HUTCH NEWS

Molecular magnification

New SLAP technique allows researchers to study tiny tissue samples and develop tools for targeted treatment

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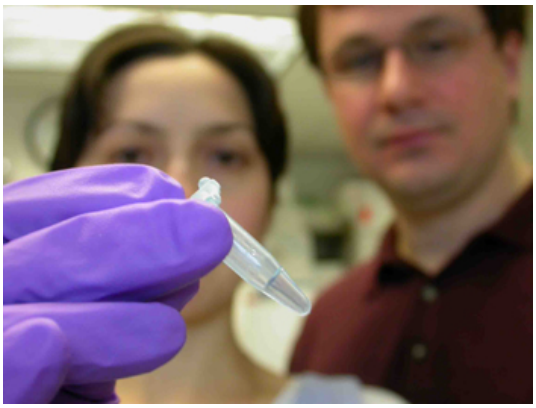

Dr. Derek Stirewalt and Era Pogosova-Agadjanyan inspect a tube of RNA, which will be prepared for analysis with a new technique called SLAP.
photo by Todd McNaught

A new technique developed in the Clinical Research Division could help scientists learn more about the genes that lead to cancer and develop better tests for diagnosis and treatment. The method may allow researchers to analyze genes from microscopically small pieces of human tissue, such as tumor biopsies, which have been difficult to study using conventional means.

The system enables scientists to conduct experiments designed to hone in on the sets of genes that are switched on or off in normal or cancerous cells. Such experiments are conducted using a tool known as a microarray, or DNA chip, which analyzes many or all of the genes in a cell simultaneously. Microarray experiments typically cannot be performed on tiny pieces of human tissue, which contain only a fraction of the number of cells needed to conduct the tests with high accuracy.

The new technique, nicknamed SLAP-which stands for Single-Stranded Linear Amplification Protocol-makes it possible to reproducibly and reliably perform microarray experiments using snippets of tissue that contain as few as 100,000 cells. The center has recently filed for patent protection on the procedure.

Dr. Derek Stirewalt, a clinical research associate in Dr. Jerry Radich's lab, led the study, which appears in the February issue of Genomics. Co-authors included Era Pogosova-Agadjanyan and Paula Ladne, research technicians in the Radich lab; Dan Hare, technician in the Genomics Shared Resource; Dr. Najma Khalid, research associate in the Public Health Sciences Division; Dr. Olga Sala-Torra, postdoc in the Radich lab; Dr. Lue Ping Zhao, investigator in the PHS Division; and Radich.

The technique can be used to conduct studies on any cell type. Stirewalt developed the method in order to conduct studies that compare normal white blood cells with cells from leukemia patients.

"Our lab is using microarrays to develop a better classification system for tumors based on the sets of genes that are turned on or off at different stages of the disease," Stirewalt said. "Microarrays may also allow us to find tumor-specific genes that might be good drug targets, which are not expressed in normal cells."

Amplification approaches

These and related studies have been hampered by the very tiny amounts of human tissue that are often available from tumor biopsies or samples of blood or other body fluids. Standard microarray methods do not permit a reliable investigation of minute numbers of cells. Although amplification techniques have been developed to help with such investigations, the existing methods tend to bias the results, Stirewalt said.

"This is a real challenge for many analyses," he said. "It's not a problem if you are working with cells that can be grown in the laboratory, which can be expanded into large quantities. But if you want to analyze small pieces of tissue dissected from a tumor, for example, it's very difficult

with the existing methods."

Microarrays permit researchers to analyze the expression of thousands of genes simultaneously. The experiments provide a snapshot of all genes that are switched on, or expressed, as well as those that are turned off. A comparison of normal breast tissue and breast tumors, for example, may reveal genetic clues about how cancer develops as well as the differences unique to subtypes of breast cancer. Ultimately, doctors may base treatment decisions on these kinds of tests. The microarray experiments also help researchers determine genes that are uniquely expressed in cancer cells. Such genes could be effective targets for new drugs that attack only cancer cells, sparing healthy cells from destruction.

Stirewalt said that most microarray experiments require scientists to use about 5 to 10 micrograms of RNA, which can be obtained from roughly 10 million cells. RNA is an intermediate molecule in the process of making proteins and provides a readout of genes that are active in a cell. A piece of tumor tissue might only contain about 100,000 cells, or 1 percent of the RNA that is required to obtain accurate results.

In order to conduct microarray experiments with small tissue samples, researchers must first make many copies of the RNA they obtain from the limited numbers of cells. Stirewalt said that two methods for boosting the amount of RNA have been available for the last several years.

"Both of these techniques have limitations when used with very small starting samples," he said. "One method, which uses a procedure called in vitro transcription, has good reliability, but has limited robustness of amplification and problems generating complete RNA molecules, which means you can lose parts that might be important to your study. The other technique utilizes a polymerase chain reaction (PCR) method of amplification. Although it is much more robust, it has been associated with increased amplification biases, which could limit the reliability of the microarray results."

SLAP method

The SLAP method merges the two amplification approaches and attempts to incorporate the positive aspects from each of the other two techniques. Stirewalt said that when compared to the older in vitro transcription method, SLAP is more robust and yielded highly reproducible and reliable results when used with tiny amounts of starting material.

"Microarray analysis is a very powerful technology for cancer research, but it's been limited in its application because of the difficulties in obtaining sufficient amounts of sample." Stirewalt said. "SLAP may help to overcome that."

Stirewalt said that several labs at the center are using the method and researchers at the University of Washington, Harvard University and the University of Michigan have expressed interest as well.

"We expect that it will be useful for asking basic biological questions about cancer, which is the first step in the development of new tests to accurately diagnose and stage cancer."

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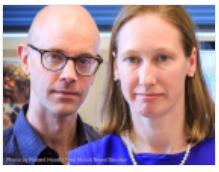

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