INSIDE STANFORD MEDICINE

Volume 10, No. 9 ■ May 7, 2018 ■ Published by the Office of Communication & Public Affairs

The new cryo-EM facility provides advanced tools for exploring the machinery of cells.
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Professor's lung tissue used in cancer study

By Krista Conger

PAUL SAKUMA

James Spudich (left) donated healthy and cancerous tissue from his lungs to help advance the research of his longtime friend Mark Krasnow.

It was around 3 p.m. on a Thursday last October when James Spudich and Suzanne Pfeffer poked their heads into the office of Mark Krasnow, on the fourth floor of the Beckman Center for Molecular and Genetic Medicine, interrupting a meeting he was having with a colleague.

"Can we talk to you for a minute?" Pfeffer said.

Impromptu conversations were nothing new for the three. The longtime colleagues and friends have a lot to talk about. Spudich, PhD, whose research focuses on understanding the molecular forces that drive muscle contractions, earned a doctoral degree from Stanford in 1968 under Arthur Kornberg, MD, a founding member of the Department of Biochemistry. Krasnow, MD, PhD, who studies lung development and disease, completed his postdoctoral work under former department chair David Hogness, PhD. Krasnow, Spudich and Pfeffer, PhD, are all professors of biochemistry and have chaired the department. (Pfeffer, who joined the department in 1986, is completing her second stint as chair.)

But this time, the topic was more personal. Spudich, 76, had come to share some unexpected news: He had lung cancer. "I was wondering if you were looking for any human tissue samples for your research," he said.

Shocked and saddened, Krasnow asked when Spudich would undergo surgery to have the tumor removed.

"I told him it was scheduled for 8 a.m. the next morning," Spudich recalled. "I remember Mark went kind of pale."

Spudich had no way of knowing it, but the meeting he'd interrupted between Krasnow and assistant professor of pediatrics Christin Kuo, MD, had been called to discuss how Krasnow could broaden his research, which he had been conducting mainly in mice and small primates called mouse lemurs, to include human cancers. But the logistical and legal hoops that would need to be cleared prior to any kind of human-based research were daunting, and Krasnow knew it would likely take months to obtain the necessary approvals.

Kuo, a specialist in pulmonary medicine, had been working with Stephen Quake, PhD, professor of bioengineering and of applied physics and co-president of the Chan Zuckerberg Biohub, and postdoctoral scholar Spyros Darmanis, PhD, a group leader at the Biohub, to study the development and function of lung neuroendocrine cells using single-cell RNA sequencing. As a pulmonary fellow, Kuo had worked with Krasnow to conduct her initial studies in mice. But in 2016 she'd established her own laboratory in the Department of Pediatrics and had begun to develop the methods and key reagents to efficiently isolate human lung cells. Not only did she have all the necessary protocols and approvals in place to begin her study, she had also already established a collaboration with Joseph Shrager, MD, professor and chief of thoracic surgery, who was slated to perform Spudich's procedure the next morning.

Within moments, the researchers had initiated a remarkable series of events that would, over the next 16 hours, lead to the beginning of what will likely be the world's largest study of healthy and diseased human lung tissue. Dubbed the "lungome," the effort has brought together researchers and clinicians from throughout the Beckman Center, See SPUDICH, page 6

Researchers use CRISPR to genetically edit coral

By Hanae Armitage

Coral reefs on the precipice of collapse may get a conservation boost from the gene-editing tool known as CRISPR, according to researchers at the School of Medicine and their collaborators.

The scientists found, for what appears to be the first time, definitive evidence that the CRISPR-Cas9 gene-editing tool could be a potent resource for coral biologists. Phillip Cleves, PhD, a postdoctoral scholar at Stanford, is a geneticist whose efforts to delineate gene function in animals resides squarely within the marine invertebrate realm — namely, corals.

"Up until now, there hasn't been a way to ask whether a gene whose expression correlates with coral survival actually plays a causative role," Cleves said. "There's been no method to modify genes in coral and then ask what the consequences are."

PHILLIP CLEVES

Acropora millepora coral at the Australian Institute of Marine Science.

The study was published online April 23 in the *Proceedings of the National Academy of Sciences*. Cleves is the lead author. John Pringle, PhD, professor of genetics at Stanford, and Mikhail Matz, PhD, associate professor of integrative biology at the University of Texas-Austin, share senior authorship.

The damage of coral bleaching

In the late 1990s, the ocean's coral reefs experienced the first big wave of something called coral bleaching, a bleak event in which ocean conditions — most prominently increasing temperatures — kill off or "bleach" parts of the reef, turning once-vibrant colors bland and damaging the entire reef ecosystem.

Cleves' work, conducted in collaboration with researchers at UT-Austin and the Australian Institute of Marine Science, sprouted from a conversation at an international coral meeting that aimed to concretely understand the genes behind coral survival. Are there some genes that render corals more resilient to spikes in ocean temperatures? Or perhaps a gene that helps establish new coral colonies? Scientists had hypothesized answers to these questions, but to truly know, Cleves wanted to create a technique that could allow coral biologists to answer such questions more rigorously.

"We want to use CRISPR-Cas9 with the express interest to start understanding what genes are See CORAL, page 6

Scientists identify fear and courage 'switches' in brain

By Bruce Goldman

Researchers at the School of Medicine have identified two adjacent clusters of nerve cells in the brains of mice whose activity level upon sighting a visual threat spells the difference between a timid response and a bold or even fierce one.

Located smack-dab in the middle of the brain, these clusters, or nuclei, each send signals to a different area of the brain, igniting opposite behaviors in the face of a visual threat. By selectively altering the activation levels of the two nuclei, the investigators could dispose the mice to freeze or duck into a hiding space, or to aggressively stand their ground, when approached by a simulated predator.

People's brains probably possess equivalent circuitry, said Andrew Huberman, PhD, associate professor of neurobiology and of ophthalmology. So, finding ways to noninvasively shift the balance between the signaling strengths of the two nuclei in advance of, or in the midst of, situations that people perceive as threatening may help See COURAGE, page 4

Inaugural chair of Emergency Medicine Department appointed

By Tracie White

Andra Blomkalns, MD, professor of emergency medicine at the University of Texas Southwestern Medical Center, has been appointed the inaugural chair of the Department of Emergency Medicine at the School of Medicine. Her first day will be Sept. 15.

Andra Blomkalns

"Dr. Blomkalns has a passion for innovation, entrepreneurship and the translation of academic discovery to the patient bedside," said Lloyd Minor, MD, dean of the School of Medicine. "As a distinguished clinician and mentor, she is also deeply committed to our tripartite mission of patient care, research and education. I'm delighted she will be joining us."

Blomkalns will lead the department as preparations unfold for the final phase of construction at the new Stanford Hospital, which is scheduled to open in late 2019 and which will include a new, larger space for emergency care. (Sam Shen, MD, clinical associate professor of emergency medicine, and Mary Hawn, MD, professor and chair of of surgery, have been serving as interim co-chairs of the department.)

Blomkalns is the division chief of general emergency medicine and vice chair for academic affairs and business development at UT Southwestern's Department of Emergency Medicine. She also is on the intellectual property advisory committee and serves as the clinical liaison to the office for technology development.

"I'm thrilled to be coming to Stanford," said Blomkalns, a native of New Orleans. "My passion for technology development and medical device innovation fits perfectly with Stanford's leadership in this arena. Stanford has the unique capacity to become the home of the world's leading experts in emergency care innovation."

During her three-year tenure at UT Southwestern, Blomkalns' academic work focused on the evaluation and improvement of the process for technology development and commercialization. She earned an MBA at the University of Texas-Dallas this year with a concentration in innovation and entrepreneurship.

'Proven innovator and leader'

Previously, Blomkalns served as residency program director and vice chair for academic affairs in emergency medicine at the University of Cincinnati. Her research has focused on cardiovascular emergencies, obesity and dietary influences on health and disease.

"We are incredibly fortunate to have a proven innovator and leader like Dr. Blomkalns joining our institution," said David Entwistle, president and CEO of Stanford Health Care. "She has a distinguished record as a compassionate clinician and an accomplished researcher in academic medicine. As we plan for the opening of our new Stanford Hospital in 2019, her appointment as the inaugural chair of the Department of Emergency Medicine comes at a critical time. We are incredibly pleased to have such an outstanding leader join us to help us advance the emergency care services that we offer to patients in our community."

Blomkalns received her medical degree in 1997 from Louisiana State Health Sciences Center in Shreveport. She is former president of the Society for Academic Emergency Medicine.

Dennis Lund, MD, interim CEO and chief medical officer of Stanford Children's Health, said, "We welcome Dr. Blomkalns to Stanford Medicine and excitedly anticipate her arrival to oversee the development of the Department of Emergency Medicine. We know that she will be an excellent leader and partner in the emergency care of children."

The search committee was led by Leslee Subak, MD, professor and chair of of obstetrics and gynecology, and Odette Harris, MD, professor of neurosurgery.

