Zebrafish Take the Stage in Cancer Research

By Mary Beckman

Cancer biologists are dashing a little striped fish as bait to net answers to many unresolved cancer research questions. Commonly known as the zebrafish and found in your local pet store, the species Danio rerio is helping scientists investigate a wide variety of malignancies, including breast and colon cancer, melanoma, and leukemia. And researchers hope the small translucent vertebrate will help make some processes, such as angiogenesis or genomic instability, more transparent.

“There’s quite a bit of interest in this new animal model,” says breast cancer researcher Richard Klemke, Ph.D., of the University of California, San Diego. He takes advantage of the fish’s thin skin to watch fluorescent tumor cells as they migrate through tissues to find out why some cancers become so invasive.

Molecular oncologist Thomas Look, M.D., of Dana-Farber Cancer Institute in Boston, says that the zebrafish field has grown substantially in the last 7 years, due partly to its advantages over mammals such as mice. “A mating pair can make 100–300 embryos a week,” he says. His Harvard University colleague and sometime collaborator Leonard Zon, M.D., agrees. “My fish facility has about 150,000 fish in it. You would need an entire building to house that number of mice,” he says.

Zebrafish have long been the darling of developmental biologists. Their see-through eggs, which hatch in about 4 or 5 days, have allowed scientists to track early events in embryonic development. The embryos—each about the size of the white sugar dots on chocolate nonpareil candies—can be used in experiments in large numbers. This advantage allows researchers to test anticancer drugs easily, says cancer biologist Ronald DePinho, M.D., also at Dana-Farber. They can also engineer cancer and other complex human diseases in zebrafish while maintaining thousands of “subjects,” he says. “There is an unprecedented opportunity to conduct medium-throughput ‘physiological’ screens for novel agents.” Also, zebrafish can live 4 or 5 years, allowing some cancer biologists to study when and how cancer develops normally during the fish’s lifetime as a parallel for human disease.

One Fish ... Two Fish ... Red, Green, and Blue Fish

In 2003, Look and Zon inserted a mouse cancer gene into zebrafish chromosomes to generate the first transgenic fish, which resulted in leukemia. To visualize the oncogene c-myc in the animals, they engineered a green fluorescent protein onto the end of mouse c-myc and overproduced it in zebrafish immune cells. Under a microscope, the bright green cells can be seen right through the swimmers’ skin. The animals came down with colorful lymphomas after about a month and died of leukemia around 90 days of age. “The model was very reminiscent of T-cell lymphoma progression to leukemia in mammals,” Look says.

What Look considers a distinct advantage of zebrafish appears, at first, counterintuitive: Zebrafish have no bone marrow, making their blood cells instead in the kidney. Look says that helps researchers focus on what happens in leukemia cells. “In humans and mice, leukemia crowds out normal stem cells. Their problems are due to lack of normal cells.” Instead of dying of anemia, fish die of unimpeded cancer cell growth. “We’re interested in what makes leukemia grow and regress, so we’re more interested in leukemia cells and not how they might suppress normal cells,” he says.

UC–San Diego’s Klemke put human cancer cells glowing blue, red, and green in zebrafish in 2006 to study how breast cancers metastasize. First, his group engineered the human breast cancer cell line MDA to produce a fluorescent protein of one color and then engineered some MDA cells to overproduce another color for the fluorescently tagged protein RhoC, which appears in aggressive breast cancers. The team injected these cell lines into fish whose blood vessels glowed green because of yet another fluorescent protein and then looked through a microscope at the advancing cancer.

They found that the cancer cells moving to invade—the RhoC cells—changed shape and didn’t chew through tissue to get around, as some cells do. Instead, the cells found existing openings in blood vessels and they “squirt and bleb through the holes,” Klemke says. “This has big implications for trying to treat invasive breast cancer. If we block one type of movement, will the cells switch to the other?” In the same study, the team also found that the VEGF protein can puncture tiny holes in blood vessels, near where the RhoC tumor cells dock. Klemke says this finding suggests that VEGF and RhoC work in concert to help cancer cells get into the bloodstream.

