

Thinking outside the cell

Anti-estrogen drugs like tamoxifen help fight breast cancer by keeping the natural hormone away from the many breast cancer cells that rely on estrogen to spark rapid tumor growth. But not all breast cancer cells respond to estrogen, so not all breast cancer patients are helped by such drugs. To decide whether to use them or other chemotherapy and radiation, doctors use a test that is only about 70 percent accurate.

That test screens the nuclei of breast cancer tumor cells for estrogen receptors, molecules that “dock” with the hormone and enable the cells to respond to its signal by multiplying. UTMB scientist Cheryl Watson thinks she knows why the tests are unreliable: because they’re failing to detect some estrogen receptors. What’s more, Watson, a professor of human biological chemistry and genetics and the associate director of research for the Center for Interdisciplinary Research in Women’s Health, thinks she knows where to find the undetected molecules: not in the cell’s nucleus, as orthodox science insists, but in its membrane—as far from the nucleus as it’s possible to get and still be in the cell.

The notion that cell membranes might harbor estrogen receptors has intrigued Watson for the last fifteen years, ever since a collaborator, former UTMB researcher Bahiru Gametchu, found that another kind of hormone receptor could be detected in cell membranes. Gametchu was attempting to use fluorescent antibodies to label glucocorticoid receptors in leukemic cancer cell nuclei. That required using a detergent or other chemical to punch holes in the cells’ membranes, through which the antibodies could pass. One day he forgot to poke holes in the cell membranes. Still, when he peered through his microscope he saw tiny, glowing spots scattered on the surface of the cells—evidence that, despite his omission, the added antibodies had labeled something.

Gametchu shared his surprise with Watson, who recalled that Clara M. Szego, a UCLA researcher, had claimed in the early 1970s that receptors like those in the nucleus also existed in the membranes of cells. That could explain Gametchu’s glowing dots. Szego’s theory remains highly controversial. Watson, however, was fascinated by its possible implications for estrogen receptors, the focus of her own research. She has since become one of the idea’s foremost proponents, featuring it among other theories in a book she edited, *The Identities of Membrane Steroid Receptors...And Other Proteins Mediating Nongenomic Steroid Action*, published in January 2003.

The presence of estrogen receptors in cell membranes could explain an effect Watson has seen when she adds estrogen to rodent pituitary tumor cells: the cells suddenly release large amounts of prolactin, a powerful reproductive hormone capable of inducing lactation and maternal behavior. Like Szego, who observed similar swift cellular changes, Watson is skeptical that hormones binding to

receptors in the nucleus could cause such a quick response, since that would require that many RNAs and proteins be made from scratch. Instead, Watson theorizes that estrogen, by binding with membrane estrogen receptors, unlocks the cell’s external doors, prompting prolactin stored inside the cell to be released.

Watson believes that if she can continue convincingly to demonstrate that estrogen receptors identical to those in the nucleus exist in the cell membrane (and she acknowledges that other theories may also explain these data), her work could lead to better treatment for breast cancer. One outcome could be a better test for determining the likely result of anti-estrogen therapy, one that screens cell membranes as well as cell nuclei for estrogen receptors. “The idea,” Watson says, “is to eliminate the unnecessary treatments and get on to the effective ones.”

—Ann T. Lemon



Professor Cheryl S. Watson: on the trail of better treatments for breast cancer.

An inside job

The subject lies sedated on a table in a UTMB hospital operating room. Nearby, seven gastroenterologists observe video and computer monitors as a thin, round flexible instrument is carefully pushed through the subject's mouth, snaking down through the glossy pink esophagus and gently entering the shimmering walls of the stomach.

The instrument is an endoscope, a long, cylindrical tool that uses tiny surgical devices and fiber optics to perform diagnostic and therapeutic procedures within the gastrointestinal (GI) area without requiring invasive surgery. The subject is a pig. And the observing gastroenterologists are world-class leaders in endoscopic therapy research conducting an experiment to find out if a common human ailment, gastro-esophageal reflux disease (also known as acid reflux disease), can be treated non-invasively.

The researchers watch the monitor as a tiny set of pincers grasps the lower esophageal sphincter where the esophagus meets the stomach. The pincers pull on the sphincter to bring it farther into the stomach. From another tendril of the endoscope, a needle with thread pokes through the stomach's lining to suture the area, leaving a flap that should now act as a valve that will allow food into the organ but keep acids and other contents from backing up into the esophagus.

If perfected, this technique may let doctors replace the current treatment for gastro-esophageal reflux disease—major surgery—with a five- to ten-minute procedure. That kind of revolutionary development was one of the goals envisioned several years ago when the Olympus Optical Corporation of Tokyo—the largest manufacturer of endoscopes in the world—assembled this team of researchers to create the next generation of endoscopic procedures and instruments.

