HARVARD CATALYST

THE HARVARD CLINICAL AND TRANSLATIONAL SCIENCE CENTER

Advanced Microscopy – Zooming in on the Big Idea in Pathology

Funded Projects

In this initiative, the Harvard Catalyst Reactor Program invited investigators to submit innovative pilot grant applications that utilized the advanced Zeiss microscopes at the Harvard Center for Biological Imaging (HCBI), with a focus on advancing an area of clinical investigation centered on pathology. Applications were required to (1) address areas of pathology that cannot be addressed using current technology applied in clinical practice or (2) provide substantial improvements on current practice. The awarded projects center on the development of new diagnostics and biomarkers or provide new insight into the pathophysiology of disease.

This funding opportunity was open only to investigators who attended a training/educational event at HCBI or met with HCBI personnel.

11 pilot grants were awarded in amounts of up to $40,000 for each one-year project.

Funding decisions for the advanced microscopy pilot grants were announced in February 2014.

Novel Mechanism of Atherosclerotic Plaque Rupture: Zooming in on the Genesis of Microcalcifications

Principal Investigator: Elena Aikawa, MD, PhD, Brigham and Women's Hospital

Co-Investigators: Joshua D. Hutcheson, PhD, Brigham and Women's Hospital
Frederick J. Schoen, MD, PhD, Brigham and Women's Hospital

Rupture of vulnerable atherosclerotic plaques is the leading cause of myocardial infarct and stroke. Classically, atherosclerotic plaque vulnerability has been attributed to a reduction of collagen in the fibrous cap; however, recent studies have identified microcalcifications in the collagenous fibrous cap that contribute to biomechanical plaque failure. Calcifying matrix vesicles released by cells (e.g., smooth muscle cells, macrophages) within the plaque contribute to the formation of microcalcifications, but the mechanisms of microcalcification genesis are unknown. A major limitation in the field, which hinders current clinical practice and research progress, is the inability to identify and visualize the early processes that lead to microcalcifications. To overcome this limitation, we will use super-resolution imaging available at the HCBI along with a near-infrared calcium tracer to visualize the nucleation of microcalcifications within our recently developed controllable three-dimensional collagen hydrogel system that recapitulates the fibrous cap. We will then extend these analyses to samples of human atherosclerotic plaques to connect our in vitro model findings to pathological in vivo processes. Overall, this approach will address areas of pathology that cannot be addressed using current technologies applied in clinical practice and provide new insights into the pathobiology of plaque rupture. The long-term goal of the project is to develop a simple imaging tool that can be used by pathologists to diagnose rupture prone plaques.

Systematic Analysis of Breast Neoplasia with Lightsheet Microscopy

Principal Investigator: Andrew Beck, MD, PhD, Beth Israel Deaconess Medical Center

Co-Investigators: Nicholas Knoblauch, Beth Israel Deaconess Medical Center
Doug Richardson, PhD, Harvard Center for Biological Imaging
Stuart Schnitt, MD, Beth Israel Deaconess Medical Center
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Breast cancer is a leading cause of cancer death among women. The current system for diagnosing breast cancer based on the pathological analysis of microscopic images has changed little over the past several decades. Recently, a new state-of-the-art microscopy platform (fluorescent lightsheet microscopy) has been developed, which enables the rapid acquisition of high-resolution three-dimensional (3D) images directly from tissue samples up to several millimeters in thickness. The primary aim of our project is to determine the ability to perform fluorescent lightsheet microscopy directly on archival patient tissue samples and to use the method to identify 3D morphological hallmarks of breast carcinogenesis. To achieve these goals, we will perform lightsheet microscopic analysis on a total of 175 breast tissue samples, including: normal breast, usual ductal hyperplasia, atypical ductal hyperplasia, ductal carcinoma in situ, and invasive ductal carcinoma. We will develop and implement machine learning-based image analysis procedures to measure quantitative 3D morphologic phenotypes from the images, and we will perform statistical analyses to identify lightsheet microscopy-derived features significantly associated with progression of breast neoplasia from normal breast to intra-ductal proliferative lesions to invasive breast cancer. If successful, this project will lay the ground work for future larger scale studies to use lightsheet microscopy to identify novel breast

tissue-based 3D quantitative morphologic phenotypes to predict future breast cancer risk (in the setting of benign and pre-invasive breast lesions) and to predict patient survival (in the setting of invasive breast cancer).

