MIP technology reveals important copy number changes in pediatric cancers

The University of Utah’s Joshua Schiffman, MD, discusses the use of molecular inversion probe (MIP) technology for studying copy number alterations in pediatric cancers

Scientists at the University of Utah, led by Dr. Joshua Schiffman, are using MIP technology to identify unique genetic aberrations that can distinguish between different types of pediatric cancers. Their work has uncovered patterns of copy number aberrations and regions of allelic imbalance that could be used to guide risk stratification and future treatment.

In 2009, Dr. Schiffman collaborated with Affymetrix using a 24K MIP panel and GeneChip® 30K Universal Tag Arrays to analyze 45 pediatric leukemia samples in order to detect unique copy number aberrations. Their study identified 69 regions of copy number changes, including unique patterns of copy number loss in samples with a deletion of the CDKN2A gene. These patterns differentiated between two similar subtypes of acute lymphoblastic leukemia (ALL), precursor B-cell ALL and precursor T-cell ALL.

Recently, Schiffman and colleagues used Affymetrix MIP Copy Number Services to perform a genome-wide analysis of copy number alterations in different malignancy grades of pediatric astrocytomas. The study identified several genomic amplifications that characterized the different tumor grades. Specifically, the study revealed distinct BRAF gene rearrangements that occurred in grade 1 versus grade 2 to 4 tumors and indicated BRAF mutation as a frequent mutation target in pediatric astrocytomas. They also found that BRAF mutations were significantly associated with homozygous CDKN2A deletions, suggesting the possibility of a new subset of pediatric astrocytomas.

"With the MIP assay, we can easily correlate patient outcome with higher copy numbers," said Schiffman. "Once we collect enough samples and enough outcome data, we’ll better understand the relationship between high copy number value and clinical outcome in many different types of cancer."

Schiffman recently spoke with Jessica Parra, Associate Marketing Manager at Affymetrix, about his use of the MIP copy number platform to study different forms of pediatric cancers.

Joshua Schiffman, MD, is an assistant professor of pediatric hematology and oncology and adjunct assistant professor of oncological sciences at the University of Utah. He is also the Medical Director for the High Risk Pediatric Cancer Clinic. Dr. Schiffman received his MD from Brown University School of Medicine and completed a pediatric residency, pediatric chief residency, and pediatric hematology and oncology fellowship at Stanford University. Currently, Dr. Schiffman’s research focuses on identifying pediatric patients who are at risk for developing hereditary cancer, understanding the molecular basis of their predisposition, and working to prevent the development of cancer.
cancers. The two discussed:
• The advantages of being able to analyze formalin-fixed, paraffin-embedded (FFPE) samples
• The MIP copy number platform design and the linear dynamic range of the assay
• The clinical importance of being able to identify copy number changes that can characterize different forms of pediatric cancers

“Some of our experiments have samples that are up to 15 years old, and we’re still able to get very clean copy number data from very little DNA, as little as 75 ng.”

Experimental design and sampling strategy

Parra: Can you tell us about the different cancers you’re studying? What are some of the challenges you’ve encountered and how did the Affymetrix platform address some of these challenges?

Schiffman: I focus on pediatric cancers. The three main cancers that our laboratory is studying at the moment are acute lymphoblastic leukemia (ALL), Ewing’s sarcoma, and pediatric brain tumors. We’ve also started some collaborative pilot studies in Burkitt lymphoma, a type of pediatric lymphoma.

The biggest challenge in studying pediatric cancers is sample availability. It’s very difficult to obtain large numbers of intact samples because children have fewer cancers than adults. It’s especially difficult to get brain tumor or Ewing’s sarcoma samples. Often what happens clinically is that a small biopsy will be taken and the patient will be treated with chemotherapy followed by surgical resection. At the time of resection, the tumor may be somewhat necrotic because it’s already been treated with chemotherapy. In other cases, the patient will die and a large enough piece of tumor will never have been obtained or stored for subsequent analysis.