Fish Disease Illuminates Humans

Zon is also using zebrafish to investigate melanoma. As people keeping an eye on their freckles can attest, melanomas start out looking like a mole. In human moles, the gene BRAF is always on in 80%–90% of the cells, whereas BRAF is usually off in normal cells. When Zon’s group engineered fish epithelial cells to pump out human BRAF, the zebrafish got moles—but no melanoma.

Then Zon mated the BRAF fish to animals with a mutant p53 gene, the most common cancer-causing gene. The fish carrying both BRAF and the mutant p53 did get
“What that told us is that you need two events to get melanoma,” Zon says. “That was a very nice story.” Now he is looking for as yet unidentified cancer stem cells—the parent cells of tumors thought to give rise to more disease. By separating out single malignant cells and transplanting the individuals into fish, he hopes to determine which cells serve as architects of cancer.

Zebrafish scientists have also found that ribosomal genes might play a bigger role in cancer than previously thought. Researchers Nancy Hopkins, Ph.D., and Jacqueline Lees, Ph.D., at the Massachusetts Institute of Technology in Cambridge looked to see whether fish carrying certain kinds of mutations known as “recessive embryonic lethals” had higher rates of cancer. These embryonic lethal genes normally kill embryos when both chromosomal copies are mutant. But when one gene copy is normal and the other mutant in people, those individuals might have higher rates of cancer.

The researchers noticed that some fish carrying one copy of some embryonic lethal genes died younger than healthy fish and had malignancies growing in a variety of tissues. Eleven of the 12 mutated genes turned out to code for ribosomal proteins, molecules involved in protein production inside cells. “That’s never been suspected except in rare instances. It’s kind of an astounding result,” Look says.

Other researchers are using zebrafish genetics to examine genomic instability, the tendency of chromosomes to fall to pieces in cancer cells. Geneticist Keith Cheng, M.D., Ph.D., at Pennsylvania State College of Medicine in Hershey randomly mutated zebrafish genes and then screened for embryos with higher rates of genomic instability. He could “see” genomic instability by looking at the embryonic eye: Mutations that increase chromosomal damage also made the cells lighter in color, so fish with mosaic eyes carried genomic instability mutations.

“And you don’t have to kill the embryo,” Cheng says. “I can look at a bowlful of embryos and find mutations.” And find them he did. The screening came up with 12 mutations, the strongest of which caused a 10-fold increase in cancers in middle-aged fish. Cheng is also using zebrafish to investigate which genes are responsible for the histological appearance of cancers under the microscope and how such genes might contribute to cancer.

**Development to Malignancy**

The cumulative knowledge of zebrafish development has given cancer scientists a jumping-off point to study colon cancer and processes such as blood vessel formation. For example, molecular biologist Stephen Ekker, Ph.D., of the University of Minnesota, Twin Cities, is trying to understand how blood vessels develop in embryos as a model for what happens as tumors grow. “Most tumors have weak or leaky vessels. The ones that get really big make the most normal blood vessels,” he says.

But you can’t block blood vessel formation in mammals because they need the vessels for their placentas, a structure absent in eggs. By deleting genes one at a time in zebrafish and watching what happens to the blood vessels developing in embryos, Ekker and his group found a gene called syndecan-2 that is required for the formation of new blood vessels. He is also exploring whether certain Alzheimer drugs that fight cancer do so by converting the artery-like vessels in tumors to more veinlike vessels.

Colon cancer researcher David Jones, Ph.D., at the University of Utah in Salt Lake City studies the formation of zebrafish intestines. Eighty-five percent of colon cancers carry mutations in a gene called adenomatous polyposis coli (APC). Mutant APC in fish causes epithelial cells lining the gut to be stuck in an immature state, and his group reported in 2004 that APC mutants failed to make retinoic acid. “The fish now become our patients,” he says. “We give them retinoic acid and their intestines develop more normally.” He adds that side effects of retinoic acid could limit its usefulness in treating colon cancer.

From Cheng’s bowlful of embryos to Zon’s 150,000-critter facility, cancer biologists are happy to have the aquarium dwellers in their labs. “At the beginning we didn’t know how good it would be. It turns out to be a fantastic system for studying cancer,” Zon says.