Haroldo Silva, Research Scientist,
OncoSENS Lead,
SENS Research Foundation

Investigator Profile

Simplifying a telomeric 3D fluorescence colocalization assay for high-throughput screening of cancer cells

"The problem that we encountered was that, while the image quality was good for all instruments, most couldn't achieve the throughput desired. With the ImageXpress Micro from Molecular Devices, however, we found good image quality and automation that enabled, hands-down, the fastest assays in terms of data acquisition and analysis"

– Haroldo Silva

Telomeres are repetitive DNA sequences at the end of chromosomes that shorten as cells replicate. To prevent telomere exhaustion and support indefinite proliferation, about 85–90% of cancers express telomerase, an enzyme that catalyzes the synthesis of telomeric DNA.

While anti-telomerase drugs are progressing through clinical trials, some tumors (10–15%) also contain cells that preserve their telomeres not by telomerase, but by a less-understood mechanism called alternative lengthening of telomeres (ALT). In these tumors, anti-telomerase drugs will potentially end up selecting for ALT-positive cells and will not eradicate a patient's cancer. Currently, little is known about the ALT pathway and few labs are studying it.

Haroldo Silva's lab is developing the first truly high-throughput versions of the assays commonly used to screen for genes and drugs relevant to the ALT pathway.

What is the state of the ALT field today?

We know all or most of the genes involved in the telomerase pathway. We know very little about ALT and few labs are studying it. We don't know what gene or genes to knockdown or overexpress to specifically regulate the ALT pathway. If you look at PubMed, telomerase-based studies average about 800 to 900 papers annually, while those on the ALT pathway average 35 to 40.

The lack of robust and functional ALT-relevant assays is partially slowing progress and restricting the size of the field. Since labs are quantitating the existing assays in different ways, it has been challenging to compare the data from one lab to another. Current methods do not allow this small field to study phenomena at the level and scale we need. We want to change that.

MOLECULAR DEVICES

We believe at the SENS Research Foundation that developing high-throughput, high-content methods to detect and measure ALT activity can help accelerate the pace at which this niche field can find drugs, molecules, and genes that are relevant to the ALT pathway.

What specifically is challenging about existing assays for ALT activity?

The ALT cancer pathway is studied using two main assays: the C-circle assay and the APB assay. The C-circle assay identifies abnormal circular DNA structures that are present in tremendous quantities in ALT cancer cell nuclei. The APB assay quantifies the frequency of colocalized fluorescently-labeled telomeric DNA probes and antibodies against ALT-associated promyelocytic leukemia (PML) nuclear bodies (APBs).

The APB assay is particularly problematic. When labs count colocalizations manually, methodologies or thresholds vary widely and it takes a long time to count enough cells to make valid conclusions. The best, most-detailed method to study APBs was developed by a German group that performed very careful image acquisition using 3D confocal microscopy and volumetric reconstruction for data analysis using supercomputers. While robust, it is not scalable.

We need to standardize the APB assay, namely the methods, the metrics, and the parameters that we measure so that labs can communicate more easily and understand collective results. Our work here is to do this simply, without either manual counting or the extreme of lengthy confocal microscopy image acquisition and 3D reconstruction.

How did you go about developing a high-throughput APB assay?

Inspired by the very detailed 3D analysis, we knew exactly the parameters that we needed to replicate, such as the average number of APBs per nuclei or the shift in the size of PML foci when localized to telomeres. We then tested three different high-content microscopy systems to see which allowed us to mimic, or rather reproduce, those data in a much faster, much simpler way.

The problem that we encountered was that, while the image quality was good for all instruments, most couldn't achieve the throughput desired. With the ImageXpress® Micro from Molecular Devices, however, we found good image quality and automation that enabled, hands-down, the fastest assays in terms of data acquisition and analysis. A few clicks set everything for acquisition and analysis, so there are no delays. That's one of the key factors in effortlessly using that instrument for the APB assay.

The high-content version of the APB assay that we developed consists of several steps. First, we seed the cells in 96-well plates and set up many different treatments and conditions across the plate. We culture the cells for a few days and stain for a telomeric DNA-binding protein (e.g. TRF2), nuclear DNA, and PML. We put the cells in the ImageXpress Micro, which automatically acquires three-color fluorescence images from every single well in the plate. The MetaXpress® software then analyzes all the images acquired for the colocalized signals between TRF2 and PML in the cell nucleus that indicate APBs.

What have you accomplished with your high-throughput APB assay?

Before the HCS, we could do a few slides; say 8 to 12 slides of 8 wells each, in a week from start to finish, collecting data from a few hundred cells at best. Using the assay we developed on the ImageXpress, if we really pushed it, we can do five to ten 96-well plates in one week of work and collect data from hundreds of thousands individual cells.

I can be a lot more ambitious now. Before, it would take me two weeks to see how two or three different conditions affected ALT cells using the APB assay. Now we're saying, let's look at these 20 different things, or 30, or 50 depending how we designed the experiment. It's completely changed how much we think we can accomplish with the work we're doing here.

What's next for your lab?

Our most immediate goal with the HCS APB assay is to study ALT activity by screening a library of about 1,200 drugs that are already approved by the FDA. We are trying to jump ahead by skipping the biology temporarily, screening as many compounds as we can to see if by chance we stumble upon a molecule that has a significant impact.

The next step in our ALT assay development efforts is to automate the entire process, from seeding the cells on plates to acquiring and analyzing the images. The speed automation provides can help small research groups like ours achieve more within a shorter timeframe. Before we made a list of 10 different genes that seem to be related to ALT activity; now we're looking at doing the whole genome. Another application for the system is using RNA interference to find which genes are responsible for regulating the ALT pathway. Once you make a high-content assay, it can be applied in many different ways, from basic research to drug development.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com
Check our website for a current listing of worldwide distributors