"We are excited about the selection of Dr. Blomkalns," Harris said. "She brings energy and vision as assets to a fantastic department poised for progress and growth. Her leadership is well-anticipated."

"Her passion for strategic innovation will inspire novel science and new collaborations," added Subak.

The Department of Emergency Medicine at Stanford was established in 2015. Previously it was a division in the Department of Surgery. **ISM**

Differences in young, older people's immune cells linked to environment

By Bruce Goldman

Discoveries by School of Medicine investigators may help explain why older people's immune systems often don't work so well, why different people's immune systems age at different rates, and why the environment matters more than heredity in generating these age-related differences.

The findings, which could lead to new ways of putting the brakes on aging and disease, were enabled by a technological innovation: a fast, accurate way to tell what proteins single cells are being instructed to produce, and the degree to which separate cells of the same type have received differing versions of those instructions. Using this new method to analyze hundreds of millions of immune cells one by one, the researchers found that older people's immune cells get a fuzzier set of marching orders than do those of younger people.

In a study published April 26 in *Cell*, the scientists focused on chemical marks affixed to proteins called histones, which closely associate with DNA in the cell nuclei of all living creatures that aren't bacteria or closely related one-celled organisms.

It's known that these so-called epigenetic marks are more than mere graffiti. "They're instructions rendering stretches of DNA — and the genes residing in those stretches — alternatively accessible or off-limits to the massive mobile molecular machines that read our genes. Ultimately, they orchestrate the production of the proteins our genes encode," said PJ Utz, MD, professor of immunology and rheumatology. Utz shares senior authorship of the study with Purvesh Khatri, PhD, assistant professor of biomedical informatics and of biomedical data science, and with basic life science research associate Alex Kuo, PhD. Lead authorship is shared by basic life science research associate Peggie Cheung, PhD, and postdoctoral scholar Francesco Vallania, PhD.

Epigenetic influence

Proteins are the workhorses that carry out the bulk of a cell's activities, so a cell's identity and agenda are intimately tied to the types and amounts of proteins that are active inside it. While virtually every cell in your body contains the same DNA, your skin cells, fat cells and nerve cells differ vastly from one another in their protein content and, therefore, in their function. By specifying which genes are to be active or quiescent, the constellation of epigenetic marks along a cell's DNA largely directs and defines the cell's overall behavior.

These marks, moreover, are in flux; unlike our more-or-less unchanging genes, they can be rapidly affixed to or expunged from histones upon a cell's exposure to pathogens, nutrients, growth factors or hormones, or upon changes in the cell's internal state — for example, when it's time for the cell to undergo division, or as the cell ages. The cell's protein output, and its work agenda, change in response.

"Barring the odd mutation or some fraying of the tips of your chromosomes, your DNA stays essentially the same as you get older," said Khatri. "But while for the most part our genes don't change much as we age, how active each of them is can change quite considerably in either direction over time."

PJ Utz

Purvesh Khatri

In particular, the numerous types of white blood cells in our immune systems show marked changes in gene-activation levels as we age. We also know that as we age, our immune system usually doesn't work so well, Khatri noted.

"The immune system plays a prominent role in all kinds of diseases," he said. "By focusing too heavily on genetics, we're ignoring the implications of human immunology and environmental influences that act on it."

The Stanford team hypothesized that aging-related changes in immune cells' genes might arise from flux in the pattern of epigenetic marks on the cells' histones. They set out to determine whether and how much, for any given immune cell type, these patterns diverged between different people or between different individual cells of the same type in any single person's blood.

Analyzing single cells

To make these determinations, the scientists modified a technique called mass cytometry. This method allows multiple features of a single cell to be characterized simultaneously as specialized molecular barcodes that have been attached to it strike a detector, revealing not only the cell's identity but also its state — for example, immature versus mature, or activated versus quiescent. The cells are incinerated and their remains flung at a detector in rapid-fire sequence. Although the cells themselves have gone up in smoke, their incombustible barcodes hit the detector and are identified and catalogued. In this way, the individual identities and states of huge numbers of cells can be quickly ascertained.

For the study, Kuo and Cheung spent more than a year designing molecular barcodes that would permit mass cytometry to specify the amounts of each of 40 different types of epigenetic marks and 30 additional identifying features in 22 different immune cell types, and more than another year conducting experiments with them. In all, the ensuing experiments generated some 21.7 billion data points. Vallania devised specialized techniques for analyzing this huge bolus of information.

The researchers found that for many of the immune-cell types, older peoples' cells bore, on average, substantially more histone marks than those of younger ones. In addition, older people showed more cell-to-cell variation in how much their histones were marked up than did younger people.

Then, to assess environmental versus genetic influences on histone marking patterns, the researchers obtained blood samples from identical and fraternal twin pairs. Identical twins share the same DNA sequences. They also share a common intrauterine environment, and, if raised together, reasonably similar childhood environments; fraternal twins, although their DNA is no more similar than that of typical siblings, share their intrauterine and, if raised together, childhood environments.

Histone-marking patterns between older identical twins diverged substantially more from one another than those in younger twin pairs. The differences between older identical twins were effectively equal to the differences between genetically unrelated people. Data analysis indicated that the histone-mark divergence among older people comes from nonheritable factors, such as food, infections and city of residence.

Medications targeting the enzymes that affix some histone marks are approved for some cancer indications. Utz and Khatri are now examining histone-marking patterns of other diseases to see if any are characterized by elevated or diminished levels of specific types of marks. They speculate that histone-mark analysis may lead to drugs that, by reversing histone-mark deviations from the healthy state, could treat diseases characterized by those deviations. **ISM**

INSIDE STANFORD MEDICINE

Inside Stanford Medicine is published monthly in July and December and semi-monthly the rest of the year.

is produced by
Office of Communication & Public Affairs
Stanford University
School of Medicine
3172 Porter Drive
Palo Alto, CA 94304
Mail code 5471
(650) 723-6911
http://med.stanford.edu/news/

Paul Costello
Chief communications officer
Susan Ipaktchian
Director of print & Web communications
John Sanford
Editor
Robin Weiss
Graphic designer

Send letters, comments and story ideas to John Sanford at 723-8309 or at jsanford@stanford.edu. Please also contact him to receive an e-mail version of *Inside Stanford Medicine*.

STANFORD MEDICINE

Descendants of Henrietta Lacks discuss her famous cell line

By Tracie White

Henrietta Lacks was a poor, African-American tobacco farmer and mother in the 1950s when physicians, following protocol at the time, took a tissue sample of her cells without her knowledge just prior to treatment for cervical cancer.

Lacks died a horrible death a short time later, at the age of 31, her body ravaged by those rapidly metastasizing cells. But those same cells, preserved in that tissue sample, would live on, reproducing in labs around the world and changing the face of science and medicine.

Today, those cells make up HeLa, the first immortalized cell line, which has been the subject of more than 74,000 studies, yielding insights into cell biology, vaccines, in vitro fertilization and cancer. But virtually no one knew the story behind those cells until the publication of the bestselling book *The Immortal Life of Henrietta Lacks* in 2010.

"Her doctor, before treating her, cut a bit of her cervix tissue, and for reasons that stayed a mystery for many years, her cells just never died," said Rebecca Skloot, the author of the book, at a discussion on campus April 19 that included two members of the Lacks family: Jeri Lacks-Whye, Henrietta's granddaughter, and Alfred Carter Jr., her grandson.

The event was hosted by the Stanford Storytelling Project and Medicine and the Muse.

"Everything we know about our grandmother came from the book," said Lacks-Whye. "We have a better sense of who she was as a person, as a mom, as a wife. They say she loved to wear red nail polish, that she never left the house without a neatly pleated skirt, loved to cook, had hazel eyes, a small waist, size 6 shoes. It's a great honor to know our grandmother as a person rather than just as HeLa cells."

Skloot worked for years alongside Henrietta's daughter, Deborah Lacks, whose determination to uncover the true story of her mother resulted in a book that has changed not only the lives of the family she left behind, but the course of science as well. In 2013, the National Institutes of Health set up a panel with three Lacks family members, including Lacks-Whye, to review requests to conduct genomic research on HeLa cells. The goal is to keep the family informed and protect their privacy, said Russ Altman, MD, PhD, a member of that panel and a Stanford professor of bioengineering, of genetics, of medicine and of biomedical data science."Deborah was desperate to know what her mother was like," Skloot said. "She was an infant when her mom died. And she would say things like, 'Can you look in these cells and tell me what my mother's favorite color was?' She was worried that research on these cells would hurt her mother in the afterlife. She'd say things like, 'Can she rest in peace if you are shooting bits of her off to the moon?'"Scientists who began doing research on the Lacks family in the 1970s to try to find out more about the HeLa cell line had no

JAKE WARGA

Rebecca Skloot, Jeri Lacks-Whye and Alfred Carter Jr. discussed the legacy of Henrietta Lacks on April 19.

idea how to respond to Deborah's questions, Skloot said. One gave her a medical school book on genetics, and said, "Here, read this." The family's lack of understanding of science and the medical field resulted in much fear and anger that was only exacerbated by the researchers' inability or unwillingness to bridge this enormous communication gap.