Zooming in on the Big "Eye-dea" in Retinopathy: Live Imaging Ocular Angiogenesis

Principal Investigator: Katie Bentley, PhD, Beth Israel Deaconess Medical Center

Co-Investigator: Claudia Prahst, PhD, Beth Israel Deaconess Medical Center

Retinopathies, which often cause blindness and are characterized by abnormal growth of excessive, leaky and bulbous new blood vessels, include 1) retinopathy of prematurity in premature infants 2) diabetic retinopathy, the main cause of blindness in working-aged Americans and 3) age-related macular degeneration, the most common cause of sight loss in older people. To date, it is not known why the new blood vessels grow in an abnormal manner. During development, blood vessels grow in a well-defined process generating a regularly connected, stable network. In order to develop new drugs against these debilitating diseases, the reason for the disrupted new vessel growth has to be better understood.

The growth of new blood vessels is fundamentally a three-dimensional problem, where cells move and connect throughout the tissue to develop an extensive tubular network. To date no one has imaged this process in its full three dimensions throughout the different layers of eye tissue, as deep imaging techniques were previously unavailable. By comparing diseased and normal tissues using light sheet microscopy, this study will help unravel how and why vessel growth degenerates in retinopathy conditions and provide a new testbed to investigate the effects of new therapeutic targets. We call this observation method the big Eye-dea (Dimensional Environment Analysis) as we for the first time will preserve the real three-**D**imensional and normal/diseased tissue **E**nvironment of the eye while **A**nalyzing changes in vessel growth with state-of-the-art computational techniques.

Human Focal Cortical Dysplasia: A Brainbow Connectomics Approach

Principal Investigator: David Clapham, MD, PhD, Boston Children’s Hospital

Co-Investigators: Shu-Hsien Sheu, MD, PhD, MPH, Beth Israel Deaconess Center

Focal cortical dysplasia (FCD) is a common pathology finding in medically refractory seizure foci. Microscopically, it is characterized by disrupted cortical layering, dysmorphic neurons, and balloon cells. However, how these changes contribute to the initiation of seizures remains obscure. Two of the main limitations of our current pathological evaluations are: 1. Lack of structural data on the neuronal circuitry. For example, we do not know if the circuitry is altered when cortical lamination is abnormal. We also do not know if dysmorphic neurons receive or send out aberrant connections. 2. The scarcity of pathological studies on inhibitory interneuronal populations, which modulate the activity of pyramidal neurons and may play a critical role in epileptogenesis. Indeed, a class of inhibitory neurons — the parvalbumin-positive (PV+) interneurons, has been linked to seizures in animal models. However, how well these data extrapolate to human disease is not known. We propose a novel way to analyze the PV+ interneuron circuitry in humans to test the hypothesis that the inhibitory circuitry is altered in FCD. We sought to transfect human FCD surgical samples with Brainbow adeno-associated viral vectors. This approach would label the neurons in a wide variety of colors, enabling reconstruction of dendritic and axonal arbors. The advanced microscope at HCBI will greatly

facilitate the imaging of these multicolor samples. This will mark the very first time for a type of human cortical neurons to be analyzed in a near-saturated manner, and the resulting data will provide the first structural evidence for alterations in the inhibitory circuitry in epilepsy.

Advancing from Two to Three Dimensions in Digital Pathology

Principal Investigator: Beverly Faulkner-Jones, MD, PhD, Beth Israel Deaconess Medical Center

Co-Investigator: Charles Law, PhD, Kitware, Inc.

Pathologists render a diagnosis by reading thin tissue sections mounted on glass slides. An ability to infer three-dimensional (3D) structure from two-dimensional (2D) tissue sections is crucial for diagnosis and for full characterization of many disease processes, but this depends on the skill and experience of the individual pathologist. With advances in digital methods, it is now feasible to have a new workflow in which pathologists are presented with aligned whole slide images (WSIs) of serial sections. The expectation is for faster evaluation, greater objectivity, and improved diagnostic accuracy. A limitation of current slide scanning systems is that they are designed primarily for acquiring and viewing 2D WSIs. Thus we have two major aims in this project: 1) validate the usefulness of the 3D approach, and 2) develop and deploy web-based tools for aligning and viewing the WSI datasets. Our application will be 3D analysis of needle core renal biopsies. Our current protocol already calls for serially sectioning through a renal needle core biopsy. Here we will generate multiple WSI image datasets that we can use for direct comparison with the current approach of viewing glass slides on conventional microscopes. Our web-based tools will be integrated into an open-source digital pathology platform that we have developed and use for routine teaching of residents. Ultimately our goal is to have tools that are automated and easy to use so that they can be used routinely in clinical work.