The scientists came from institutions including Johns Hopkins University, the Mayo Clinic, the Medical University of South Carolina, Chinese University of Hong Kong, and UTMB. They called themselves the Apollo Group, after NASA's Apollo moon-landing program.

"When we started off, we really didn't know what we were going to accomplish," says UTMB physician Jay Pasricha, who joined the group in 1997, just before he left Johns Hopkins to direct UTMB's Division of Gastroenterology and Hepatology. "We didn't know if any of the projects would be feasible at all, but we did feel confident that in the process of trying we would create new instruments, new paradigms, and new ways of looking at things, the way the Apollo project that went to the moon did."

"The GI tract, second only to the skin, is probably the most accessible organ in the body," Pasricha explains. "Now, with modern technology in endoscopes, there's virtually no part of the GI tract that you can't treat. The



Associate Professor Jay Pasricha: building a better endoscope.

ability to reach it with a scope opens up the field for a lot of interventions previously thought purely diagnostic procedures."

Pasricha believes that endoscopic research can dramatically change the way we currently view surgical procedures of the gastrointestinal system. It's a goal he has been working toward throughout his career, trying to reduce pain, scars, and hospital stays for patients who suffer both common and uncommon diseases of the GI system. He has developed tools that use freezing techniques to kill or remove tumors and suturing devices that allow surgeries to be performed without cutting through a patient's skin and muscle.

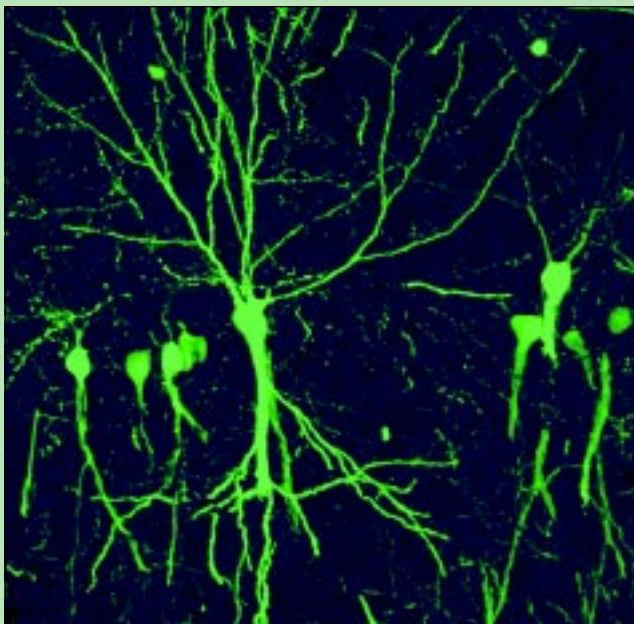
In January 2002, in an effort to broaden the range of endoscopic research, he got together with UTMB GI investigators G.S. Raju, Manoop S. Bhutani, Massoud Motamedi, Guillermo Gomez, Shu-Yuan Xiao, and Alexander Oraevsky to create the Center for Endoscopic Research, Training, and Innovation (CERTAIN). CERTAIN is intended to be an interdisciplinary group that will combine the skills of internationally known gastrointestinal researchers to not only improve current endoscopic therapy methods but also increase patient access and service.

Says Pasricha: "The field of therapeutic endoscopy is going to really be the mainstay by which we treat GI diseases in the future."—*Jennifer Reynolds-Sanchez*

Branching out in the brain

Because adult nerve tissue usually doesn't regenerate, repairing damage done to the central nervous system by disease and injury is one of the most daunting challenges of medical science. One proposed solution is implanting stem cells—those that can become any cell type in the human body—with the goal that they reproduce and replace damaged brain and spinal-cord tissue. Unfortunately, stem cells placed in adult brains and spinal cords normally don't develop into nerve cells; instead, they tend to turn into glial cells, which support neurons but do not themselves transmit nerve impulses.

In the December 2002 issue of *Nature Neuroscience*, however, a team led by UTMB Assistant Professor of Anatomy and Neuroscience Ping Wu reported that it had found a way to make the majority of human fetal neural stem cells injected into rat brains and spinal cords transform themselves into nerve cells. What's more, those



nerve cells were specifically suited to the particular regions of the brain and spine in which they were implanted.

The secret was a special mixture of nutrients and growth factors similar to those found in the developing central nervous system. The researchers used this medium to "prime" the neural stem cells prior to implanting them. The cells glowing green as a result of the fluorescent protein used to label them seen in the accompanying picture (a confocal microscope image) are the descendants of a long-established line of fetal neural stem cells. They started life in a dish, and then were injected into a rat's hippocampus, where they took on the distinctive branched "pyramidal" architecture of the other neurons found there.