Carter, who was in prison when Skloot was researching the book, said that it was "heartwarming" when she came to visit him, and they worked together on fact-finding for the family history.

The book, he said, only further added to his love and pride for his mother, Deborah, and the strength she had.

"Right before my mom passed away, she told me she was scared. I said 'Don't be scared. I got my strength from you.' She said, 'For real?' I promised her I would continue to do her work when I got out."

A pre-med student in the audience asked, "What can future scientists and physicians do to address the bioethical issues surrounding scientific progress?" Carter responded, "Just keep in mind that these are human beings that you are dealing with. Try to talk to them in a way that they can understand. Just know that they're human." ISM

Scientists find possible autism biomarker in cerebrospinal fluid

By Erin Digitale

Autism diagnosis is slow and cumbersome, but new findings linking a hormone called vasopressin to social behavior in monkeys and autism in people may change that. Low vasopressin in cerebrospinal fluid was related to less sociability in both species, indicating the hormone may be a biomarker for autism.

A paper describing the research, which was led by scientists at the School of Medicine and UC-Davis, was published May 2 in *Science Translational Medicine*.

NORBERT VON DER GROEBEN

Karen Parker and her colleagues found that low levels of the hormone vasopressin in the cerebrospinal fluid of both monkeys and humans was related to less sociability.

"Since autism affects the brain, it's really hard to access the biology of the condition to know what might be altered," said Karen Parker, PhD, associate professor of psychiatry and behavioral sciences at Stanford and the lead author of the new study. "Right now, the diagnosis is based on parents' reports of their children's symptoms, and on clinicians observing children in the clinic."

The study's senior author is John Capitanio, PhD, professor of psychology at UC-Davis.

Autism, a developmental disorder characterized by impaired social abilities, affects 1 in 68 U.S. children. Research has shown that early, intensive behavioral treatment is beneficial. Yet many children don't receive a timely diagnosis. A biological test, with a specific lab measurement indicating autism, could make diagnosis faster.

Difficult condition to study

Not only is the biology of autism difficult to study in people, but many research animals are unsuited to autism research, Parker said. For instance, mice often fail to show behavioral changes in response to gene mutations that cause autism in people.

> A biological test for autism could make diagnosis faster.

So the researchers looked for autism biomarkers in rhesus monkeys, a species whose social capabilities are closer to those of humans. The monkeys had been raised by their mothers in social groups in a primate research colony at UC-Davis. From 222 male animals, the scientists selected 15 with naturally low sociability and compared them with 15 monkeys with naturally high sociability on several biological parameters.

The scientists measured levels of two hormones, oxytocin and vasopressin, in the monkeys' blood and in their cerebrospinal fluid, which bathes the brain. Both hormones are peptides implicated in a variety of social roles, including parental care and bonds between mates. Some prior studies have hinted that these hormones may also be involved in autism.

Monkeys in the less social group had significantly less vasopressin in their cerebrospinal fluid than monkeys in the more social group. These vasopressin levels accurately predicted the frequency with which individual monkeys participated in social grooming, an important social activity for rhesus monkeys. Vasopressin levels in blood were not different between the two groups. In a second group of 10 monkeys, whose cerebrospinal fluid was sampled four times over four months, the scientists showed that vasopressin levels in the fluid were stable over time.

The researchers also compared vasopressin levels in 14 boys with autism and seven age-matched children without autism. (Vasopressin levels were tested in the children's cerebrospinal fluid, which was collected via lumbar puncture for medical reasons; their families agreed to allow some fluid to be used for research.) Children with autism had lower vasopressin levels than children without autism, the study found.

"What we consider this to be at this point is a biomarker for low sociability," Capitanio said.

The researchers now want to test a larger group of monkeys for vasopressin levels to determine whether the hormone levels can distinguish monkeys with low social abilities from others with a wide range of social ability. And they want to explore whether low vasopressin could be detected before symptoms of impaired social ability emerge.

"We don't know if we see really low cerebrospinal fluid vasopressin before you see behavioral symptoms of autism," Parker said. "Ideally, it would be a risk marker, but we haven't studied that yet."

Parker is a member of the Stanford Child Health Research Institute, the Stanford Neurosciences Institute and Stanford Bio-X.

The study's other Stanford authors are Joseph Garner, D.Phil., associate professor of comparative medicine; research scientist Ozge Oztan, PhD; former research coordinator Sean Berquist; Sonia Partap, MD, clinical associate professor of neurology and neurological sciences; and Antonio Hardan, MD, professor of psychiatry and behavioral sciences.

Scientists from UC-Davis, UC-San Francisco and the Sutter Neuroscience Medical Group in Sacramento also contributed to the study.

The research was funded by grants from the National Institutes of Health, the Simons Foundation, the Mosbacher Family Fund for Autism Research, Stanford Bio-X, the Weston Havens Foundation, Stanford's Child Health Research Institute, the Katherine D. McCormick Fund and the Yani Calmidis Memorial Fund for Autism Research.

Stanford's Department of Psychiatry and Behavioral Sciences also supported the work. ISM

Protein mimic developed to help injured lungs breathe

By Bruce Goldman

A Stanford researcher has bioengineered an effective protein mimic that restored breathing capacity to the injured lungs of rats, according to a new study.

This synthetic product could lead to better, cheaper treatments for acute lung injury in humans. When used in the rats, it equaled or outperformed a costly natural counterpart in several physiological measures, the study said.

A paper describing the research was published online May 1 in *Scientific Reports*.

Imagine the force you'd need to blow up a balloon whose surface area nearly matched that of a tennis court. To make things challenging, imagine further that the balloon is made of exquisitely delicate material. That balloon is your lungs, and every breath you take is a miracle. What makes it possible is a thin coating of a soaplike film, or surfactant, that lowers the tension of the lung's inner surface, radically reducing the amount of force required to inhale. Without this surfactant, you couldn't breathe.

JOEL SIMON IMAGES

Annelise Barron and her colleagues synthesized a protein mimic that could lead to better treatments for acute lung injury in humans.

"Lung surfactant is endowed with amazing biological properties," said Annelise Barron, PhD, associate professor of bioengineering. "The key to this is the presence, in the surfactant, of two special proteins whose structures uniquely enable them to cut surface tension." But those same amazing structural properties, she said, also make these proteins difficult to synthesize and purify, and relatively unstable in solution, limiting shelf-life and increasing price.

"One of them contains the most hydrophobic, or fat-resembling, stretch of chemical constituents of all known human proteins," Barron said. "It's really hard to work with because that fatty stretch makes it tend to clump up, ruining its activity."

With the new study, Barron, who's been working on stable, synthetic substitutes for these special proteins for two decades, appears to be nearing success. She shares senior authorship of the study with Ruud Veldhuizen, PhD, associate professor of medicine and of physiology and oncology at the University of Western Ontario, whose team performed the animal experiments. The study's lead author is Ann Czyzewski, PhD, Barron's former graduate student at Northwestern University, where Barron worked before coming to Stanford.

The first big challenge

Breathing is the first big challenge a newborn faces. Each year in the United States, some 20,000-30,000 infants born too early to produce their own natural surfactant are treated with an animal-derived variety, which is expensive. It's only in richer countries that this life-saving therapy occurs: The cost of a single vial containing enough animal-derived natural surfactant to coat the tiny lungs of a newborn infant is prohibitive in developing countries.

An adult's lungs are more than 20 times bigger than a baby's — their surface area approaches that of a tennis court, Barron said — pricing natural surfactant out of reach for many life-threatening cases of acute lung injury, which affects 200,000 adults annually in the United States. Surfactant dysfunction can result from severe lung infections, including bacterial and viral pneumonia; lung-collapsing trauma from impacts, such as those that occur in a car accident; inhalation of water in near-drownings; or aspiration of foreign materials in a drug overdose.

Ironically, attempts to keep patients breathing via mechanical ventilators often cause a surfactant-depleting lung infection, Barron said. "People who wind up on ventilation in intensive-care units are intubated. A breathing tube is inserted into their nose and threaded down to their lungs," she said. "If that intubation lasts for more than three days, their chance of acquiring a lung infection rises to 100 percent." Even four hours spent on a ventilator spells a 1-in-6 chance of lung infection.

Until recently, the only way to obtain functional surfactant was from the lungs of cattle or pigs. "You get only a tiny amount per animal," Barron said. "And whatever you've collected, you have to purify very carefully, as the material is so fragile you can't treat it with high heat to kill microbial pathogens."

In recent years, a somewhat cheaper substitute has become available. It contains a water-based dispersion of fatty lipids along with short protein snippets that, to an extent, mimic the surface-tension-reducing capabilities of their natural counterparts, the surfactant proteins. While much better than nothing, this mixture is not quite as effective as animal-derived surfactant.