We chose to use the MIP copy number platform because of its ability to interrogate FFPE samples and provide very clean copy number data. Some of our experiments use samples that are up to 15 years old, and we’re still able to get very clean copy number data from very little DNA, as little as 75 nanograms. Because only a small amount of DNA is required for the MIP assay, we are able to get enough DNA from a very fine needle aspirate or biopsy. This lets us study very rare samples, from relapses or metastatic lesions, which are often unavailable from biobanks. Studying these types of samples gives us information about the molecular genetics of tumor resistance. Because the assay works on FFPE samples, which is the way all clinical samples are stored, we have access to samples that would be otherwise unavailable.

Furthermore, the use of FFPE samples provides the advantage of knowing the clinical data from the patient’s history. Rather than having to wait years to accumulate samples and to see if the patient relapsed, we have the samples and we already know the outcome. We’re able to link our clinical data to molecular data, which enables us to identify prognostic markers and possibly identify new therapeutic targets.

Parra: Can you describe your experimental design and sampling strategy?

Schiffman: We found that the MIP copy number platform yields the best data when we include the highest numbers of matched normal samples. Whenever you perform high-resolution genomic microarray studies, you want to include as many normal samples as possible. Ideally you’d like those normal samples to be from the same patient. However, not every tumor biobank collects normal samples and keeps them as fresh-frozen specimens.

In many pediatric cancers, we perform staging at the time of diagnosis and often take a bone marrow aspirate or a lymph node biopsy to look for metastasis or to make sure a patient is in remission. These samples are often stored in paraffin blocks and contain normal cells that can be used for comparison. We always try to include paired normals in our studies so that we
can confirm that any copy number changes are not artifacts or carryover of normal copy number variation from the germline. We also can use normals from other patients from the same tissue source even when we don’t have paired samples.

**Parra:** How do you deal with the normal contamination in your samples?

**Schiffman:** For solid tumors, we’re able to detect copy number changes in tumor cells if there’s as much as 50 percent contamination with normal cells. The linear data and the allele-specific copy number data from the MIP platform makes it very obvious when there is a mix of normal and tumor cells.

**Parra:** What have you found to be the clinical implications of loss of heterozygosity with or without any kind of copy number change?

**Schiffman:** This is a really hot area of research right now. We’re finding that there are several samples that have regions of copy-neutral loss of heterozygosity, or what’s called uniparental disomy. For a particular gene or region, there are runs of homozygosity where the allele is identical. That may be a problem if there is a mutation of a particular tumor suppressor gene in that region and you have two identical copies with the mutation. These regions can be linked with clinical outcomes. We are currently collecting clinical data and correlating it with the unique copy number changes or runs of homozygosity we’re seeing in the different subsets of tumors.

**Platform design and data analysis**

**Parra:** You mentioned that the MIP copy number data from the ALL samples is very clean. Can you explain how the data compares to that of other genetic techniques?

As we started to collect and correlate clinical data, we started using the Nexus Copy Number software program from BioDiscovery, Inc. We have found that this program works well with the format of the MIP data set. One of the advantages of the Nexus Copy Number software is that it does a great job of identifying copy number changes. We required at least three to five probes consecutively that were either amplified or deleted before we considered it an accurate call. Unfortunately, we didn’t have a lot of success with our own software.

**Schiffman:** One of the major advantages of the MIP assay is the platform design. Once the molecular inversion probe hybridizes to the genomic DNA and then ligates closed with the complementary nucleotide, the probe is then circularized. Ultimately the probe itself is then amplified, rather than the genomic DNA. This reduces the chance of false positive results, decreases cross-hybridization of the sample to the microarray chip, and reduces the overall noise of the assay, all contributing to the high specificity of the assay. Additionally, it allows you to look at data in a linear format without having to look at a log transformation, and you can pick out very distinct copy number changes.

**Parra:** What are the benefits of the linear dynamic range of the platform and what does it allow you to see in the cancers that you’re studying?

**Schiffman:** It allows us to reliably detect copy number changes, amplifications as high as 80, sometimes even higher. We have correlated these high copy number changes with the expression of the involved genes. This instills confidence that the increased copy number changes we see are real.

There are some cancers that have shown correlation of higher copy number changes with increasingly worse prognosis. With the MIP assay, we can easily correlate patient outcome with higher copy numbers. Once we collect enough samples and enough outcome data, we’ll better understand the relationship between high copy number value and clinical outcome.

