Scripps Research Scientists Develop Molecular Test Providing a New Pathway for Identifying Obesity and Diabetes Drugs

Scientists at The Scripps Research Institute have designed a new molecular test that will allow researchers to look for potential drugs targeting a human metabolic enzyme believed to stimulate the appetite and play a role in diabetes.

The new test, which the scientists call a simple assay, will allow researchers to look through hundreds of thousands of compounds for those that have potential to block the action of an enzyme known as ghrelin O-acyltransferase (GOAT). If drugs can be found that safely suppress the action of GOAT, they may help people who have clinical problems with appetite, obesity, and diabetes.

“There hasn’t been a simple screen until now,” says Kim D. Janda, Ph.D., a professor in the Departments of Chemistry and Immunology and Microbial Science, member of The Skaggs Institute for Chemical Biology, and director of The Worm Institute for Research and Medicine at Scripps Research.

Janda and Amanda Garner, Ph.D., a research associate in his laboratory, described the new approach—which may also prove useful for investigating other enzymes involved in a variety of diseases—in an advance, online Early Edition of the journal Angewandte Chemie on September 15, 2010.

Even though GOAT was only discovered recently, in 2008, scientists had speculated for years that it had to exist. After all, its target (ghrelin, or the “G” in the acronym GOAT) had been known for more than a decade.

Ghrelin, a small peptide hormone that is mainly produced in the stomach, signals hunger, typically before meals. Ghrelin has been associated with suppressing tumor growth.

Team Led by Scripps Research Scientist Identifies New Gene for Memory

> The Findings Could Shed New Light on Human Learning and Neurological and Psychiatric Disorders

A team led by a Scripps Research Institute scientist has for the first time identified a new gene that is required for memory formation in Drosophila, the common fruit fly. The gene may have similar functions in humans, shedding light on neurological disorders such as Alzheimer’s disease or human learning disabilities.

“This is the first time we have a new memory and learning gene that lies outside what has been considered the most fundamental signaling pathway that underlies learning in the fruit fly,” said Ron Davis, chair of the Scripps Research Department of Neuroscience who led the study. “Since many of the learning and memory genes...”
with Prader-Willi syndrome, a common genetic cause of childhood obesity in which patients have exceptionally high ghrelin levels.

The biology of ghrelin is quite intricate. For ghrelin to impact the body’s metabolism, it must move from the stomach to the brain, where it acts on neurons. GOAT acts as a “passport” checkpoint station for the processing of ‘pro-ghrelin.’

“Without GOAT’s modification of ‘pro-ghrelin,’ ghrelin’s entry into the brain, and thus its medicinal implications, are nullified,” said Janda. “That’s why GOAT has been coveted as a promising therapeutic target for obesity and diabetes.”

However, the task of finding drug-like compounds that target GOAT has been complicated by the fact that the enzyme is difficult to work with. As a membrane-bound protein, GOAT cannot easily be purified to homogeneity and has to be suspended in a fatty environment.

Most critically, however, no user-friendly high-throughput screening tests had been developed for this class of enzymes. Current assay technology for targets similar to GOAT rely on radiolabeling techniques, which are labor-intensive and require additional safety precautions.

When Janda and Garner set out to develop an assay to monitor GOAT’s activity, they aimed to change this situation.

The scientists were particularly interested in designing a fluorescence-based assay for GOAT. In contrast to radiolabeling techniques, fluorescence assays are safe, can easily be performed in a high-throughput format, and require only a simple light source.

In developing their new approach, the team was inspired by enzyme immunoassay strategies commonly used in biology, chemistry, and medicine, and by “click chemistry,” a concept developed at Scripps Research. Because the amount of ghrelin modified by GOAT is minute, the scientists also used the principle of multi-turnover signal amplification for accurate detection.

In the end, the team succeeded in designing and creating the new technology—which they call “catalytic assay using enzyme-linked click chemistry assay” (cat-ELCCCA).