Synthetic mimics of two proteins

Barron's designer polymers, called peptoids, have specific sequences and helical structures that mimic key bioactive portions of the two important proteins, surfactant proteins B and C, found in the lungs. The mimics, which she calls pB and pC, resemble the proteins. But their component building blocks differ subtly from those of proteins in a way that makes them extremely resistant to breakdown by heat or naturally occurring bodily enzymes called proteases. In addition, they are much less inclined to aggregate into clumps and lose their bioactivity than their natural counterparts. They can be synthesized at one-quarter to one-third of the cost of obtaining the surfactant from animals or of the available synthetic version.

Most important, the rodent study indicated that a surfactant containing pC was superior to the animal-derived surfactant in oxygenating blood, which is the lungs' main purpose.

The trial pitted the two designer substances against the animal extract and against a control solution. The researchers administered different surfactant candidates to anesthetized rats whose lungs had been rinsed to rid them of their own natural sur-

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Courage

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people with excessive anxiety, phobias or post-traumatic stress disorder lead more normal lives.

"This opens the door to future work on how to shift us from paralysis and fear to being able to confront challenges in ways that make our lives better," said Huberman, the senior author of a paper describing the experimental results. It was published online May 2 in *Nature*. Graduate student Lindsey Salay is the lead author.

Perilous life of a mouse

There are plenty of real threats in a mouse's world, and the rodents have evolved to deal with those threats as best they can. For example, they're innately afraid of aerial predators, such as a hawk or owl swooping down on them. When a mouse in an open field perceives a raptor overhead, it must make a split-second decision to either freeze, making it harder for the predator to detect; duck into a shelter, if one is available; or run for its life.

To learn how brain activity changes in the face of such a visual threat, Salay simulated a looming predator's approach using a scenario devised some years ago by neurobiologist Melis Yilmaz Balban, PhD, now a postdoctoral scholar in Huberman's lab. It involves a chamber about the size of a 20-gallon fish tank, with a video screen covering most of its ceiling. This overhead screen can display an expanding black disc simulating a bird-of-prey's aerial approach.

Looking for brain regions that were more active in mice exposed to this "looming predator" than in unexposed mice, Salay pinpointed a structure called the ventral midline thalamus, or vMT.

Salay mapped the inputs and outputs of the vMT and found that it receives sensory signals and inputs from regions of the brain that register internal brain states, such as arousal levels. But in contrast to the broad inputs the vMT receives, its output destination points were remarkably selective. The scientists traced these outputs to two main destinations: the basolateral amygdala and the medial prefrontal cortex. Previous work has tied the amygdala to the processing of threat detection and fear, and the medial prefrontal cortex is associated with high-level executive functions and

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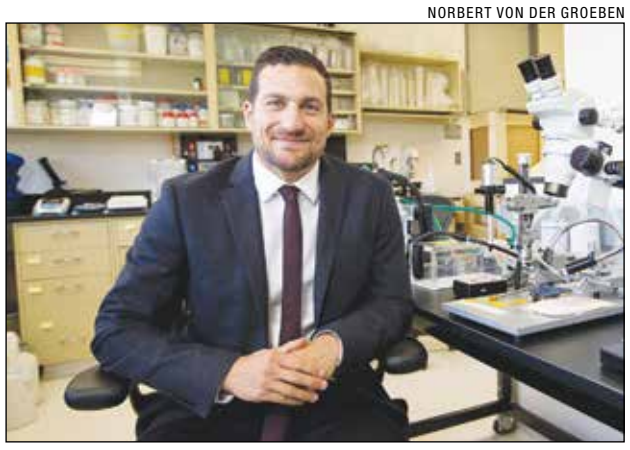


Andrew Huberman and his colleagues have found two clusters of brain cells in mice that are linked to either a timid or a bold response to a visual threat.

anxiety.

Further inquiry revealed that the nerve tract leading to the basolateral amygdala emanates from a nerve-cell cluster in the vMT called the xiphoid nucleus. The tract that leads to the medial prefrontal cortex, the investigators learned, comes from a cluster called the nucleus reuniens, which snugly envelopes the xiphoid nucleus.

Next, the investigators selectively modified specific sets of nerve cells in mice's brains so they could stimulate or inhibit signaling in these two nerve tracts. Exclusively stimulating xiphoid activity markedly increased mice's propensity to freeze in place in the presence of a perceived aerial predator. Exclusively boosting activity in the tract running from the nucleus reuniens to the medial prefrontal cortex in mice exposed to the looming-predator stimulus radically increased a response seldom seen under similar conditions in the wild or in previous open-field experiments: The mice stood their ground, right out in the open, and rattled their tails, an action ordinarily associated with aggression in the species.

This "courageous" behavior was unmistakable, and loud, Huberman said. "You could hear their tails thumping against the side of the chamber. It's the mouse equivalent of slapping and beating your chest and saying, 'OK, let's fight!'" The mice in which the nucleus reuniens was stimulated also ran around more in the chamber's open area, as opposed to simply running toward hiding places. But it wasn't because nucleus reuniens stimulation put ants in their pants; in the absence of a simulated looming predator, the same mice just chilled out.

In another experiment, the researchers showed that stimulating mice's nucleus reuniens for 30 seconds before displaying the "looming predator" induced the same increase in tail rattling and running around in the unprotected part of the chamber as did vMT stimulation executed concurrently with the display. This suggests, Huberman said, that stimulating nerve cells leading from the nucleus reunions to the prefrontal cortex induces a shift in the brain's internal state, predisposing mice to act more boldly.

Another experiment pinpointed the likely nature of that internal-state shift: arousal of the autonomic nervous system, which kick-starts the fight, flight or freeze response. Stimulating either the vMT as a whole or just the nucleus reuniens increased the mice's pupil diameter — a good proxy of autonomic arousal.

On repeated exposures to the looming-predator mockup, the mice became habituated. Their spontaneous vMT firing diminished, as did their behavioral responses. This correlates with lowered autonomic arousal levels.

Human brains harbor a structure equivalent to the vMT, Huberman said. He speculated that in people with phobias, constant anxiety or PTSD, malfunctioning circuitry or traumatic episodes may prevent vMT signaling from dropping off with repeated exposure to a stress-inducing situation. In other experiments, his group is now exploring the efficacy of techniques, such as deep breathing and relaxation of visual fixation, in adjusting the arousal states of people suffering from these problems. The thinking is that reducing vMT signaling in such individuals, or altering the balance of signaling strength from their human equivalents of the xiphoid nucleus and nucleus reuniens may increase their flexibility in coping with stress.

Huberman is a member of Stanford Bio-X and of the Stanford Neurosciences Institute.

Life science research professional Nao Ishiko also co-authored the study.

The study was funded by the National Institutes of Health.

Stanford's departments of Neurobiology and of Ophthalmology also supported the work. **ISM**

SLAC, Stanford open facility for cryogenic electron microscopy

By Glennda Chui

A new facility for cryogenic electron microscopy, or cryo-EM, has opened at the Department of Energy's SLAC National Accelerator Laboratory.

Built and operated in partnership with Stanford University, it's equipped with four state-of-the-art instruments for cryo-EM, a groundbreaking technology whose rapid development over the past few years has given scientists unprecedented views of the inner workings of the cell.

The facility is the first to open as part of the Stanford-SLAC Cryo-EM Initiative, and it is one of the most advanced in the world in terms of the number of high-end instruments and level of expertise it makes available. One of the goals of the cryo-EM initiative is to get evermore detailed 3D images of DNA, RNA, proteins, viruses, cells and the tiny biological machines within the cell.

The facility allows researchers to prepare samples, collect data at high speed and assess the quality of that data on the fly so they can make the best use of their experimental time. They can carry out experiments in person or remotely.

"This facility is the result of several years of work and planning by faculty and other leaders at SLAC and Stanford, and it's a great example of the opportunities our partnership brings us," said Stanford President Marc Tessier-Lavigne, PhD. "Cryo-EM has become an essential tool for research, especially in structural biology, and we're excited that this state-of-the-art facility is finally here."

Detailed view of life's machinery

Cryo-EM is a version of electron microscopy, which was invented in the 1930s. These microscopes use beams of electrons rather than light to form images of samples. Because the wavelength of an electron is much shorter than the wavelength of light, electron beams reveal much smaller things.

In the mid-1970s, scientists came up with the idea of freezing samples to preserve the natural structure of biological specimens and reduce damage from the electron beam, and cryo-EM was born. The technology slowly evolved, and then a few years ago took a giant leap, thanks to dramatic advances in detectors and software. In 2017, three scientists were awarded the Nobel Prize in chemistry for their roles in developing cryo-EM.

