Research Report

Dr. Karl Deisseroth – Stanford
D. H. Chen Professor
Professor, Psychiatry and Behavioral Science
Professor, Bioengineering
Member, Bio-X

The H. L. Snyder Medical Foundation has supported Dr. Deisseroth's work since early 2004. Since then, he has published more than 120 papers in scientific journals.

Dr. Deisseroth's team is making a systematic and rigorous scientific effort to crack the code of depression and develop new and effective treatment strategies. The Depression Optogenetics Center at Stanford (DOCS) applies bioscience and engineering to develop tools and approaches for understanding and treating psychiatric diseases.

The center taps a relatively new, revelatory tool. In optogenetics, created by Dr. Deisseroth, light-sensitive genes are bioengineered into specific neural circuits. Flashes of light then turn neurons on or off, and researchers can observe a live animal's behavior and brain activity to learn what the circuit does. The light is delivered through painless optical fibers.

DOCS have already been able to fundamentally advance the science of depression in the first year of a 10-year project. During the past year, the team has developed and applied key optogenetic tools for fundamental research into the circuit basis of depression.

In humans and other complex organisms, the neural mechanisms that help answer the question "Is it worth my effort?" can fail, leading to severe brain disorders such as depression. The will to act is believed to arise in the prefrontal cortex, the part of the brain that helps plan and coordinate action. Signals to act then zip through the brain in a series of electrical signals, passing from neuron to neuron along branching pathways until reaching the nerves that directly stimulate movement.

In a series of DOCS papers partially funded by the H. L. Snyder Medical Foundation, Dr. Deisseroth presented the first real circuit-dynamics understanding of depression symptoms. In a *Nature* paper published in November 2012, he reported how his team used optogenetics to identify the brain signaling pathways that control the willingness to meet challenges, or the anticipation that action might be worthwhile in a difficult situation. Disruption

in these pathways appears to occur in cases of depression and other severe brain disorders where motivation to act deteriorates.

In December 2012, the team reported their success in inducing as well as relieving depression-like symptoms in mice by controlling just a single area of the brain known as the ventral tegmental area, or VTA. The VTA is known to be a source of dopamine and a central player in the brain's internal motivation and reward systems. This was the first time that well-defined types of neurons within a specific brain region have been directly tied to the control of myriad symptoms of major depressive illness.

In addition to his groundbreaking research, Dr. Deisseroth is also an inpatient/outpatient attending physician in the Department of Psychiatry and Behavioral Sciences at Stanford, focusing clinically on treatment-resistant depression and autism-spectrum disease. He treats these conditions and comorbid symptoms using, among other clinical tools, medications and high-speed neural stimulation. http://med.stanford.edu/profiles/Karl_Deisseroth/

His work can be reviewed at: http://www.stanford.edu/group/dlab/about_pi.html

Dr. Seung Kim – Stanford

Professor, Developmental Biology
Member, Stanford Cancer Institute

Research in Dr. Kim's laboratory focuses on the developmental biology of the pancreas, a vital organ of the digestive and endocrine systems. A major goal is to translate the lab's studies into novel diagnostic and therapeutic strategies for common pancreatic diseases, particularly diabetes mellitus and pancreatic cancer.

Researchers have been working toward functional restoration of diseased organs through replacing or regenerating the pancreas's islets of Langerhans, endocrine systems that secrete insulin. A deficiency of insulin-producing islet beta cells underlies diabetes. However, islet replacement has been limited by the inadequate understanding of mechanisms controlling islet formation and growth.

To meet this challenge, Dr. Kim's group has pioneered new approaches to create, expand, and regenerate islets. They discovered endocrine cells, including insulin-producing cells, in the fruit fly *Drosophila* that have the same ancestry as mammalian islet cells. Subsequently, they generated novel genetic screens to

identify evolutionarily conserved programs that control the development, expansion, and reprogramming of islet cells.

The team has identified new methods, based on FACS (fluorescence-activated cell sorting), to purify specific classes of cells that generate the pancreas and islets during mouse and human development. This provides a powerful platform to accelerate the use of pancreatic and embryonic stem cells for islet studies and replacement.

Dr. Kim's lab has elucidated new molecular pathways that control proliferation of beta cells in physiological settings or islet tumors. They envision that modulation of these pathways will be useful for stimulating expansion of functional islets for diabetes, and for treating neuroendocrine cancer.

Lab members anticipate that their unique approaches to islet and pancreas biology will culminate in diagnostic and therapeutic paradigms for human diseases. Their efforts in just a few years have created unprecedented opportunities for harnessing knowledge about the molecular and cellular basis of pancreatic development and growth with the goals of restoring pancreas islet function and treating endocrine cancers. Their work has revealed mechanisms underlying islet development, as well as adaptations and disease pathogenesis. The discoveries may help provide the tools and expertise needed to produce islet regeneration therapies for type 1 diabetes, improve treatments and tests to mitigate or prevent type 2 diabetes, and generate new therapeutic strategies for neuroendocrine cancers.

