Philadelphia College of Osteopathic Medicine Examines Embryonic Cells to Find the Origin of Muscles With Image Analysis Software

Kisha D. DeSandies and Gabriella Madden

Tens of thousands of Americans suffer from muscular dystrophy (MD), a disease that gradually deteriorates a person's skeletal muscle. While there is no effective cure, scientists know MD is caused by a genetic defect and are searching for treatments that will stop or retard the deterioration of muscle. In June, American and British researchers announced the success of a long-term treatment that repairs the genetic defect in Duchenne muscular dystrophy, the most common childhood form of MD.

Last fall, these researchers injected a short strand of nucleic acid into the shin muscle of a six-week old golden retriever – which had a genetic defect that leads to Duchenne MD in dogs – in order to maintain normal levels of dystrophin, the muscle protein missing in Duchenne MD. Their goal was for the nucleic acid to trigger the dog's system to correct the genetic defect. Eleven months later, the injected muscle continues to show normal levels of dystrophin.

Researchers in the Anatomy Department at the Philadelphia College of Osteopathic Medicine (PCOM) are taking an alternative approach to muscle research. With funding from the National Institute of Health, they are studying the origin of muscle at the embryonic stage. Their findings could eventually help in the treatment of muscle-related diseases, like MD.

For ten years, the PCOM's research team – led by Chief Investigator, Mindy George-Weinstein and Senior Research Assistant, Jackie Gerhart – has been investigating when and how embryonic cells become programmed to form muscle. They use chick embryos as an animal model, because they can be obtained in unlimited quantities and in many ways resemble human embryos.

The scientific image analysis software Image-Pro Plus, made by Media Cybernetics in Silver Spring, Md., has enhanced PCOM's research, enabling them to more accurately view and analyze images under the microscope and clearly document their findings.

Researching Muscle Development

Before becoming muscle, embryonic cells go through a number of divisions and migrate throughout the embryo to produce a sufficiently sized muscle mass in the proper locations. The question that emerges with these and other embryonic cells is, if all cells have the same DNA, how do they turn on the genes to form muscle instead of cartilage and nerves?

"It's very important to know how cells become programmed to form muscle in order to develop methods for identifying and isolating precursor cells to implant into diseased tissues," says Gerhart.

The research team is searching for the muscle marker, MyoD, in the cells of young chick embryos. MyoD is responsible for turning on other genes that enable a muscle cell to contract.

To detect MyoD, researchers have developed a Fluorescent In Situ Hybridization (FISH) procedure, which uses extremely sensitive and precise probes called DNA dendrimers to localize messenger RNA for MyoD in the early embryo. The dendrimers, developed by Genisphere, Inc. (Philadelphia, Pa.), contain fluorescent tags and a recognition sequence for mRNA. These reagents have allowed them to detect the presence of MyoD immediately after the egg is laid, in the epiblast layer of cells that give rise to the entire embryo.

Normally, fluorescent images can be photographed using a 35mm camera attached to a microscope. However, processing

the film and printing the negatives can take several days, and it is difficult to tell if a quality image has been captured until after printing.

Research Challenges

The team encountered problems photographing early muscle precursor cells because the cells contained low levels of MyoD. When the stained specimen was viewed under a fluorescent light microscope, the light faded the fluorescence, usually within 10 seconds to a minute, making it undetectable. This fading is called photobleaching.

Typically, the chick embryo gives off a natural fluorescent background color. Therefore, when the sample is stained, there is little to no contrast between the sample color and the stain, making it difficult for the team to clearly identify MyoD.

"We couldn't capture the images quickly enough to document the presence of MyoD," says George-Weinstein.

Racing against the clock, researchers snapped as many photographs as possible before the sample is fully bleached, in hopes of getting one photo they can use for publication. However, most of the photos were not useable and the team was forced to start the process over. Furthermore, in order to assemble multiple photographs in a single figure for publication, prints had to be cut and pasted and the composite re-photographed and printed. The whole reproduction process took about a week and often reduced the quality of the prints.

The team's inability to save images for later use was also frustrating. Only one person could view the image because it would fade by the time the second person got to the microscope.

Image Analysis Software

Two years ago, the team began to search for a more effective way to view, analyze, and document their research. The research team was introduced to scientific image analysis software during a microscope demonstration. Image analysis software allows researchers to capture images directly from a digitally equipped microscope and transfer the images onto a computer screen where they can enhance the image quality for better analysis. They are also able to record and store data and images electronically.

The team decided this technology would be beneficial to their work and in addition to a Nikon E800 microscope and Optronics DEI 750 camera, they purchased the image analysis software Image-Pro Plus.

George-Weinstein and Gerhart say the new microscope and Image-Pro Plus have virtually eliminated the team's documentation problems and allows them to see finer details of their work.

Image-Pro Plus' standard image filters enable the team to reduce the natural background color of the tissue sample and highlight the MyoD markers clearly. This gives them a more accurate view to identify MyoD and changes in cells.

"Before we could only see a small portion of what was really there," Gerhart says. "With the new scope and computer imaging system, we're probably seeing close to 100 percent of the signal."

Image-Pro Plus also allows the team to capture an image in two seconds. The quick capture time allows for minimal light exposure, reducing the amount of photobleaching. Since the fluorescent signal remains stronger and appears much brighter on the image, multiple researchers are able to view the image on a computer screen and immediately determine its quality.

Another benefit of using image analysis software is that the team can save an image onto their computer hard drive, disk or

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CD-ROM, allowing them to go back and manipulate an image in a matter of seconds to enhance contrast and color and label for identification. Printing the image straight from the computer saves them the time of printing from negatives and preserves the high resolution of the image. They can also make publication quality photos with four to six images on one sheet, or overlay different images in minutes.

"A lot of journals are requiring you to submit manuscripts electronically and the imaging system helps us do it faster," says George-Weinstein. "We can provide better resolution of an image because it doesn't have to be rescanned or reproduced."

Future of Scientific Image Analysis Research

Weinstein says using an image analysis software system not only makes their research more efficient, but also helps them keep up with the changing technology. She and the other researchers in the Anatomy Department of the Philadelphia College of Osteopathic Medicine plan to continue in their quest to find the answers to the mysteries of embryonic development. Using Image-Pro Plus, the team hopes to decode the riddle of how and when muscle cells arise during embryonic development and find the location of those muscle cells in adults. ■

About the authors: Kisha DeSandies and Gabriella Madden are writers at Strategic Communications Group, in Silver Spring, MD. They can be reached at Tel: 301-408-4500 or Email: kdesandies@gotostrategic.com or gmadden@gotostrategic.com