When implanted into the spinal cord and the medial septum of the brain (a region thought to be linked to memory, learning, and cognition), most of the stem cells developed into cholinergic nerve cells, which make the neurotransmitter acetylcholine and are vital to motor function, learning, and memory. "Cholinergic neurons are what degenerate in disorders like Alzheimer's and Lou Gehrig's disease, as well as being damaged in spinal and brain trauma," Wu says. "Until now, nobody's been able to get a significant number of cholinergic neurons from primarily cultured stem cells, but using this primer, when we transplant stem cells we can get over 55 percent of such neurons." Before the breakthrough can be translated from the lab bench to patients' bedsides, Wu says, crucial questions must be answered in animal experiments, including whether the new neurons will make the proper connections with muscles, whether they will actually work to restore function, and whether they cause cancers. "We have to sort out those critical issues," she says, over several years. "Then, hopefully, we can think about clinical applications that will help patients with neurological disorders and brain and spinal cord injuries."

—Jim Kelly

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Combating bioterrorism

With a faculty that includes top experts on tropical and emerging infectious diseases, a strong program to develop countermeasures against biological terrorism, and a nascent Biosafety Level Four (BSL-4) lab—the country's only such facility on a university campus—UTMB already plays a role in national biodefense initiatives.

That role could grow if a 900-plus-page proposal submitted in February to the National Institute of Allergy and Infectious Diseases (NIAID) succeeds.

UTMB is seeking funding to construct a National Biocontainment Laboratory (NBL), a component of the \$1.75 billion NIAID biodefense program proposed by the Bush Administration after September 11 and the deadly anthrax attacks beginning the following month. Estimated to cost (including equipment) between \$160 million and \$190 million—about two-thirds of which would be supplied by the federal government—the NBL would house a large complex of BSL-2, -3, and -4 labs, adding more than 48,000 square feet of new lab research space.

NIAID would provide the lion's share of the lab's construction costs. The facility would be owned and operated by UTMB and committed to NIAID-supported biodefense research to develop countermeasures against potential biological threats: better diagnostic tests, better therapeutics, and better vaccines for the prevention of infection. The UT System Board of Regents would authorize \$40 million in revenue bonds to pay most of the local share, to be repaid out of the "overhead" portion of grants for sponsored research undertaken in the facility. UTMB is also seeking philanthropic support for a portion of the costs.



In this campus model, the proposed seven-story National Biocontainment Laboratory (NBL) is shown in blue, dwarfing the Biosafety Level Four (BSL-4) lab now under construction, shown in yellow. The Keiller Building, immediately to the left, provides access to the BSL-4 and also would serve as the initial entrance for workers in the NBL. Across the street to the left of Keiller is Old Red, the original medical school building.

If the proposal succeeds, Dean of Medicine Stanley Lemon noted, UTMB will help nurture a new generation of infectious-disease researchers. "Nationally there is an incredible need for well-trained research scientists to address these issues, which are certainly going to be with us into the rest of this century," Lemon said. "Not just bioterrorist attacks but new infections that continue to emerge—West Nile for one example, AIDS for another, Legionnaires' disease for another."

NIAID plans to fund no more than two NBLs, and competition to be selected as a site for the facility is expected to be fierce. To be considered for an NBL, UTMB must also be selected by NIAID as a Regional Center of Excellence (RCE) in biodefense and emerging infectious diseases, a designation that carries with it about \$50 million in federal funds over five years. To compete for an RCE, UTMB is leading a coalition of academic and government institutions in Texas, Arkansas, Louisiana, Oklahoma, and New Mexico. NIAID may ultimately fund as many as ten RCEs; one of the purposes of the NBL is to support the new regional centers by providing high-containment laboratory facilities now available at only a few locations in the United States.

Work in the NBL will focus on biological agents with a high potential for use by terrorists, which the NIAID has classified into Category A, B, and C pathogens. Examples of Category A pathogens include the bacteria responsible for anthrax and bubonic plague, as well as hemorrhagic fever viruses like Ebola virus; Category B includes typhus, West Nile virus, and Venezuelan equine encephalitis; and Category C includes influenza virus and multi-drug

resistant tuberculosis. Many of those are diseases UTMB scientists are either already studying or plan to study in the new BSL-4 lab, which is expected to be completed this summer.

"One of the important things about biological warfare is that many of the agents that are used are things that are out there in nature, that are local problems all over the world," said C.J. Peters, director for biodefense at UTMB's Center for Biodefense and Emerging Infectious Diseases and holder of the John Sealy Distinguished University Chair in Tropical and Emerging Virology. Work done in the NBL would be directly applicable to fighting those agents, whether they emerge naturally or are unleashed by bioterrorists.—Jim Kelly