“This new assay technology is both highly sensitive and reproducible,” noted Janda, “making it an excellent assay for high-throughput screening.”

Janda and Garner are now further developing the assay so that researchers can screen thousands to millions of compounds at once against the activity of GOAT.

---

**Memories, continued**

originally identified in the fruit fly are clearly involved in human neurological or psychiatric diseases, this discovery may offer significant new insights into multiple neurological disorders. We’re definitely in the right ballpark.”

The study shows that different alleles or mutant forms of the gene, known as *gilgamesh* (*gish*), are required for short-term memory formation in *Drosophila* olfactory associative learning—learning that links a specific odor with a negative or positive reinforcer.

Because *Drosophila* learning genes are known to be conserved in higher organisms including humans, they often provide new insights into human brain disorders. For example, the *Drosophila* gene known as *dunce*, which Davis helped identify several years ago, provided clues to the genetics of the devastating psychiatric condition of schizophrenia. Recent studies have revealed that the human version of the *dunce* gene is a susceptibility determinant for schizophrenia. In a similar way, any new learning gene identified in *Drosophila*, including *gilgamesh*, may provide new clues to genes involved in human neurological or psychiatric disorders.

“We’re still early in the process of making connections between *Drosophila* memory and learning genes and the pathology of human disease,” Davis said, “but it’s already clear that many of these genes will provide important conceptual information and potential insights into human brain disorders. In addition, there is every reason to believe that their gene products will one day become the target of new drugs to enhance cognition. Uncovering this new gene and its signaling pathway helps bring us that much closer to this goal.”

To identify the new gene, Davis and his colleagues used a novel screen for new memory mutants, looking for lines that showed abnormal learning when only one of two copies of the gene was mutant.

“We used a dominant screen because we realized that behavior such as learning and memory are very sensitive to gene dosage,” Davis said. “That is, the mutation of just one copy of a gene involved in behavior is often sufficient to produce an abnormality.”

The formation of new memories occurs, in part, through the activation of molecular signaling pathways within neurons that comprise the neural circuitry for learning, and for storing and retrieving those memories.

One of the things that makes the function of *gish* so interesting, Davis noted, is the fact that it is independent of mutations of the *rutabaga* gene, a *Drosophila* memory-learning pathway that is known to be essential for memory formation. The *rutabaga* mutants convert ATP, the energy chip of cells, into cyclic AMP or cAMP, which plays a critical role in olfactory learning in *Drosophila*.

“The cAMP pathway is the major signaling pathway used by *Drosophila* neurons to turn on other enzymes and genes

*continued on page 3*
Selective Inhibition of BMK1 Suppresses Tumor Growth

A Scripps Research study describing a newly developed pharmacological inhibitor is providing detailed insight into how an enzyme that has been implicated in multiple human malignancies regulates a known tumor suppressor. The research may have broad application for treating human cancers.

Mitogen-activated protein kinases (MAPKs) are enzymes that regulate multiple cellular activities, including proliferation and cell survival. Mutations in MAPK signaling pathways have been shown to play a significant role in many types of cancer. Of the four different MAPKs that have been identified in mammalian cells, ERK1/2 and BMK1 exhibit significant structural similarity. In fact, recent research has shown that some pharmacological compounds which have been considered to be specific inhibitors of ERK1/2, also interfere with the lesser known BMK1.

“It is critical that results using the common MAPK inhibitors be reevaluated using more specific inhibitors of the BMK1 and ERK1/2 cascades.”

However, so far there has been no specific small-molecule inhibitor of BMK1 that is effective both in cells and animals,” explains senior study author, Dr. Jiing-Dwan Lee, from The Scripps Research Institute in La Jolla, California. “More importantly, the lack of this kind of BMK1 inhibitor has hampered the understanding of the physiological/pathological roles of BMK1.”

