Microarray-Based Cytogenetic Testing Illuminates Congenital Abnormalities and Cancer

The Infinium CytoSNP-850K BeadChip enables cytogenetics researchers to scan the entire genome for common or rare genomic alterations.

Introduction

Trilochan Sahoo, M.D. is an expert in the development, evaluation, and implementation of microarray-based cytogenomic technologies. A medical doctor by training, his interest in human genetics began early on in his career during his post-doctoral fellowship at Duke University. “My research was focused on identifying the cause of a group of Mendelian genetic disorders,” Dr. Sahoo said. “I moved to Baylor College of Medicine for a clinical cytogenetics fellowship, an area that was on the verge of becoming empowered by genomic analysis tools. I was very fortunate to work with some of the leaders of human genetics at Duke and Baylor. I have not regretted giving up clinical medicine to dive into molecular cytogenetics and human genetics.”

In April 2014, Dr. Sahoo was appointed Director of Cytogenetics at CombiMatrix, a diagnostics company that offers DNA-based testing for the detection of genetic abnormalities. As a full-scale cytogenetic and cytogenomic laboratory, CombiMatrix offers chromosomal microarray analysis, standard and customized FISH (fluorescence *in situ* hybridization), and high-resolution karyotyping to clinicians and researchers.

iCommunity spoke to Dr. Sahoo about how cytogenetic analysis has changed over the course of his career, the use of microarray-based cytogenetic testing for everything from congenital disorders to cancer diagnoses, and the explosion of knowledge made possible through whole-genome microarray technology.

Q: Could you tell me about CombiMatrix, its history, and the services you offer?

Trilochan Sahoo (TS): CombiMatrix was founded in 1995 and was initially focused on producing and providing proprietary microarrays using oligonucleotide or BAC clones, two of the early methods for manufacturing microarrays. More recently, CombiMatrix has become a significant national provider of microarray-based molecular cytogenomic services. In recent years, it has shifted its focus from providing oncology genetic testing to developmental testing, with an emphasis on pre- and post-natal testing and miscarriage analysis using Illumina SNP-based microarrays.

Trilochan Sahoo, MD, is Director of Cytogenetics at CombiMatrix.

Q: What is the biggest market for microarray-based cytogenomic analysis?

TS: We have been using microarrays and similar tools for about a decade, beginning with small scale approaches developed at a few academic centers. Today, it’s routine to perform postnatal cytogenetic testing to look for causes of developmental disorders and congenital abnormalities. In the last few years, there has also been an explosion of microarray studies evaluating prenatal samples to look for causes of abnormal ultrasound findings or abnormal maternal serum screening.

Using microarrays for the genomic investigation of cancer has occurred more recently. In the past, cancer genetic studies were performed using conventional tools such as chromosome analysis and FISH. One of the main benefits of the new generation of cancer genomics tools, including microarrays and next-generation sequencing (NGS), is that they can identify genetic mutations rapidly and interrogate the entire genome. There is also significant interest in using microarray-based technologies to identify structural variation.

Q: What is the value of cytogenetic research?

TS: Classical cytogenetics looks at chromosomes using cell biology or FISH methods. It has enabled the identification of major chromosomal abnormalities, such as Down syndrome, that rely on the identification of extra chromosomes. Classical cytogenetic tools can also detect large deletions or duplications of chromosomes that give rise to congenital disorders in a prenatal or a postnatal situation. Additionally,

these tools can detect recurrent gross chromosomal abnormalities, many of which are known to contribute to the evolution of malignant disorders, such as variety of cancers. They've also enabled us to study cancer progression.

Microarrays have greatly improved the ease with which we can evaluate the entire genome. They've enabled us to identify abnormalities or genomic imbalances at a high resolution and at a scale that we couldn't imagine a few years ago.

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Q: How has cytogenetic analysis changed recently?
TS: We knew that genetic changes in cancer happen, particularly chromosomal rearrangements and loss or amplification of specific genes. However, we didn't realize the nature, variety, and magnitude of these abnormalities. That's because the traditional tools, such as chromosome analysis and FISH, could only identify large or major abnormalities.