Other platforms don’t provide accurate results with copy number changes higher than about 60. Affymetrix scientists have done some very nice studies to measure the linear dynamic range of the MIP assay. One of the advantages of using the MIP platform is its ability to accurately detect very high changes in copy number, to values of 60 copies or higher per gene.

**Parra:** Can you describe your data analysis workflow? How easy is it to handle the large amounts of data?

**Schiffman:** We’ve been working with several different analysis platforms. Originally we designed our own software analysis program to try to identify copy number calls. We required that any particular probe had to have a call rate 90 percent or greater and that the standard deviation of that probe within the normal samples had to be 20 percent or less. We also required at least three to five probes consecutively that were either amplified or deleted before we considered it an accurate call. Unfortunately, we didn’t have a lot of success with our own software.

**Parra:** What methodologies will you be using for validating copy number changes?

As we started to collect and correlate clinical data, we started using the Nexus Copy Number software program from BioDiscovery, Inc. We have found that this program works well with the format of the MIP data set. One of the advantages of the Nexus Copy Number software is that it does a great job of correlating clinical features with genomic data. The program tells us which genes are involved with statistically significant overlapping signaling pathways within different subsets of samples. We are now able to tell some really interesting stories about which genes are involved in specific pathways within different clinical subsets of samples, and it’s all at the push of a button.
**Schiffman:** We routinely use real-time quantitative PCR, or RT-qPCR as it’s called, to validate the genes in which we’re really interested, as this method is considered the gold standard. However, recently we’ve started trying to validate our findings with the SNP Array 6.0 and we’ve found very good overlap between the two platforms.

We’ve also validated some of our findings with FISH probes. This technique offers the value of actually seeing the copy number increases in the cancer cells. We’ve been able to validate our MIP data with both FISH and RT-qPCR and we’ve seen very good correlation.

**MIP technology and the future**

**Parra:** How do you think the use of MIP copy number technology will change the future of patient care?

**Schiffman:** I think that MIP copy number technology is going to have a tremendous impact on translational medicine. The ability to go back in time and analyze literally an unlimited number of samples for any cancer of interest and to be able to obtain clinical data on those same specimens is going to be unbelievably powerful. We’re now going to be able to look at tens of thousands of samples and correlate clinical outcomes and clinical features with molecular data. This is going to lead directly to new developmental therapeutics in the pipeline, new targets, and new discovery of oncogenes and tumor suppressor genes.

I believe the ability to obtain molecular data from the hundreds of thousands of cancer patients who are diagnosed with cancer every day around the world is going to yield a goldmine of clinically relevant information. All of the patients around the country, and the world, who are clinically diagnosed with cancer have clinical samples stored in paraffin. Now, with the proper institutional review board approval, these diagnostic samples can be obtained for molecular analysis. There is an unlimited amount of information just waiting for physicians and scientists in the form of samples archived in paraffin. There is really nothing I can see that is going to prevent correlating the molecular and clinical data from these samples and translating this information back to patient treatment.

**Parra:** How do you see MIP Copy Number Services integrating with other technologies?

**Schiffman:** The MIP assay is a great complement to traditional microarray technology. The more high-resolution genomic platforms you can use to analyze copy number changes, the more confidence you have in your data. I think that MIPs should be incorporated with other assays to validate genomic findings. It is very cost prohibitive and labor intensive to do real-time qPCR on hundreds of different copy number changes across hundreds of samples. If you can run another genome-wide assay, like the MIP assay, in addition to your original microarray study, you can validate your markers in a very quick and affordable manner.

The ability of MIPs to work with such a small amount of DNA and to provide such consistently accurate results holds great promise for future clinical applications. The assay can be performed in just a few days, which is potentially enough time to impact risk stratification and patient treatment.

MIP Copy Number Services is a fantastic product. The assay is perfect for physician scientists who are interested in analyzing clinical samples to learn more about the molecular basis of cancer. It’s really one of the only assays currently available that will give you high-performance, high-quality results on archived FFPE samples.

**Further reading**