To help them pursue these goals, they are actively collaborating with other Stanford faculty, particularly Drs. Roel Nusse, Gerald Crabtree, Joanna Wysocka, and Irving Weissman. In addition, they have regular joint group meetings with laboratories of the UCSF Diabetes Center led by Drs. Matthias Hebrok and Michael German. http://med.stanford.edu/profiles/Seung_Kim

Dr. Judith Shizuru – Stanford

Associate Professor, Medicine (Blood & Marrow Transplantation)
Member, Stanford Cancer Institute

Dr. Shizuru and her team continue their research to make bone marrow transplantation (BMT) a more effective and safer modality for curing many life-threatening disorders. Diseases currently cured by BMT include: various cancers; non-cancerous conditions resulting from defects in

blood formation, such as sickle cell anemia and severe combined immune deficiency (i.e., bubble boy disease); and autoimmune diseases like childhood diabetes, systemic lupus erythematosus, arthritis, and multiple sclerosis.

Her recent published work examines the details of how the transfer of donor blood cells can cure autoimmune diabetes and arthritis. Other recent studies focus on delineating the basic biology of stem cell engraftment, wherein transplanted blood stem cells begin making healthy blood cells. Before new cells can be transplanted, diseased or faulty cells need to be removed, which is typically done with the toxic modalities of radiation and chemotherapy. With deeper understanding of engraftment, Dr. Shizuru's team aims to use substances that target and eradicate the delinquent cells with fewer side effects than the standard procedures.

In addition to the fundamental science work, she and her colleagues are making significant progress in the translation of these concepts to patient studies in the following areas:

Treatment of Severe Combined Immunodeficiency Disease (SCID): Dr. Shizuru heads a disease team that includes basic stem cell scientists and adult and pediatric transplant experts. This team is testing a monoclonal antibody that will be used to prepare children with SCID to accept donor stem cell transplants from their parents. This antibody will be the first biologic agent used for the purpose of eliminating faulty cells to make space for donor cells to engraft. The clinical trial is scheduled to begin in the summer of 2015.

Breast Cancer Treatment: Her team is in the final stages of production of the monoclonal antibodies that will permit the purification of human blood-forming stem cells. These antibodies will separate healthy blood-forming stem cells from the cancer cells that contaminate standard grafts. The pure stem cells will be used in a clinical trial led by Dr. Shizuru for the treatment of metastatic breast cancer.

Immune Tolerance Induction: Dr. Shizuru and her surgical colleagues are now routinely performing co-transplantations of donor-matched blood cells and kidneys. Unlike patients undergoing standard kidney transplantation who must continue on life-long immune suppressive drugs, many of the patients treated in this study have been able to come off these medications and have maintained excellent kidney function.

In the coming year, Dr. Shizuru and her team are looking forward to the commencement of the breast cancer study and to the development of next-generation reagents that can be used to target cells in recipients to allow blood-forming stem cells to engraft. http://med.stanford.edu/profiles/Judith_Shizuru/

Dr. William Hahn – Dana Farber
Co-Director of the Center for Cancer Genome Discovery
Deputy Chief Scientific Officer
Chief of the Division of Molecular & Cellular Oncology
Associate Professor of Medicine at the Harvard Medical School.
Research Interests

The Hahn laboratory has developed systematic approaches to discover and characterize mutations that program cancer development in order to derive a deeper understanding of the molecular networks that lead to malignant transformation and to drive the translation of these findings into clinically useful therapeutics. By combining work in experimental cancer models with comprehensive analyses of patient derived tumors, the overarching goal of their research is the development of new types of clinical trials focused on targeting specific cancer targets in selected patients with embedded molecular endpoints.

Their prior work has addressed the regulation of cellular lifespan, both in normal and malignant human cells. Both cell cycle regulatory proteins and telomerase regulate replicative lifespan, and alterations in each of these mechanisms are commonly found in human cancers. Telomerase plays a key role in cellular immortalization; expression of telomerase in many cells is sufficient to achieve immortalization, a hallmark of cancer. Using telomerase to immortalize human cells, they have shown that such immortalized cells are now susceptible to transformation by the combination of oncogene activation and inactivation of tumor suppressor pathways in vitro. Using oncogenes, dominant inhibitors of tumor suppressor proteins, and telomerase, they have now created models of human breast, lung, prostate, and ovarian epithelial cancers of defined genetic constitution that recapitulate many of the characteristics of spontaneously arising human tumors. They are using these models to understand the molecular basis of specific cancer phenotypes including androgen independence in prostate cancer, invasion and metastasis.