Now that we have whole-genome tools such as microarrays and NGS, we can analyze the entire genome from a few malignant cells at a resolution that allows us to identify abnormalities and look for rearrangements with a very high degree of precision. It's now quite easy to identify recurrent actionable abnormalities. In fact, we often identify multiple abnormalities when we analyze cancer samples with these tools. We are now slowly trying to understand what fraction or subsets of these abnormalities are truly actionable.

With microarrays, we can scan the entire genome and find a link to the phenotype in a significant fraction of samples. There are a few things that we still cannot define or identify with microarrays, such as balanced rearrangements. In balanced rearrangements, all genetic information is present in the right amount, just in a different order or in the wrong location. Fortunately, balanced rearrangements, more often than not, actually do not lead to a phenotypic abnormality.

Q: What are FFPE samples and what makes them difficult to work with?
TS: Formalin fixed and paraffin embedded (FFPE) is the usual method of preserving tissue that is acquired by surgical methods or biopsy. It's the common way of processing cancer samples, particularly solid tumors. During sample processing, there is significant denaturation and degradation of the DNA, which makes retrieving good quality, high-molecular weight DNA a challenge.

The good news is that companies like Illumina have addressed this challenge and have developed sample preparation solutions that allow some degree of repair of this damaged DNA. Illumina sample preparation kits and workflows enable us to obtain adequate amounts of quality DNA in a high fraction of FFPE samples so we are able to perform the downstream analysis. Over the years, we have perfected the processing and extraction of DNA from FFPE samples to perform microarray analysis using the Illumina CytoSNP BeadChip platform.

Q: Which CytoSNP BeadChip are you using and what does it enable you to assay?
TS: The CytoSNP-850K BeadChip is a whole-genome microarray. CombiMatrix actually contributed to its design, along with a consortium of cytogenetic labs. The CytoSNP-850K BeadChip is composed of 850,000 empirically selected single nucleotide polymorphisms (SNPs), which cover the entire genome at a high density. It has enriched coverage for over 3,000 genes of known cytogenetic relevance in both constitutional and cancer applications. It allows us to identify copy number changes, such as deletions or duplications at 20 kb (20,000 base pairs) or more, which is very high resolution.

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Q: How well does it cover genes of cytogenetic interest?
TS: Because there was significant contribution from a group of cytogenetics labs and clinical geneticists, I think there is very good coverage. The CytoSNP-850K BeadChip is a great microarray for cytogenomics. It covers those parts of the genome that we know undergo recurrent alterations leading to genetic disorders, as well as the rest of the genome that we're still studying and discovering. The resolution that the CytoSNP-850K array provides for the entire genome has allowed us to easily identify the well-known deletions and duplications. Over the last few years, tools such as CytoSNP-850K BeadChip have also enabled the identification of a whole series of new syndromic disorders.

Q: What types of abnormalities do you see in cancer samples?

TS: We basically have to redefine our concepts of how much genomic alteration happens when there is a malignant transformation that gives rise to a specific cancer. This realization is causing clinicians to re-examine how they should manage patients with these cancers. This is because when there are multiple abnormalities occurring in a cancer, the traditional therapeutic regimens that are followed often might not work.

> "Arrays such as the CytoSNP-850K BeadChip have enabled us to discover phenotype links in a high fraction of samples and have been of huge advantage in research, leading to an explosion in our knowledge about the genetic basis of cancer and other diseases."

Q: How has the quality of the CytoSNP-850K data helped your lab?

TS: The CytoSNP-850K BeadChip is an excellent platform to scan the entire genome for common or rare genomic alterations. Arrays such as the CytoSNP-850K BeadChip have enabled us to discover phenotype links in a high fraction of samples and have been of huge advantage in research, leading to an explosion in our knowledge about the genetic basis of cancer and other diseases. From a basic biology perspective, we are slowly beginning to understand different pathways and how the loss or gain of some genes affects growth and evolution of the physical parts of the body, or the brain.

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