Dr. Lee and colleagues discovered that promyelocytic leukemia protein (PML), which is a known tumor suppressor, is inhibited by BMK1. “Previous reports had implicated ERK1/2 in the regulation of PML,” says Dr. Lee. “However, in our study we found that that BMK1 interacts with PML and suppresses its antitumor actions.” To further investigate the BMK1-PML interaction, the researchers developed a compound called XMD8-92 that was remarkably selective at inhibiting BMK1.

Treatment with XMD8-92 blocked tumor cell proliferation and significantly inhibited tumor growth in mice. Importantly XMD8-92 had no obvious negative effects on the animals.

“These results demonstrate that the BMK1 pathway can be blocked effectively by a small-molecule inhibitor without apparent adverse effects and, more importantly, BMK1 inhibition is a very effective way to prevent cancer development in animals,” concludes Dr. Lee.

“As BMK1 is expressed in most tumor cells, our results suggest that cancer therapies targeting BMK1 may be useful for treating diverse types of human tumors.”

Memory, CONTINUED

that are necessary for memories to form,” Davis said. “In fruit flies, memory and learning revolves around mutants of this pathway. It is fundamental to the process.”

In the new study, gish provided an answer to a longstanding problem in Drosophila learning and memory research—the unexplained residual memory performance of flies carrying rutabaga mutations, which indicated the existence of an independent signaling pathway for memory formation. While other memory mutants have been identified, until the discovery of gish none have been shown to reduce the residual learning of mutant rutabaga flies.

Interestingly, the study found that the gish gene encodes a kind of casein kinase (which help regulate signal pathways in cells) called Iγ (CKIγ). This is the first time that this specific kinase has been cited as having a role in memory formation.

The identification of all signaling pathways that are engaged in specific neurons during memory formation and how they interact with one another to encode memories is an issue of great importance, Davis said, one that needs more exploration for a deeper understanding of memory formation and memory failure in humans.

“The truth is that we have an extremely sketchy understanding of what causes diseases like Alzheimer’s,” Davis said. “We need to understand a lot more than we do now about normal brain functions like memory and learning before we have a high probability of succeeding in the development of a cure.”
In 1924, “Miss Ellen,” as she was affectionately known, broke her hip and was confined to a La Jolla sanitarium that was far from ideal. She decided to replace it with a first class hospital, and in that year, she and her brother, George funded the 44-bed Scripps Memorial Hospital along with The Scripps Metabolic Clinic — the precursor to the modern Scripps Research Institute. The early focus was on caring for patients with diabetes, a disease that afflicted the Scripps family, and on researching treatment for diabetes and medical conditions that might befall seniors. Miss Ellen passed away in 1932, but the institutions she helped found lived on, almost eight decades after her death.

“Miss Ellen always tried to stay fit,” said Doug Dawson, executive director of the Ellen Browning Scripps Foundation. “She was very careful about what she ate. She would sleep outside on her porch, believing that it would ensure a long and healthy life. She had a passion for scientific research. Her broken hip really propelled her interest in health even further, to the point of starting her own hospital and research center right next to her cottage!”

Miss Ellen’s principles were straightforward. She believed in education and free speech, she was an advocate of women’s suffrage and rights long before women had a prayer of getting the vote, and she abhorred discrimination and privilege.

An avid reader, Miss Ellen became one of the first women in the United States to attend college, graduating from Knox College in Illinois in 1858. She even landed on the cover of Time Magazine at a time when women made few appearances there.

Miss Ellen founded, endowed, or bankrolled many of the region’s most cherished institutions, including the Scripps Institution of Oceanography, Scripps College, the San Diego Zoo, the Bishop’s School, and the La Jolla Women’s Club. She disliked the word “philanthropist,” and referred to her gifts as investments.

The late Ellen Revelle, Ellen’s grand-niece, once remembered her namesake as a “wonderfully warm, simple person. She didn’t spend money on herself. She was self-consciously working class, and she never tried to play the social game. She dressed really simply, wearing old hats, and just didn’t care what she wore.”