In parallel to these studies, they continue to investigate the pathways perturbed by telomerase and SV40 small t antigen in human cell transformation. Their recent work in telomere biology focuses on a new function of telomerase in transformation distinct from its role in immortalization. In addition, they have shown that SV40 small t antigen contributes to transformation by perturbing the serine-threonine phosphatase PP2A, and they are studying the consequences of this interaction for transformation.

With their colleagues at the Broad Institute, they have developed genome scale tools to perform somatic cell genetics in mammalian cells. Specifically, Hahn's lab is a founding member of the RNAi Consortium (TRC), which is dedicated to the production, validation, and use of genome scale RNA interference reagents. Together with colleagues in the DFCI Center for Cancer Genome Discovery and Center for Cancer Systems Biology, they have created an integrated cancer genomics platform that combines whole genome methods in patient derived tumors with functional genomic validation studies in experimental models. Their recent pilot studies provide proof-of-principle evidence that these technologies are sufficiently mature to be deployed within the context of a translational effort to discover and validate cancer targets. Their goal is to use this platform to drive the development of new clinical trials focused on targeting specific cancer targets in selected patients with embedded molecular endpoints.

For details about Dr. Hahn and his research, start at this web site:
http://research4.dfci.harvard.edu/hahnlab//index.html

Dr. Matthew Freedman – Dana Farber
Associate Physician at Dana Farber
Associate Physician at Harvard Medical School
Associate Member of the Broad Institute
Investigator for the Howard Hughes Medical Institute

Dr. Freedman's cross cutting work includes several multi-disciplinary efforts that continue to provide new insights into the genetic and epigenetic underpinnings of cancer.

Dr. Freedman was among the original faculty members at Dana-Farber's Center for Functional Cancer Epigenetics (CFCE). The CFCE is an integrative research center which explores the key role that epigenetic alterations and abnormal transcriptional regulation play in the development and progression of cancer. A better understanding of these alterations will lead to more accurate more timely cancer diagnoses and will expand the potential to contribute to the knowledge required for the development of new therapeutics exploiting epigenetic mechanisms. Dr. Freedman is also a founding member of Dana-Farber's Center for Cancer Genomics Discovery, another research center in which faculty use cutting edge technologies to identify mutations, copy number alterations, and epigenetic modifications in cancer genomes.

Employing powerful technologies such as Chromatin Immunoprecipitation followed by next-generation-sequencing (ChIP-seq), DNase hypersensitivity mapping, and gene expression profiling (RNA-Seq), Dr. Freedman continues to focus on overcoming the challenges presented by the discovery that the vast majority of cancer risk variants reside outside of our DNA's protein coding regions. In collaboration with other world-class faculty with expertise from a variety of disciplines including cell biology, physiology, cancer biology, human genetics, and computational biology, he leverages methods from these fields to identify the genes and pathways driving cancer risk.

The Freedman lab is currently at the forefront of the pursuit of expanding our understanding of the role of the androgen receptor (AR) across various prostate cancer "states": from normal tissue to tumor to advanced disease. This emerging field of study is a novel approach to the roles of AR, which is an undisputed factor in cancer development and progression. While pharmacologically turning off the androgen axis has been the mainstay of therapy for advanced prostate cancer for over six decades, relapse inevitably occurs. Therefore, new tools are needed to understand how tumors become resistant to therapy.

Dr. Freedman's lab has developed a method to perform AR ChIP-seq in human prostate tissue. This development is particularly significant because over 99% of ChIP-seq studies are performed in cell lines. However, cell lines almost certainly do not fully capture human biology and our early data confirm this notion. AR ChIP-seq in human tissue allows us to map the exact AR binding locations across the entire genome. Charting these locations in advanced disease states, and understanding which genes AR binding influences, will enable us to improve prostate cancer treatment. Using this novel method, Dr. Freedman has already made some important discoveries about the pathways driving prostate cancer. His lab is now working closely with clinicians to collect clinical samples that will provide insights into why some men ultimately fail anti-androgen therapy. Support from the H. L. Snyder Medical Foundation has enabled this critical work to rapidly move forward.

As a result of his innovative work, Dr. Freedman publishes prolifically in some of the nation's top peer-reviewed scientific journals. In the past year alone, he has published five major studies, including one in *Cell*. Additionally, some of his most ground-breaking work on *MYC*, a well-known cancer-causing gene, has recently been validated by other labs, and the paper detailing this discovery has been cited over 250 times. This work was also supported in part by the H. L. Snyder Medical Foundation.

It has become increasingly evident over the past several years that further investment in this field is vital to the future of cancer research. As such, Dana-Farber will be dedicating substantial resources to a Center for Germline Cancer Studies, of which Dr. Freedman will be the Director.

