



PHOTOGRAPH by Sara Coburn

Killing cancer?

By Donna Self

Not Yet. More than a million people are diagnosed with cancer every year. While a cure remains beyond our reach, Mote scientists have discovered something that kills cancer cell lines in the lab. The process is decades away from any applied research but it could be a hand up in the search for new treatments.



PHOTOGRAPH by Dick Dickinson

Mote scientists Dr. Carl Luer and Dr. Cathy Walsh know *something* is killing the cancer cells they study in the lab.

But what? How does it kill the cancer cells in a Petri dish, and why? And what could it mean decades down the road to people with cancer?

Luer and Walsh have spent their professional careers in Mote Marine Laboratory's Center for Shark Research trying to find out why sharks and rays rarely get cancer and what it might mean for humans who do. The reward for every victory — large and small — has been a new set of questions, a new line of study and a new search for ways to pay for the scientific analysis needed to answer the questions.

"It's not the kind of research where you all of a sudden find something. It's more like a result of weeks and months and years of research that you realize you have something," says Luer from behind a desk overrun with coffee mugs, piles of papers and dried skate purses.

The American Cancer Society estimates that in 2004, nearly 1.4 million people — roughly four times the population of

Sarasota County, Fla. — will be diagnosed with cancer and that 563,700 people will die from the disease. Most people with cancer are treated with surgery, radiation and chemotherapy. But some oncologists are now looking at immunotherapy as a fourth mode of treatment.

That's where Luer and Walsh have set their sights as well.

THE LONG HAUL

Luer's research into sharks started 25 years ago when the biochemist arrived at Mote in the summer of 1979 with the ink still damp on his University of Kansas Medical Center Ph.D. He joined a staff of about 20 and worked on a table in director emeritus Perry Gilbert's office. His lab was in a trailer.

During graduate studies, he took and taught numerous comparative vertebrate biology courses, all of which included information on sharks. "I got to appreciate them on both a molecular level as well as a whole-animal level," he says. "Too often, people who perform either molecular or behavioral studies don't see the relationship there. I'm pleased that I can see both ends of the spectrum."

Working with cancer

To test activity of the secreted substance, Dr. Carl Luer and Dr. Cathy Walsh work with three cancer cell lines obtained from the American Type Culture Collection. Two are human cell lines: breast cancer and malignant melanoma. A third, a fibrosarcoma, comes from mice.

Walsh and Luer take immune cells from the epigonal organs of sharks and put them in culture. Using a centrifuge, they separate fluid from the shark cells.

The fluid has a high salt concentration that would kill the mammalian cancer cells, so the salt is removed. Then the fluid is freeze-dried to a powder form, placed in a medium friendly to the mammalian cancer cells and left for three days to grow.

Walsh and Luer add a dye that shows the difference between live and dead cells. The consistent result: About 80 percent of the cancer cells die.

Walsh and Luer have a patent pending with the U.S. Patent and Trademark Office on the secreted products and on the process of producing the secreted products.

Knowing about sharks led Luer to study the low incidence of disease, especially cancer, in elasmobranchs — the subclass of cartilaginous fishes that includes sharks, skates and rays. “We went to the literature and found that the impression that sharks didn’t get cancer was based primarily on anecdotal observations,” Luer says. “There had been little, if any, experimentation; it was just difficult to document any reasonable occurrence of sick sharks. The specimens used in prior studies were usually dead, and we wanted to be able to study live animals, so my first goal was to establish the use of elasmobranchs as lab animals.”

WHO NEEDS RATS?

Luer picked nurse sharks and clearnose skates for several reasons.

Nurse sharks survive well in captivity and because of their sedentary nature, don’t require large holding spaces. They are also relatively docile and can be collected as newborn pups. They grow slowly, so it takes three or four years before they become too large to handle.

The downside is that it takes about 20 years for nurse sharks to become sexually mature, so animals old enough to reproduce are too large to be kept as experimental animals.

Clearnose skates, whose biochemistry and physiology closely resembles those of sharks, complement nurse shark studies. As sexually mature specimens, they’re relatively easy to maintain and breed in captivity. Since skates are egg-layers, mothers don’t have to be sacrificed for embryonic specimens. Luer could establish a controlled population, much like the lab rat population that can be bought with a complete genetic history — a sort of rat pedigree.

After picking his animals, Luer set out to explore whether sharks and skates really were resistant to cancer and other diseases.