According to Molly McCain, an historian who is researching a biography on Miss Ellen that she plans to finish in a few years, “she was a gentle, kind and deeply generous woman who was very interested in people and the people around her.”

“She was a big believer that people could raise themselves up through education,” McCain continued. “She hoped her gifts would help ordinary people to educate and advance themselves.”

“She wanted to change the world, one life at a time,” said Dawson.

Although she intended to give away her entire fortune during her lifetime, the residue of her estate was used by her nephew Robert Scripps in 1933 to establish the Ellen Browning Scripps Foundation which continues the legacy of her philanthropy today. Dawson has served as executive director of the foundation for almost 40 years. The foundation has granted an estimated $80 million to $150 million over the past 80 years to many things Miss Ellen supported in her day: health care, education, animals and the environment.

Several Scripps family members are integrally involved with the Foundation, serving on the board of trustees, conducting site visits, and participating in philanthropic decisions — and like Miss Ellen, they do not seek fame or glory for their philanthropy.

“The foundation perpetuates the philanthropic integrity of Ellen Browning Scripps in its work,” said Dawson. “It is an honor to be associated with the incredible Scripps family and its rich legacy worldwide.”
“We fund services across the board, just as Miss Ellen did,” Dawson continued. “This includes science, education, and the arts — every activity that is part of a human being’s life. Like Miss Ellen, who was a businesswoman first and integral to the success of the Scripps Howard newspapers, we consider ourselves shareholders in these organizations and look for a positive return on our investment in humankind.”

Over the years, The Ellen Browning Scripps Foundation has provided approximately $3 million in support to Scripps Research, including its highly-ranked graduate school and its high school student summer internship program. Many interns from the program have gone on to attend colleges including Harvard University, the California Institute of Technology, and the Massachusetts Institute of Technology.

“While Miss Ellen was generous towards all causes, her priority was children and education, and the foundation’s gifts towards Scripps Research reflect those priorities,” said Dawson. “She felt that the youth were our future.”

“In the last generation or two, our country has fallen behind in the academic and professional pursuit of science, math, and engineering,” Dawson continued. “We need to continue concentration in these areas at home, and our funding of incredible organizations like Scripps Research and Achievement Rewards for College Scientists is a big step in this area.”

“We’ve been extremely pleased with our investments at Scripps Research. When it comes to stewardship, there are thousands of good nonprofits, but only a handful are great ones that are deserving of a fair share of the philanthropic dollar — Scripps Research definitely falls in this category. They are very effective and diligent in their stewardship for today’s philanthropic investor.”

Dawson himself is committed to the work of Scripps Research through his role on the Scripps Research California Council. “All of the institutes on the Torrey Pines Mesa are doing incredibly profound work, but I am extremely impressed with the cutting-edge and relevant science being performed at Scripps Research,” he said. “I am pleased to serve on the Council as an emissary to bring Scripps Research’s message to the world, especially to philanthropic investors in the community we serve — this will ultimately make the institute more successful.”

“I am extremely impressed with the cutting-edge and relevant science being performed at Scripps Research.”
—Doug Dawson
Carlos and his colleague Scripps Research President Richard A. Lerner developed a new class of drugs in 2002. Their work offers a groundbreaking way to physically combine antibodies, which are large, soluble molecules that remain in the body for long periods of time, with small molecule drugs and peptides, which can kill disease-causing cells, but may be expelled from the body too quickly to be effective as a therapy. These hybrid molecules, called “chemically programmed antibodies,” have the desired properties of each – killing disease-causing cells and staying in circulation long enough to dramatically enhance the drug’s effectiveness.

Empowered by these compelling results, Carlos and Dr. Lerner founded CovX, a biotechnology venture, which was later acquired by Pfizer to develop this unique and powerful class of antibodies.