Today, cryo-EM generates 3D images at nearly atomic resolution of viruses, molecules and complex biological machines inside the cell — such as the ribosomes, where proteins are synthesized. By flash-freezing these tiny things in their natural environments, scientists can see how they are built and what they do in much more detail than before, stringing thousands of images together to create stop-action movies and even taking virtual "slices" through cells, much like miniature CT scans. Meanwhile, cryo-EM instruments have become easier to use and much more accessible.

"In biology, everything is dynamic, always moving around and changing. Cryo-EM lets you capture snapshots of proteins and other biological nanomachines as they assemble, carry out their work and disassemble again," said Wah Chiu, PhD, professor of bioengineering and of microbiology and immunology at Stanford and of photon science at SLAC. Chiu is one of two faculty members hired last year who bring decades of experience in cryo-EM research and technology development.

"A protein may multitask, changing its shape for each of its functions," Chiu said. "From how it looks, you can determine how to modify its shape and consequently its functions — for instance, if you want to block its activity with a vaccine or develop a medication that fits into a particular pocket and triggers a response that fights disease." He added that his own ambition is to be able to look at these biological machines at work while they're still inside the cell, without having to remove or purify them.

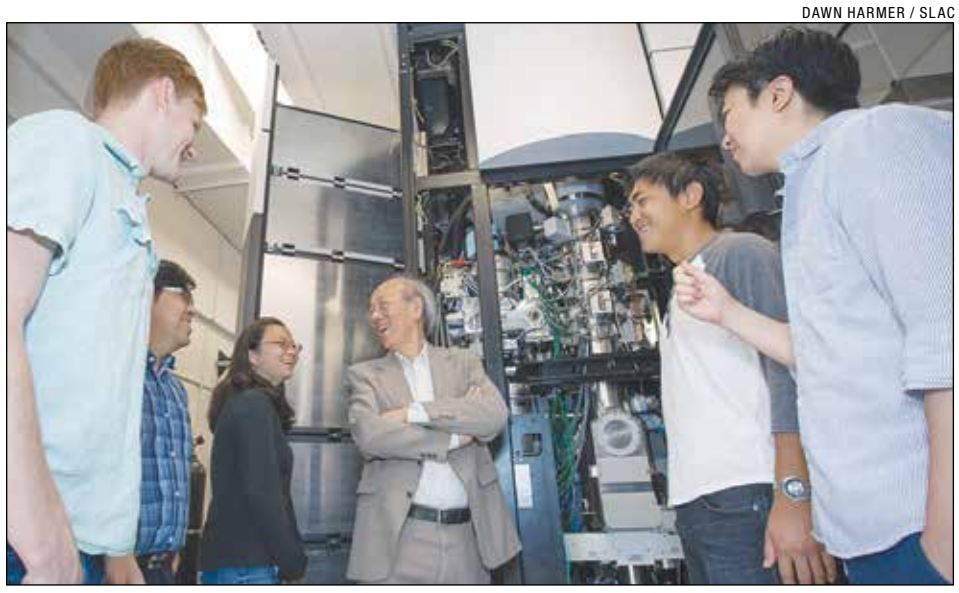
Georgios Skiniotis, PhD, professor of molecular and cellular physiology and of structural biology, arrived at the Stanford School of Medicine last year from the University of Michigan. He specializes in studies of complex receptors on the cell's outer membrane that are important targets for drug development, but whose structure and function are still poorly understood. Cryo-EM makes them much easier to study in detail.

"Many refer to it as the cryo-EM revolution, but I view it more as accelerated evolution because most of these concepts and tools have been steadily evolving for many years," Skiniotis said. "Without any sense of exaggeration, the technology offers unprecedented imaging capabilities. Cryo-EM can be used for so many purposes."

In battery research, for instance, scientists working with an older cryo-EM instrument at the School of Medicine recently captured the first atomic-level images of fingerlike growths called dendrites that can pierce the barrier between battery compartments and trigger short circuits or fires.

ANDY FREEBERG / SLAC

DAWN HARMER / SLAC



(Top) Graduate student Patrick Mitchell pours liquid nitrogen into a device used to freeze samples prior to study. (Bottom) Wah Chiu and members of the cryo-EM team stand next to a cryo-EM instrument.

Technology and the human touch

One big advantage of locating the new facility at SLAC is the lab's long history of technology development, including computing and data analysis, said Soichi Wakatsuki, PhD, a professor of photon science at SLAC and of structural biology at the School of Medicine, who leads the lab's Biosciences Division. "Adding cryo-EM to our established X-ray approaches will help us build the fundamental understanding that researchers need to design biofuels and biomaterials, predict how carbon cycles through geological and living systems and tease out the complicated interactions of fungi and other organisms in the root zones of plants, which are important for understanding natural ecosystems as well as improving soil fertility," Wakatsuki said.

Both Chiu and Skiniotis have been collaborating with Stanford scientists on research projects over the years, and that's even easier now that they — and the cryo-EM facility — are close by, Chiu said.

"I think the environment of physicists, engineers, computational scientists and chemists around the SLAC and Stanford campuses can inspire us to think differently," he said. "There's no doubt in my mind that this will lead to new approaches in specimen preparation, data collection, image processing — in the whole pipeline."

Chiu brings with him two National Institutes of Health research programs he started while at Baylor College of Medicine. One is a regional consortium for cryo-EM data collection. It makes instruments available to cryo-EM scientists across the United States who would not otherwise have access to the technology. The other, a national center for 3D electron microscopy of macromolecules, is focused on cryo-EM technology development that is driven by the biomedical projects of collaborators around the globe, as well as on training and serving the global community of cryo-EM researchers.

Path to the initiative

Roger Kornberg, PhD, professor of structural biology and 2006 Nobel laureate in chemistry, has used cryo-EM for many years in research on how the cell carries out instructions in DNA, and has advocated for expansion in this area. Investments from the School of Medicine initiated the drive to expand access to this technology for the Stanford research community and, along with contributions from the President's Office and SLAC, enabled the hiring of Skiniotis and Chiu.

"We were extremely pleased to have received this tremendous support," said William Weis, PhD, professor and chair of structural biology. Weis and Axel Brunger, PhD, professor of molecular and cellular physiology and of neurology and neurological sciences, and Norbert Pelc, ScD, professor of bioengineering, played a major part in recruiting the new faculty members "This is a wonderful achievement for the whole interdisciplinary team that has been working diligently to develop this program," said Pelc, the former chair of bioengineering. "It is also a great opportunity for scientists from SLAC, Stanford and other institutions who will have access to this leading-edge technology. We all will benefit from the discoveries they will make."

Brunger, the former chair of molecular and cellular physiology, added, "Cryo-EM now enables the study of biomolecules in their native environment, such as in membranes, and ultimately, to study them in their cellular context. Further developments in technology are conceivable and could revolutionize the fields of cell biology and molecular neuroscience." **ISM**

Mimic

continued from page 4

factant, and then assessed several physiological outcomes at various time points. The solution containing pC was equal to or better than the animal surfactant in every outcome — a "shocking result," according to Barron.

"This opens up new frontiers," Barron said. "Our relatively simple synthetic mimic of a very complex material will have a much longer shelf life and can be made in large amounts at reasonable prices. Reasonable enough, we hope, that it may for the first time be possible to conduct a clinical trial in adults in intensive care units who've been intubated and who might benefit substantially from surfactant replacement. And it would, finally, also be available to premature babies in developing countries like Bolivia, where my father was born."

Further preclinical and clinical trials lie ahead before that day comes, she added.

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Other Stanford co-authors are former postdoctoral scholar Michelle Dohm, PhD; former graduate student Maruti Didwania, PhD; research engineer Jennifer Lin, PhD; and research technician Lauren Broering.

Researchers at the University of Western Ontario and Northwestern University also contributed to the study.

The study was funded by the National Institutes of Health, the National Science Foundation, and the deans of both the School of Medicine and School of Engineering, and was also supported by the Molecular Foundry at Lawrence Berkeley National Labs.

Stanford's Department of Bioengineering, which is jointly operated by the schools of Medicine and of Engineering, also supported the work. **ISM**

Spudich

continued from page 1

Stanford Bio-X and the Biohub to perform a kind of unprecedented molecular poking and prodding of Spudich's lung tissue — analyzing gene expression profiles, signaling pathways, cellular architecture and immune responses — to identify for the first time exactly what goes wrong when lung cells become cancerous.

'A remarkable story'

"This is a massive attack on a human disease with the most modern of research tools on one tumor from one patient," said Krasnow, who estimates the project will cost $1 million or more. "In five or 10 years, I believe that the knowledge we will learn from this study will guide the standard of care for all lung adenocarcinoma patients. It's a remarkable story."

The effort isn't intended specifically to help Spudich, whose prognosis is good because his cancer was caught early and removed quickly. Rather, it promises to offer an intimate glimpse into the earliest steps of cancer development in human lung tissue and will likely lead to better diagnosis and treatment of lung adenocarcinomas.

In the hours leading up to the surgery, the researchers struggled to balance their excitement about the scientific opportunity with their concern for a colleague. To say James Spudich is well-known in the Stanford Medicine community would be an understatement.

