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CANCER RESEARCH CENTER

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Chromosomal Clues

From analyzing liquid samples to solving the puzzles of karyotyping, Cytogenetics Lab helps doctors diagnose disease and monitor treatment

Holding a vial of bone marrow — which contains the clues for saving a patient's life — is Aija Alberg, research technician in the Cytogenetics Lab. Photo by Michelle Hruby

By MARITA GRAUBE

A three-inch-tall vial stands in a wire rack on a lab countertop. The thick, red liquid inside, extracted from bone marrow, awaits analysis. In it lie crucial clues for saving a patient's life.

Ferreting out these clues every day, the Cytogenetics Lab of the Seattle Cancer Care Alliance helps physicians determine a diagnosis or prognosis and monitor the progress of therapy.

The lab works mainly with samples from hematopoietic stem-cell transplant patients. Hematopoietic stem cells, found in blood and bone marrow, produce blood and immune system cells. Patients with leukemia or other potentially fatal blood disorders may be treated with stem-cell transplantation.

With a bone-marrow sample, the cytogenetic technologists study the patient's dividing cells and the chromosomes within them. Each of a human's 23 chromosome pairs brims with genetic information. Abnormalities such as an extra, missing or rearranged chromosome may signal disease.

New technologies

With anxious patients awaiting results, samples must be processed rapidly. To keep up with the pace, the lab draws on its staff expertise and new technologies.

Formerly in the basement of the center's Columbia Building on First Hill, the Cytogenetics Lab moved to the seventh floor of the Alliance clinic in January 2001.

"We helped design the lab with the architects," said Lori Klebeck, lab supervisor. "It's laid out from start to finish for efficient specimen flow."

Klebeck moves through the lab, showing her team's course of action from the moment a sample arrives at the lab's counter to the final product of a completed report for the physician.

First, samples of blood or marrow grow in culture. Then a chemical solution disrupts cell division so that the cells are frozen in time. The idea is to catch a cell in metaphase, the stage of cell division in which the chromosomes are visible.

After a session of cleaning, rinsing and drying the samples, cells are dropped onto a slide. But this laundering process isn't a simple one.

With the wrong temperature or humidity, the chromosomes won't appear clearly under the microscope. These sensitive samples are extremely finicky, and as with the storybook character Goldilocks, everything must be "just right."

"You can't dry it too fast or too slow," Klebeck said. "It's really an art to make a slide dry evenly and at the proper rate."

After the sample dries, it bakes for an hour, gets an enzyme treatment, absorbs a purple dye and emerges ready to view.

Under the microscope, the chromosomes look like a tangle of multi-length grub worms in a bait bucket. To make sense of this mess, a cytogenetic technologist creates a karyotype, which neatly orders the 23 chromosome pairs.

The karyotype lines up the first 22 chromosome pairs, which are identified by size and shape. Then the chromosomes are paired according to dark and light banding patterns that appeared from the purple dye. Each band can hold close to 100 genes.

The remaining chromosome pair is composed of the sex chromosomes. Female cells contain two X chromosomes and male cells contain one X and one Y chromosome.

Pieces of the puzzle

With the pieces of the puzzle assembled in the karyotype, a cytogenetic technologist analyzes the chromosomes to look for possible clues of disease.

At this stage, advanced technologies enter the process to help create and study the karyotype. Booting up the computer, Klebeck clicks open a patient's karyotype. The image uploads from a camera attached to a microscope.

Similar to a photo-editing program, this karyotyping software can cut, paste and move the images of chromosomes into place on the karyotype. Before the software, Klebeck said, this was performed by hand with scissors and glue.

Once the karyotype is in place, the stained chromosomes are examined carefully for subtle differences in the banding pattern to see if parts of the chromosomes are missing, deleted or relocated.

But even the most highly trained eyes can't always detect a difference in the banding pattern. That's where another technology comes in, called fluorescence in situ hybridization (FISH), which searches deeply inside the bands of the chromosomes to uncover highly detailed information.

FISH detects specific areas of a chromosome, such as a sequence of DNA, a gene or part of a chromosome. Sometimes, as in the case of certain diseases, checking a specific gene within a

chromosome is the best way to see abnormalities that can signal disease.

Using selected DNA sequences, called probes, FISH marks an entire chromosome or single unique sequence of DNA. To help pinpoint the specific location on the chromosome, the probe contains a fluorescent reporter molecule, which illuminates brightly under a specially equipped microscope.

"FISH allows us to detect genetic changes at the molecular level," said the lab director, Dr. Eileen Bryant.

Residual disease

Different types of FISH probes help answer different questions about the health of a patient. For example, one FISH probe known to be a marker for a specific tumor helps the cytogenetic technologists monitor for minimal residual disease.

Another type of probe marks the sex chromosomes (XX and XY). This probe is routinely used to monitor hematopoietic stem-cell transplant patients who have received a transplant from a donor of the opposite sex. To monitor engraftment, the probe provides a genetic marker that tracks how many of the healthy donor cells have repopulated the marrow of a transplant patient.

Unlike the karyotype procedure that requires dividing cells, FISH also works with non-dividing cells, which are much easier to obtain.

"This makes FISH a versatile procedure," Bryant said.

One of the newest aspects of FISH technology, called spectral karyotyping (SKY), also marks and colors its targets. Like a karyotype, a SKY image displays the shapes of the chromosomes, but instead of the purple stripes of the classic banding technique, a particular color shades each chromosome pair.

Not only does the SKY imaging shine with dozens of colors, it also gives more clues to the puzzle. If parts of the chromosomes have broken off and switched places, a change called a translocation, the chromosome will no longer display one single color. Instead, a translocated chromosome may contain two or three colors, showing that something is amiss.

On a classically banded karyotype, a switch of chromosome pieces could remain undetected, but with SKY it becomes immediately visible.

Although these advancements in technology have greatly extended the staff's analytical abilities, Klebeck expresses confidence that the computers won't take the place of humans.

"Computer technology has streamlined our work," Klebeck said, "but you still need the trained eye of a technologist to make a cytogenetic diagnosis."

Bryant and Klebeck hope that other microscopists will also share their team's passion for chromosomes, as there is a high need for trained cytogenetic technologists. The work takes training and demands intensive labor, Bryant said, but it's quite interesting.

Staff interaction

Although their work is mostly individual, staff often interact, especially when interpreting a karyotype.

"We have a strategy of a team effort, and it works well for this lab group," Bryant said.

To reiterate the need for timely results, Bryant urges staff members to accompany physicians as they visit their transplant patients.

"I encourage staff to see there's a face behind the study. I think it gives them another level of appreciation of the importance of their work."

[Marita Graube, a graduate in technical communications at the University of Washington, is a writer for Northwest Science and Technology.]

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