The approach has led to a number of compounds under development against cancer, HIV-1, and metabolic disease. Carlos and his colleagues found that the hybrid molecules they developed had a profound effect on the size of tumors in mouse models, shrinking tumors of melanoma, breast, and colon cancer.

“We were able to show the chemically programmed complex had at least 1,000-fold increase in the therapeutic effect compared with the small molecule alone,” said Carlos. “With that came the idea that this was too powerful an approach not to push into human studies.”

Today, three of the hybrid compounds developed by CovX have completed preclinical work with promising results as anti-tumor agents and are progressing in clinical trials. A fourth compound to treat diabetes is also in clinical trials.

Numerous other drugs based on this intriguing technology are in early phases of development at CovX/Pfizer. This technology represents the first time catalytic antibodies have been used in human therapy.

“It’s very rare that an entirely new class of drugs is developed that can be applied in so many therapeutic areas,” said Carlos. “It has been a dream of mine as a scientist to develop drugs that make a difference. I couldn’t be more excited by these developments. I’ve envisioned a cure for a disease since I was a child, and getting closer to this realization really drives me.”

The work was made possible by the generous philanthropy of the Skaggs family, whose milestone gift opened and sustained the Skaggs Institute of Chemical Biology at Scripps Research. “Only through funding from the Skaggs Institute could such high-risk, high-reward studies be pursued in today’s funding environment – this work is often too speculative to be funded initially by the National Institutes of Health” said Carlos.

A second focus of his laboratory also involves the development of new immunotherapeutic approaches to disease. To accomplish this, his group developed phage display methods for the selection and in vitro evolution of antibodies. Carlos and his colleagues applied these to viral diseases and their current efforts focus on creating new genetic and chemical approaches to immunotherapy that include developing novel chemotherapeutics, small molecules that function in concert with antibodies, and gene therapy approaches that use intracellular antibodies. Carlos and his colleagues are applying these methods to create effective therapies for breast and ovarian cancer, melanoma, and AIDS. The phage display and synthetic antibody methodologies developed by Carlos and his colleagues are now being used broadly by a number of pharmaceutical firms, who have developed numerous drugs or improved existing drugs using the methodology.

A third focus of the lab is the design, optimization, and utilization of transcription factors for the directed regulation of gene expression. To accomplish this, Carlos and his colleagues have developed zinc finger protein based transcription factors as a robust technology for endogenous gene regulation.
They are applying this approach to provide transcription-based solutions to disease, and have licensed the technology to Sangamo Biosciences who currently have a drug in clinical trials for ALS (or Lou Gehrig’s Disease). Other far-reaching studies involving zinc finger proteins involve developing new approaches in stem cell therapies that will allow scientists to specifically and safely insert or correct genes.

Carlos started with Scripps Research as a postdoctoral fellow in 1990, after receiving his Ph.D. from Texas A&M University, and has been thriving here ever since.

“This is the place where I could do the research I wanted to do. Richard Lerner became my mentor and instilled in me his drive for fearless exploration of diverse areas of science and medicine,” said Carlos.

“At Scripps Research, you’re free and encouraged to explore your scientific passion without boundaries and departmental constraints,” Carlos continued.

“You’re not pigeonholed as a chemist or a biologist”—it’s the feature I most like about Scripps Research.”

Carlos’s work is so promising that it’s turning some important heads. He was recently named a winner of the National Institutes of Health (NIH) Director’s Pioneer Award. He was selected for the award on the basis of his proposal for future bold and high-impact work. Specifically, his proposal concerns chemically programming immunity, research that could lead to “instant immunity” vaccines for the flu, HIV-1, and cancer. The new approach would overcome a major drawback of current vaccinations – the lag time of days, or even weeks, that it normally takes for immunity to build against pathogens such as bacteria and viruses.

“NIH is pleased to be supporting scientists from across the country who are taking considered risks in a wide range of areas in order to accelerate research,” said NIH Director Francis Collins.