Many of the more than 20 people involved in this last-minute research effort had known Spudich for years. Quake had received a bachelor's degree in physics at Stanford in 1991 and had also done postdoctoral work under professor of physics Steven Chu, PhD. At the time, researchers in the Chu and Spudich labs were collaborating to build a single-molecule laser trap that Spudich would use to study the force generated by the interaction of actin filaments with myosin molecules responsible for muscle contraction. (Chu was awarded the Nobel Prize in physics in 1997 for related work.) Together, Chu and Spudich launched Stanford Bio-X in 1997 to promote interdisciplinary collaborations between researchers in Stanford's schools of Medicine, of Engineering and of Humanities and Sciences.

Christin Kuo

Heather Wakelee

Nearly everyone speaks of Spudich with admiration, not just for his prodigious scientific accomplishments — he received the 2012 Albert Lasker Basic Medical Research Award for his research into the molecular motors that drive muscle contraction — but also for his warmth and kindness.

"Everyone knows and loves Jim," Quake said. "So it wasn't surprising that our whole community mobilized immediately to make this happen."

"This was an amazing scientific opportunity that came much earlier than expected," said Krasnow, who is also a Howard Hughes Medical Institute investigator. "But, of course, there was also the realization that we were talking about our beloved colleague, mentor and friend. When that person is right in front of you, and with you, it gives this effort an urgency and a poignancy that brings science and medicine together in such a beautiful and powerful way."

Spudich, who refers to the effort as Project Lung, is far less sentimental. In fact, some might say he's unabashedly enthusiastic. "There's no place else in the universe where the biology, the biophysics and the technology — everything that we can muster to throw at these tissues — exists that will allow us to really understand the lung in unprecedented molecular detail," Spudich said. "What is going to emerge is an understanding about lung biology at a level of depth no one has previously imagined. And it's kind of special that this is my lung."

Common form of lung cancer

Lung adenocarcinoma is the most common type of lung cancer. It usually occurs in current or former smokers, but it is also the main type of lung cancer in nonsmokers. It's frequently diagnosed at a late stage, after patients report symptoms of coughing or other vague symptoms like weight loss or unexplained pain.

Spudich was lucky that his cancer was discovered early. "I was at my annual physical on Sept. 20, 2017, when my doctor asked how I was. I said I felt great, but my wife, Anna, said, 'No, he's not alright,'" Spudich recalled. "We looked at her, and she continued, 'Jim's more tired than he usually is, and he has a slight cough, and we just came back from India.' I felt both of these symptoms were unremarkable, as I'm getting older, but heeding her concern, my doctor ordered a chest X-ray."

The X-ray, taken nine days later, showed a slight cloudiness in the upper lobe of Spudich's left lung. Subsequent CT and PET scans suggested the presence of a possible adenocarcinoma, about 2 centimeters long, which was confirmed by a biopsy. Surgery to remove the lobe, containing both normal and cancerous tissue, was scheduled for the morning of Oct. 27.

"So, totally unexpectedly, I went in one month from feeling normal and fit to having to deal with lung cancer," Spudich said. "I knew it was time to talk to Mark, who is the world's expert on lung development and who was carrying out some of the most cutting-edge research on the origins of lung adenocarcinomas in laboratory mice."

At the time, Krasnow was part of a large collaboration organized by Quake and Tony Wyss-Coray, PhD, professor of neurology, to understand the diversity of cell types across many tissues as part of the Chan Zuckerberg Biohub's Cell Atlas project. Using technologies developed by Quake and others, researchers are performing comprehensive profiling of the transcriptomes of single cells, enabling them to analyze cell type and state with unprecedented sensitivity and precision in both health and disease.

"Steve [Quake], Tony and their teams were working on a mouse cell atlas," Krasnow said. "We were in charge of the lung aspect. To do so, we had been assembling what we termed 'rapid response teams' to quickly collect and analyze the mouse tissue. So we knew how to do something like this, and how to do it well. But we hadn't done it before in humans, and nowhere nearly as quickly as we had to act with Jim's tumor. Normally we had a lead time of days or weeks; now we had hours."

'A unique opportunity'

In addition to ensuring all aspects of the protocol were followed, including explaining to the patient, Spudich, exactly what would happen, Krasnow faced another hurdle: how to get funding for the surprise project.

"I immediately contacted Steve and described how this was an amazing opportunity to extend the CZ Biohub studies in mice into human lungs and lung cancer," Krasnow said. "We could analyze normal and diseased human tissue, and compare it to what we had learned in mice and mouse lemurs. And then I told him who the patient was. I don't think it took Steve even a second to say, 'Go for it.'"

That afternoon, members of the rapid response team, including Kuo and several students and postdoctoral scholars in Krasnow's lab, went to work to "humanize" their mouse studies. "Christin worked on the clinical side," Krasnow said, "getting forms and making connections with surgeons, clinicians and pathologists. Meanwhile, the students and postdocs in my lab were figuring out what was needed to be done differently with human tissue."

For one thing, the sheer amount of tissue that would be removed from Spudich would be much larger than a tiny mouse lung. Furthermore, the researchers couldn't use mouse-specific antibodies to separate the human tissue into specific cell types. And although some types of cells are relatively abundant and easy to analyze, others require meticulous care to isolate. "We're not just interested in the tumor cells themselves," Krasnow said. "We also want to understand the roles played by the stromal cells that surround the tumor, the immune cells that have infiltrated the tumor and even the endothelial cells that form the vessels that deliver blood to the growing mass. Bulk analysis, in which several cell types are combined, obscures much of the most interesting information. We wanted to sample all major cell types in the tissue in and around the tumor, and also in healthy neighboring lung."

Moving quickly

Krasnow's team pulled out all the stops to find the appropriate materials for the research.

"All of these people scurried about until late in the night to scrounge reagents and antibodies from other labs and the CZ Biohub, either here on campus or nearby," Krasnow said. He also alerted Lisa Nichols, PhD, who directs the Beckman Fluorescence Activated Cell Sorting Facility, to prepare for an influx of samples. "We had to line up time on the cell-sorting machines and find FACS operators who could be on-call after the surgery," Krasnow said. "And all of this had to be put in place by 8 a.m. the next morning."

In addition to facilitating the efforts of the rapid response team, Krasnow marshaled other lung adenocarcinoma researchers at Stanford.

"I was on the East Coast at a meeting that afternoon when I received a phone call from Mark," Maximilian Diehn, MD, PhD, associate professor of radiation oncology, recalled. "Would I be interested in collecting plasma for study?"

Diehn, who earned his doctorate in biophysics in the Department of Biochemistry at Stanford, is studying whether the presence and levels of circulating tumor DNA, or ctDNA, which is shed into the bloodstream by tumor cells, can be used to diagnose or to predict the recurrence of the disease after initial treatment. He and Ash Alizadeh, MD, PhD, associate professor of oncology, have shown that if ctDNA is detected after radiation and surgery, that patient is at high risk for recurrence.

"Jim's cancer type and stage basically fit perfectly into one of our ongoing clinical studies," Diehn said. "By collecting blood before and after surgery, and then intermittently during and after subsequent chemotherapy, we can look for the presence of ctDNA and possibly predict the chance of recurrence."

Diehn and Alizadeh have also helped to develop an assay in Stanford's Molecular Pathology Laboratory to identify the presence of genetic changes in a tumor

Coral

continued from page 1

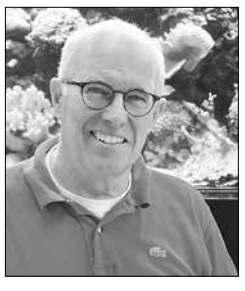
critical to coral biology," Cleves said.

CRISPR is a fast, effective tool that can be used to target and modify DNA sequences. "Breaking" genes to reveal the effects on the organism is a concept that's been the linchpin of decades of molecular biology. Now, CRISPR is helping speed up the process in many diverse animal models, but applying it to corals (don't be fooled — corals are animals, not plants) has proven tricky due in part to their infrequent reproduction. And until Cleves and his collaborators conducted this research, the use of the gene-editing tool had never been reported in corals.

"We hope that future experiments using CRISPR-Cas9 will help us develop a better understanding of basic coral biology that we then can apply to predict — and perhaps ameliorate — what's going to happen in the future due to a changing climate," Cleves said.

Spawning by moonlight

Corals pose a bit of a problem when it comes to CRISPR because of their spawning cycles. Most corals, including the *Acropora millepora* that was the focus of the study, breed only once or twice a year, during October and November in the Great Barrier Reef, cued by the rise of a full moon. During this fleeting window, corals release their sex cells into the ocean. When the eggs and sperm meet, they form zygotes, or fertilized single cells. During the narrow time window before these cells begin to divide, a researcher can introduce CRISPR by injecting a mixture of reagents into these zygotes to induce precise mutations in the coral DNA.