Dr. Freedman's work is critical to Dana-Farber's mission of eradicating cancer by focusing on the earliest events of cancer pathogenesis. He is one of the world's leaders in this area.

http://www.dfhce.harvard.edu/membership/profile/member/25/0/

Dr. Berl Oakley – University of Kansas

Irving S. Johnson Distinguished Professor
Department of Molecular Biosciences

Background: Tau is a protein that binds to microtubules in nerve cells. Microtubules are essential for axonal transport and tau stabilizes microtubules, facilitating their function. In Alzheimer's disease, tau begins to change its shape and aggregate into fibers. There is considerable evidence that aggregates or tangles of tau are important components of Alzheimer's pathology and there are a number of other neurological disorders associated with tau aggregation. Their goal is to find drugs that prevent the formation of tau fibers and aggregates or, in the best case, dissolve them. Fungi synthesize large numbers of chemicals called secondary metabolites. Many of them inhibit important biological processes in the organisms with which they compete in nature and this makes them excellent sources of compounds of medical value. They have found that the fungus *Aspergillus nidulans* produces a number of compounds that are members of, or related to, a class of compounds called anthraquinones, some of which inhibit tau aggregation and break down pre-formed aggregates *in vitro*. There are three labs involved in this effort. The Oakley lab at the University of Kansas is creating strains of *Aspergillus nidulans* that accumulate compounds predicted to inhibit tau aggregation. The lab of Dr. Clay Wang at the University of Southern California is identifying and purifying the compounds and the lab of Dr. Chris Gamblin at the University of Kansas is testing the purified compounds for their ability to inhibit tau aggregation or to disassemble tau aggregates.

Progress: So far they have purified and screened a large number of compounds produced by *Aspergillus nidulans* and have identified three compounds that have significant activity in inhibiting tau aggregation. This work has been submitted for publication (Paranjape et al., see below) and has been very positively reviewed. The reviewers suggested minor changes which they have made. It has been resubmitted and they expect that it will be accepted for publication shortly. One of the three most active compounds was a compound

discovered by the Oakley and Wang labs that they have named sperbenzaldehyde. This discovery was exciting because asperbenzaldehyde is structurally quite different from other tau aggregation inhibitors and thus is the founding member of a new class of compounds with activity against tau aggregates. The Wang lab has synthesized derivatives of asperbenzaldehyde and demonstrated that they inhibit lipoxygenases. At least one lipoxygenase is increased in patients with Alzheimer's disease and a lipoxygenase inhibitor has been shown to reduce memory impairment and reduce tau aggregates in a mouse Alzheimer's model. This gave them some hope that some of their asperbenzaldhyde derivatives might be doubly useful, inhibiting tau aggregation and inhibiting lipoxygenase activity. The experiments are ongoing but they have obtained strong evidence that some of the asperbenzaldehyde derivatives can cause disassembly of preformed tau aggregates. This is exciting because one would ultimately like to be able to disassemble tau aggregates in patients' brains.

Goals for the coming months:
1. They have purified 29 additional *Aspergillus nidulans* secondary metabolites and we plan to screen them for inhibition of tau aggregation.
2. They will work with Prof. Tom Prisinzano, of the KU Department of Medicinal Chemistry, to synthesize new asperbenzaldehyde derivatives and they will screen the compounds for activity in inhibiting tau aggregation and disassembling pre-formed tau aggregates.

Publications supported in part by the H. L. Snyder Medical Foundation:

- Chiang, Y. M., Oakley, C. E., Ahuja, M., Entwistle, R., Schultz, A., Chang, S. L., Sung, C. T., Wang, C. C. C. and Oakley, B. R. (2013). An efficient system for heterologous expression of secondary metabolite genes in *Aspergillus nidulans*. J. Am. Chem. Soc. 135, 7720-7731.

- Yaegashi, J., Praseuth, M. B., Tyan, S. W., Sanchez, J. F., Entwistle, R., Chiang, Y. M., Oakley, B. R. and Wang, C. C. C. (2013). Molecular Genetic Characterization of the Biosynthesis Cluster of a Prenylated Isoindolinone Alkaloid Aspernidine A in *Aspergillus nidulans*. Org. Lett. 15, 2862-2865.

The following manuscript supported by the Snyder Foundation has been submitted and very favorably reviewed. We anticipate that it will be accepted shortly.

- Paranjape, S. R., Chiang, Y.-M., Sanchez, J. F., Entwistle, R., Wang, C. C. C., Oakley, B. R. and Gamblin, T. C. Inhibition of tau aggregation by three *Aspergillus nidulans* secondary metabolites: 2,ω- dihydroxyemodin, asperthecin and asperbenzaldehyde. Submitted to Planta Medica.

http://www2.ku.edu/~mb/cgi-bin/viewprofile.shtml?id=27