“This is a tremendous recognition that Carlos is doing outstanding work,” said Peter Wright, chair of the Scripps Research Department of Molecular Biology. “His laboratory continues to tackle some extremely important scientific questions that lay the foundations for future therapeutics and vaccines.”

Carlos also received the Tetrahedron Young Investigator Award, Bioorganic & Medicinal Chemistry, for 2009. The award is presented to individuals under the age of 45 who have exhibited “exceptional creativity and dedication” in their fields.

In another testament to his work, according to ISI Essential Science Indicators, a web-based data file reflecting scientific papers published in the last decade, Carlos ranks in the top one percent in terms of total citations in the fields of chemistry, microbiology, and biology and biochemistry. His productivity and impact have earned him an h-index of 77 and 50 issued U.S. patents. His latest inventions are behind his founding of his third biotechnology company, Zyngenia, that aims to create ever more powerful drugs for cancer and inflammation.

Carlos was nurtured into science by his mother and he is leaving that same imprint on his own children. “I grew up in an era when men were first walking on the moon and science was the big thing. For as long as I can remember, I wanted to be a scientist, a brain surgeon, or an astronaut….and my mom was more than happy to instill these pursuits in my mind,” quipped Carlos. “I would be pleased to pass on the torch to my son and daughter, who are in college and high school. They both have an interest in science and participated in the Scripps Research summer student internship program this summer,” said a proud father.

“IT has been a dream of mine as a scientist to develop drugs that make a difference. I couldn’t be more excited by these developments.”

—Carlos Barbas
ROY SMITH APPOINTED DIRECTOR WITH AMERICAN FEDERATION FOR AGING RESEARCH

The Florida Affiliate of the American Federation for Aging Research (AFAR) has elected Roy Smith, chair of the Department of Aging and Metabolism at The Scripps Research Institute, to serve on its board of directors.

Smith will help set priorities and strategic initiatives for the non-profit AFAR Florida, established in 2008 to expand the national AFAR organization’s efforts to support aging research regionally. Since AFAR’s founding in 1981, it has granted $124 million to almost 2,600 scientists.

SCRIPPS FLORIDA GRANT SUPPORTS ADVANCED TECHNOLOGY

Scripps Research has received a grant worth nearly half a million dollars to purchase advanced technology for the Scripps Florida campus that will allow scientists to do in-depth studies of various types of proteins. The grant was awarded by the National Center for Research Resources as part of the Shared Instrumentation Grant Program.

The new technology is a LTQ Orbitrap XL mass spectrometer. The instrument, which was installed in June, is available for several national collaborations and to Scripps Research scientists for collaborative studies on protein and receptor dynamics and on transcriptional complexes that have been associated with diseases such as diabetes and cancer. In addition, it will provide training for research technicians, postdoctoral fellows, and graduate students, according to Patrick R. Griffin, chair of the Scripps Florida Department of Molecular Therapeutics.

“This high-resolution mass spectrometer is considered state-of-the-art in protein mass spectrometry,” said Michael Chalmers, senior staff scientist and manager of the HDX laboratory, “and it has been interfaced with Scripps Florida’s HDX robotic system for automated experiments.”

DANIEL BACHOVCHIN WINS CALIFORNIA BREAST CANCER RESEARCH AWARD

Daniel Bachovchin, a student in the Scripps Research Kellogg School of Science and Technology, has received a two-year award from the California Breast Cancer Research Program. His project, titled “Pharmacological Modulation of PP2A Activity in Breast Cancer,” will be conducted in the Cravatt lab.

THE SCRIPPS RESEARCH INSTITUTE

Contact Us:

- For more information about Scripps Research, visit our web page at www.supportscrippsresearch.org
- To learn more about supporting Scripps Research’s cutting-edge research, please contact:

  CALIFORNIA  (858) 784.2037 or (800) 788.4931
  burlitt@scripps.edu

  FLORIDA  (561) 228.2013
  abruner@scripps.edu