inhibiting them from focusing on the optimization of a few key diagnostic methods. In addition, as the processing of such complex data sets requires significant bioinformatics expertise, external vendors have started offering dedicated analysis solutions.

CONCLUSION

Although traditional cytology still has a place in the modern clinical laboratory, it is now starting to make way for techniques that utilize the increased resolution, accuracy and speed offered by the molecular revolution. Microarrays, next generation sequencing, and advances in automation all have the potential to further improve the accuracy and reliability of clinical research and diagnosis, and may eventually replace microscope-based methods. Furthermore, due to the complexity of analyzing the data produced by next generation sequencing analysis, more and more clinical laboratories may look to outsource the processing of samples. Suppliers of sequencing and microarrays services utilize processes built with scale-up in mind, allowing many thousands of samples to be investigated quickly and reliably. This will allow users to focus on the biological implications of their study rather than the technical details, and will minimize the need for investment in expensive hardware and teams of expert bio-informaticians.

REFERENCES

- Lejeune J, Gauthier M, Turpin R. Les chromosomes humans en culture de tissues. C R Hebd Seances Acad Sci. 1959; 248(4):602-603.
- Watson JD, Crick FHC. A structure for deoxyribose nucleic acid. Nature.1953;171:737-738.
- Gall JG, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. PNAS. 1969;63:378-383.
- Rudkin GT, Stollar BD. High resolution of DNA-RNA hybrids in situ by indirect -. Nature. 1977;265:472-474.
- Smilenov LB. Tumor development: haplo insufficiency and local network assembly.Cancer Lett. 2006;240(1):17-28.