John Pringle

Retrieving the zygotes is quite a logistical challenge, Cleves acknowledged. Fortunately, his collaborators in Australia have the timing down pat; they can predict when the moon spawn will occur within a couple of days, allowing them to take coral samples from the reef to gather zygotes for experimentation.

Cleves traveled to Australia to begin experimenting with CRISPR, targeting three coral genes: red fluorescent protein, green fluorescent protein and fibroblast growth factor 1a, a gene that is thought to help regulate new coral colonization.

Using CRISPR, the scientists made a type of genetic tweak that knocked out the genes, rendering them incapable of functioning. In the case of the red and green fluorescent proteins, determining if CRISPR worked would be easy — like seeing lights switch off. Or so they hoped. However, it turns out that there are multiple copies of red and green fluorescent-protein genes. So knocking out one copy didn't put a stop to the

MARK TUSCHMAN

JOEL SIMON IMAGES

(Above, left) Maximilian Diehn and Ash Alizadeh have shown that if circulating tumor DNA is detected after radiation and surgery, the patient is at high risk for recurrence. "Jim's cancer type and stage basically fit perfectly into one of our ongoing clinical studies," Diehn said. (Above, right) Stephen Quake, who is working on a project to map every human cell, was enlisted to help with the "lungome" effort.

that can be targeted by existing drugs or treatments.

Krasnow still had a few more experts he wanted to involve.

"Immunotherapy is a very exciting field of research right now, and we wanted to reach out to experts of the immune and blood system to systematically analyze immune cells infiltrating Jim's tumor," Krasnow said. Irving Weissman, MD, director of Stanford's Institute for Stem Cell Biology and Regenerative Medicine and of its Ludwig Center for Cancer Stem Cell Research and Medicine, fit the bill perfectly. He and Spudich are old friends.

Rahul Sinha, PhD, a former postdoctoral scholar in Weissman's lab and an instructor at the institute, came on board to collect the necessary blood and tissue samples and initiate the analysis of tumor-infiltrating immune cells.

Finally, Krasnow called professor of medicine Calvin Kuo, MD, PhD, and hematology and oncology fellow Ameen Salahudeen, MD, PHD. The two had been working to establish new ways to culture normal and cancerous human lung tissue from surgical biopsies to study human-specific biology. Together with Tushar Desai, MD, assistant professor of medicine, they had recently identified the presumed cell-of-origin for lung adenocarcinoma in humans.

"Again, we were just so fortunate to have world experts, or in many cases *the* world expert, right next door," Krasnow said.

"Jim's cancer is going to be one of the most heavily analyzed human cancers ever," Diehn said.

'A very busy patient'

By the next morning, Spudich himself was "a very busy patient," signing multiple consent forms and being briefed on what his participation in each study entailed.

"Jim's attitude was amazing," Krasnow said. "In everything he does, he has a wonderful blend of vision and hope. This was no different." Meanwhile, the rapid response team was gowned and waiting, somewhat nervously, outside an operating room at Stanford Hospital.

"These are PhD students and postdocs," Krasnow said. "I don't think any of them had ever been in an operating suite before, or even seen or touched fresh human tissue. But there they were, after being up much of the night, as Shrager removed the tissue through the tiniest of incisions."

Kuo was in the operating room to discuss with Shrager and the pathologists how to get the best samples of normal and diseased tissue for the planned analysis. "These sections were precisely annotated and hand-delivered to the lab immediately, to ensure the freshest tissues," Kuo said.

"From that point on, except for the size of the samples, it was pretty much like handling mouse tissue," Krasnow said. "Except this time it was someone they knew."

Under the care of Shrager and his team and Heather Wakelee, MD, professor of oncology and a specialist in lung cancer, Spudich recovered quickly from the surgery. "I was up and walking the halls that evening," he recalled. "I really can't say enough about Drs. Shrager and Wakelee and all the oncology care team at Stanford. They are phenomenal, and I am so thankful."

Because his cancer was caught early and removed fully, it's presumed to be cured. However, the current standard of care recommends several rounds of chemotherapy to reduce the chance of any possible recurrence. For patients with metastatic disease, the prognosis is less positive.

"In metastatic disease, chemotherapy is used to prolong life rather than cure the patient," Wakelee said. However, new approaches include an immunotherapy approach, known as checkpoint blockade, and personalized treatment based on the genetic sequence of the tumor to more precisely target cancer cells.

"Previously, we would treat all metastatic lung adenocarcinomas the same, while now we look very closely at the cancer's molecular underpinnings," Wakelee said. "Now we wouldn't think of starting treatment without understanding the genetic changes present in each patient's cancer."

Wakelee is the principal investigator for a clinical trial testing the effect of checkpoint inhibitors on the cure rates for people with early stage lung adenocarcinoma. Spudich has enrolled in the trial. After completing post-surgery chemotherapy, half the participants will receive the active drug and half will be part of a control group.

'A full participant in the project'

"It's so interesting to be the patient — I'm receiving chemo — but also to be a researcher deeply involved in the decision-making and data-analysis process. For example, I attend regular meetings of the team to decide what to do with the tissue samples," Spudich said.

"Jim's not just a patient, he's also a scientist," Krasnow said. "He started reading all our papers in the field. He wants to know everything. He quickly became not just a huge motivation for us all, both scientifically and personally, but a full participant in this project."

Raj Rohatgi, PhD, associate professor of biochemistry and of medicine and a close friend of Spudich's, facilitated Spudich's involvement in the scientific aspects of the work. "Raj is an expert in lung cancer and a close colleague of Heather Wakelee's," Spudich said. "He is extremely knowledgeable about the molecular and cell biology of the disease, and he's been invaluable to me. Mark, Raj and I confer almost daily about the latest developments in Project Lung."

In the months since the surgery, the scientists have amassed unprecedented amounts of data as a result of Spudich's impromptu visit to Krasnow's lab.

"Major results are starting to pour in," Krasnow said. "We've already learned that there are cell types in humans that are either not present or not detectable in mice, which is very interesting. We've also identified a potential driver mutation for Jim's tumor."

Diehn and his colleagues have identified a genetic change in Spudich's cancer that, although relatively rare in lung adenocarcinoma, is common in melanoma. Drugs exist that target cancer cells with that mutation when the cancer is metastatic, but they are not yet approved for use in early stage cancers.

Krasnow and his colleagues are working to identify further subpopulations of cells in the lung. "We want to get to know the tumor and what is driving it," he said. "Are there subpopulations that might be responsible for maintaining and expanding the cancer?"

Previous work in Krasnow's lab has found that a small fraction of cells called alveolar type 2 cells also have stem cell capabilities. To maintain their stem cell identity, the cells require the presence of neighboring cells called fibroblasts that secrete a signaling molecule called Wnt. "These alveolar type 2 cells are basically stem cells with a day job," Krasnow said, "and they have a private niche of just one adjacent fibroblast." If these stem cells are inappropriately activated, it's possible they could begin dividing uncontrollably and give rise to an adenocarcinoma, the researchers believe.

"We really want to know what goes on in the very earliest stages of cancer development," Krasnow said. "Are there any signs that it initiates in these alveolar type 2 cells? Intriguingly, it turns out that Jim's tumor DNA does have an oncogenic mutation in one of these activating pathways. And now we have the opportunity to study them at single-cell resolution and compare them much more carefully to normal lung cells."

Potential approach for cure

One concept Krasnow and his collaborators are pondering is the idea that it might be possible to cure, rather than treat, lung cancers with a two-pronged approach: blocking the pathway that stimulates the cells' growth while also removing the Wnt signal that is necessary to confer the cells' stem cell properties.

"This is a foundational study," Krasnow said. "We've marshaled numerous experts and techniques in an effort to truly understand for the first time the full cellular and molecular complexity of a single tumor in a way that will push us to a whole new level of potential therapies for this disease."

"It's so amazing what you can do in 2018," Spudich said. "A lot of these techniques were invented right here at Stanford, many in the Biochemistry Department in which I spent so much of my career. If someone asks me exactly what we're going to learn from this study, I would answer the same way I answered when people asked me what would come out of Bio-X when it was first proposed. 'If I could tell you that, then we have failed.' But it's very exciting to imagine that I have a chance to help unravel what causes these cancers in the first place and the molecular details that might lead to new treatments." ISM

glow altogether.

"Although we are not sure we saw convincing loss of fluorescence, DNA sequencing showed us that we were able to molecularly target both the red and the green fluorescent protein genes," Cleves said. This showed the researchers that, in one go, CRISPR could successfully alter multiple genes if the two were similar enough — a boon to genetic manipulation, as genes are often duplicated during evolution.

As for the third gene, fibroblast growth factor 1a, which only has one gene copy, post-CRISPR sequencing showed success: in some embryos, the gene was largely mutated, suggesting that CRISPR will work well to modify single-copy coral genes.