He exposed sharks and skates to two potent chemical carcinogens to try to induce cancer. But the carcinogens — aflatoxin B₁, a naturally occurring toxin produced by molds,

and methylazoxymethanol-acetate (MAM) — didn’t succeed. No tumors developed.

While Luer did learn how those carcinogens are metabolized by the animals, nearly 10 years of experiments failed to produce evidence of a single tumor. “We felt we weren’t getting to the bottom line with this approach, so in 1989, with the field of immunology exploding due to the awareness of AIDS, we decided this might be a productive area of study. We began to investigate elasmobranch immunology and hoped that understanding how their immune system functioned might explain their apparent resistance to disease.”

ENTER CATHY WALSH

Walsh came to Mote in 1991 for her first professional position after earning her Ph.D. from Clemson University. She hasn’t left.

With Walsh came new study goals: What kind of immune cells do elasmobranchs have and what is their function? Where are they produced? And, ultimately, how are these functions regulated?

The answer to the first question was that sharks and rays have essentially the same types of leukocytes, or immune cells, as humans: lymphocytes, granulocytes and macrophages.

Immune cells are produced and called to action in the face of injury or infection. In humans, they are made in the bone marrow, the spleen, the thymus and the lymph nodes. Sharks don’t have bones, so shark immune cells obviously aren’t produced in bone marrow. They also lack lymph nodes.

Elasmobranchs do have spleens that play an important role in their immune functions. They also have two other immune cell-producing organs unique to this group of fishes: the epigonal organ, located near the reproductive organs, and the Leydig organ, near the esophagus.

BUT WHAT ABOUT THE THYMUS?

The thymus is a sort of factory area where a specific type of immune cell is produced. T-cells, or thymus-derived

lymphocytes, are made there. Evidence of the suspected location of a thymus in sharks had been published nearly 100 years before Luer and Walsh started their research. But a literature search turned up confusion as to whether sharks had a thymus beyond their embryonic stage.

“After five years of dissection and looking, finding the thymus was significant for us,” says Walsh. “I remember that the first time we were absolutely certain of its location was during the dissection of a near-term blacknose shark.”

Walsh and Luer found that one of the reasons the thymus had been especially difficult to locate was that it was easily destroyed during dissection, rendering it unrecognizable. It also diminishes gradually as the animal reaches sexual maturity. In humans, the thymus is prominent until puberty, then disintegrates and is replaced with connective tissue.

ANOTHER BREAKTHROUGH

It remained extremely difficult, if not impossible, to explore the functions of shark immune cells in a live animal, and the cells didn’t live long enough in a test tube.

So Walsh came up with a method of putting shark immune cells in short-term culture, leading the way to the most recent area of study: the investigation of immune regulatory molecules that have the potential to inhibit the growth of tumor cell lines in the lab. When certain molecules secreted by the cultured shark cells are introduced to cancer cell lines, the consistent result is that about 80 percent of the cancer cells die.

With this finding comes still new questions. Luer and Walsh need to identify the secreted substance, determine if it contributes to the shark’s low incidence of cancer and explore what potential it holds for applications to human health.

“It’s still a complex mixture,” Walsh says. “We’re hoping to isolate and purify the active components. If it continues to show promise, that would be great, but that’s a long way down the road.” ■

THE MYSTERY AND THE MONEY

Identifying the “mystery fluid” that Drs. Carl Luer and Cathy Walsh have coaxed from shark cells isn’t quick or simple.

Characterizing the substance relies on sophisticated biochemical techniques to identify the types of molecules present. It’s a long process and success is proportional to funding.

“Our studies would go a lot faster if we had more funding,” Luer says. “We could have more people, newer equipment and we wouldn’t have to spend most of our time looking for money.”

The pair must compete with the thousands of cancer research projects funded by the federal government and traditional cancer research organizations each year. Funding in the past has come from the Ann and Alfred Goldstein Foundation, the Vernal W. and Florence H. Bates Foundation, the Elsa U. Pardee Foundation and the Polly Loomis Endowment for Marine Biomedical Research.

But “we have trouble convincing the conventional cancer research entities because we don’t have decades and decades of literature to support our animal model. Mammalian models have a more proven track record,” Luer says.

Individuals and organizations interested in supporting shark immunology studies can contact Mote’s Development Office at (941) 388-4441, ext 373.

Collaborators in the shark immunology work have included Dr. A.B. “Budd” Bodine at Clemson University, Dr. Gary Litman, of the University of South Florida Children’s Research Institute and Dr. Clay Smith, formerly of the H. Lee Moffitt Cancer Research Institute.