Cleves said the ultimate goal is not to engineer a genetically resilient super-coral that could populate the ocean — such a feat is currently implausible and would raise significant ethical questions. "Right now, what we really want to do is figure out the basic mechanisms of how coral works and use that to inform conservation efforts in the future," he said. "Maybe there are natural gene variants in coral that bolster their ability to survive in warmer waters; we'd want to know that."

'An all-hands-on-deck moment'

Although the current work is a proof-of-principle study, now Cleves and others are beginning to tinker with genes that are more ecologically pertinent. And he hopes that others do the same.

"I want this paper to provide an early blueprint of the types of genetic manipulations that scientists can start doing with corals," Cleves said. In the next few years, he hopes to see other groups knocking out coral genes potentially involved in bleaching, skeletal growth or the critical symbiosis with the algae that provide most of the corals' energy.

Research suggests CRISPR could aid coral conservation efforts.

Today, as much as 27 percent of the global reef ecosystem has been lost to a combination of climate change and human activities — and Cleves is feeling the urgency.

"This is an all-hands-on-deck moment," he said. "If we can start classifying what genes are important, then we can get an idea of what we can do to help conservation, or even just to predict what's going to happen in the future. And I think that makes this a really exciting time to be a basic biologist looking at the genetics of coral."

Pringle is a member of Stanford Bio-X.

The research was funded by the Simons Foundation, the National Science Foundation and the Australian Institute of Marine Science.

Stanford's Department of Genetics also supported the work. ISM

Crystal Mackall awarded $11.9 million for anti-leukemia clinical trial

Crystal Mackall, MD, professor of pediatrics and of medicine at the School of Medicine, was awarded $11.9 million by the governing board of the California Institute for Regenerative Medicine on April 26 to fund a clinical trial of immune cells genetically modified to recognize two proteins on the surface of leukemia and lymphoma cells.

Mackall directs the Stanford Center for Cancer Cell Therapy, where the trial will be conducted.

The trial will test the ability of specially modified immune cells called CAR-T cells to recognize and kill B-cell leukemia and B-cell lymphoma cells bearing one or both of two proteins: CD19 and CD22. A similar technique using CAR-T cells that target only CD19-bearing leukemia and lymphoma cells led to the approval in 2017 of two new cell therapies for the treatment of cancer by the Food and Drug Administration.

Crystal Mackall

"When a patient is told that their cancer has returned it can be devastating news," said Maria Millan, MD, president and CEO of CIRM. "CAR-T cell therapy is an exciting and promising new approach that offers us a way to help patients fight back against a relapse, using their own cells to target and destroy the cancer."

Mackall is the associate director of the Stanford Cancer Institute and the director of the Parker Institute for Cancer Immunotherapy at Stanford. Her award was one of three approved at the meeting. The others were an $8 million grant to Sangamo Therapeutics to test a new therapy for the blood disease beta-thalassemia and a $12 million grant to researchers at UC-San Francisco to develop a new treatment for children with severe combined immunodeficiency. With these awards, the stem cell institute has funded 48 clinical trials, 42 of which are active. **ISM**

OF NOTE

reports on significant honors and awards for faculty, staff and students

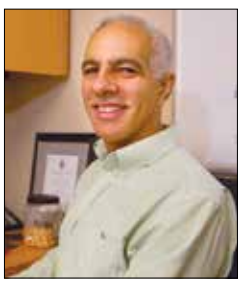
Benedict Anchang

Rebecca Aslakson

Helen Blau

Catherine Blish

David Camarillo



Michael Zeineh

Gerald Grant

Glenn Chertow

Judith Frydman

Paul Wise

Joanna Wysocka

Daniel Herschlag

Haruka Itakura

Josh Jaramillo

Roger Kornberg

Kyle Loh

Miquell Miller

Rushi Parikh

John Sunwoo

Sandra Winter

BENEDICT ANCHANG, PhD, instructor of radiology, received a grant from the Chan Zuckerberg Initiative for a one-year project to develop computational tools, algorithms, visualizations and benchmark data sets in support of the Human Cell Atlas, a collection of maps that will describe and define the cellular basis of health and disease.

REBECCA ASLAKSON, MD, PhD, was appointed associate professor of medicine and of anesthesiology, perioperative and pain medicine, effective March 1. Her research focuses on palliative care interventions and outcomes, including improving access to palliative care for surgical and intensive care unit patients.

HELEN BLAU, PhD, the Donald E. and Delia B. Baxter Foundation Professor, professor of microbiology and immunology and director of the Baxter Laboratory for Stem Cell Biology, was elected a member of the American Philosophical Society. The multidisciplinary society, founded in 1743 by Benjamin Franklin, is the oldest learned society in the United States.

CATHERINE BLISH, MD, PhD, was promoted to associate professor of medicine, effective April 1. Her work focuses on understanding human natural killer cells and their role in viral immunity, and on defining the immune mechanisms that contribute to viral susceptibility in pregnant women.

DAVID CAMARILLO, PhD, assistant professor of bioengineering; **MICHAEL ZEINEH**, MD, PhD, assistant professor of radiology; and **GERALD GRANT**, MD, associate professor of neurosurgery, were awarded a student-athlete health and well-being grant from the Pac-12 Conference. The three-year, $1.26 million grant will allow them to investigate head trauma and mental health as part of a Pac-12 initiative to improve the health, general well-being and safety of student-athletes at all conference member institutions.

GLENN CHERTOW, MD, professor of medicine and chief of the Division of Nephrology, received the National Kidney Foundation's 2018 David M. Hume Memorial Award. The award recognizes a distinguished scientist-clinician in the field of kidney and urologic diseases who exemplifies high ideals of scholarship and humanism.

JUDITH FRYDMAN, PhD, the Donald Kennedy Chair in the School of Humanities and Sciences and a professor of genetics and of biology; **PAUL WISE**, MD, the Richard E. Behrman Professor of Child Health and Society and a professor of pediatrics; and **JOANNA WYSOCKA**, PhD, professor of chemical and systems biology and of developmental biology, were among 213 new members elected to the American Academy of Arts and Sciences, one of the country's oldest honorary learned societies.

DANIEL HERSCHLAG, PhD, professor of biochemistry, was elected to the National Academy of Sciences, an organization that advises the nation on issues related to science and technology. He studies the fundamental behavior of RNA and proteins using an interdisciplinary approach to understand specific questions, such as how enzymes work, how RNA folds and how proteins recognize RNA.

HARUKA ITAKURA, MD, PhD,was appointed assistant professor of medicine, effective March 1. She uses machine learning and radiogenomic approaches to analyze cancer data to inform the development of cancer diagnostics and therapeutics.

JOSH JARAMILLO, MD, a second-year resident in general surgery, received the 2018 Pacific Coast Surgical Association Resident Global Surgery Scholarship. The $1,500 scholarship will help pay for travel and accommodation expenses for his international surgery rotation in Zimbabwe.

ROGER KORNBERG, PhD, professor of structural biology and the Mrs. George A. Winzer Professor in Medicine, was elected a fellow of the American Association for Cancer Research Academy class of 2018. The academy honors scientists whose contributions have propelled significant innovation and progress in cancer research.

KYLE LOH, PhD, assistant professor of developmental biology, has received the Fannie and John Hertz Foundation's 2018 Thesis Prize for his work, "A developmental roadmap for the diversification of human tissue fates from pluripotent cells." The prize is awarded for overall excellence and pertinence to high-impact applications of the physical sciences and includes a $5,000 honorarium.

MIQUELL MILLER, MD, resident in surgery and graduate student in health policy, was awarded the Society for Surgery of the Alimentary Tract/Society of Black Academic Surgeons Resident Research Award. The $25,000 grant will allow her to examine the relationship between physician cultural competency and coordination of care for rectal cancer patients.

RUSHI PARIKH, MD, fellow in cardiovascular medicine, received an honorable mention in the American College of Cardiology Young Investigator Awards in Clinical Investigations. Parikh was recognized for his oral presentation, "Impact of endothelin-1 on cardiac allograft vasculopathy, late mortality and re-transplantation following heart transplantation."

Xue Yuan

JOHN SUNWOO, MD, was promoted to professor of otolaryngology-head and neck surgery, effective March 1. His research interests include the immune response to cancer, the biology and developmental programs of natural killer cells, and intra-tumor and inter-tumor heterogeneity in head and neck cancer. He serves as the director of head and neck cancer research and the physician leader of the Head and Neck Cancer Care Program.

SANDRA WINTER, PhD, director of the WELL for Life research initiative and a social science research scholar at the Stanford Prevention Research Center, began her three-year term as secretary/treasurer of the Society of Behavioral Medicine, an organization of scientific researchers, clinicians and educators dedicated to better health through behavior change.

XUE YUAN, PhD, a postdoctoral scholar in plastic and reconstructive surgery, has received the Joseph Lister Award for New Investigators, which includes $8,000 from the American Association for Dental Research. Yuan won first place for her presentation, "Socket healing and immediate implant osseointegration via Wnt-responsive PDL cells." **ISM**