Studying Gastric Cancer Pathogenesis using Correlative High-Resolution Microscopy and Genomics

Principal Investigator: Susan Hagen, PhD, Beth Israel Deaconess Medical Center

Co-Investigators: Lay-Hong Ang, PhD, Beth Israel Deaconess Medical Center
Yi Zheng, PhD, Beth Israel Deaconess Medical Center

Although gastric cancer continues to be a global health issue, the management of gastric cancer remains limited. Novel therapeutic approaches to address this increasingly large unmet need necessitate the discovery of molecular targets that regulate each step in the gastric cancer (GC) cascade, which also represents an opportunity to identify biomarkers for early detection and treatment. Claudin (cldn)18 is the most highly expressed tight junction protein in human stomach, is down-regulated (no expression) in more than 80% of GCs with an intestinal phenotype, is down-regulated early in GC progression, and its attenuation is related to poor survival in advanced GC patients. Because little is known about cldn18, we aim to ascertain how the structural and molecular profile of gastric epithelial cells is affected by the attenuation of cldn18. For this, equipment in the Harvard Center for Biological Imaging (HCBI) will be used to determine, in a mouse GC model, the three-dimensional high resolution pattern of cldn18 expression in stomach from archived paraffin sections. Additionally, archived tissues from control and cldn18 knockout mice will be subjected to laser capture techniques and used to elucidate transcriptional dynamics and gene interactions that are correlated to cldn18. Strong

preliminary data from an experienced team support the feasibility of this project on instruments in the HCBI that are not available otherwise. These studies should reveal important insights into the role of cldn18 in GC pathogenesis that can be translated to early biomarker analysis in human disease.

Polycystic Kidney Disease: Visualizing Signals that Drive Cyst Initiation in 3D

Principal Investigator: Aldebaran Hofer, PhD, Veterans Affairs Boston Healthcare System

Co-Investigators: Barbara Ehrlich, PhD, Yale University
Jason Y. Jiang, PhD, Brigham and Women's Hospital
Ivana Kuo, PhD, Yale University

Autosomal dominant polycystic kidney disease (ADPKD) is a progressive disease of renal and hepatic dysfunction marked by cyst formation and organ enlargement. It is caused by mutations in two proteins, PKD1 and PKD2, that are linked to aberrations in Ca2+ signaling and loss of endoplasmic reticulum (ER) Ca2+ stores. However, the fluid secretion and cellular proliferation central to renal cyst formation depends largely on another second messenger, cyclic AMP (cAMP). How Ca2+ and cAMP signals are related to one another and how they drive the very first events during the formation of the nascent cyst is unknown. This is largely due to technical limitations that have precluded high resolution, long-term imaging of these signaling molecules concurrent with the relatively rare event of cyst formation in multi-cellular models of PKD. Here we propose to test a daring new hypothesis that may explain how loss of ER Ca2+ homeostasis in PKD mutants is linked to elevated cAMP levels, cyst formation, and epithelial cell proliferation. We will take advantage of the Zeiss Lightsheet Z.1 microscope in the HCBI core, new improved fluorescent sensors for cAMP, and 3D organ cultures to evaluate the signaling properties of individual cells lining the cysts. These methodologies will allow us to interrogate in 3D the signaling events taking place deep within the tissue over many hours. Our goal is to identify the early steps that drive cyst formation and to develop a platform for future evaluation of this process in human PKD disease variants.

Highly Multiplexed FISH for in situ Genomics

Principal Investigator: Anthony Iafrate, MD, PhD, Massachusetts General Hospital

Co-Investigator: Maristela Onozato, MD, PhD, Massachusetts General Hospital

We are entering the era where chemotherapies are chosen to precisely match the unique genetic alterations in each patient's tumor. New genomic technologies, such as next generation sequencing (NGS), are central to this approach. Our experience has been that NSG is currently inferior to Fluorescence in situ hybridization (FISH) with regard to assessing copy number. With the current FISH techniques being limited to analyzing one gene (or a few genes) at a time and the demands for complete genome analysis becoming clinically necessary, a highly-multiplexed FISH approach coupled with high throughput image capture and analysis platforms would be extremely attractive and could provide an optimal copy number screen in routine molecular pathology practice. The ability to assess accurately highly-multiplexed FISH probe sets requires imaging resources that the Harvard Catalyst grant mechanism can help provide.

Aim I of this proposal is to develop and test image acquisition and analysis platforms that enable the automated copy number analysis of a pilot panel of 30 genes simultaneously, each gene bar-coded with two or three fluorophores simultaneously hybridized on one slide of formalin-

fixed paraffin-embedded tumor biopsy sample. Aim 2 will be to validate the clinical utility of the algorithm by exploring tumor heterogeneity in primary human brain tumors (glioblastoma multiforme cases), a tumor with known multiple copy number alterations. The ultimate goal of this proposal is to provide a comprehensive genotype assay that will guide the choice of targeted therapies and that will impact how we identify, diagnose, and alter the clinical course of cancers.

Disease-specific Cellular Features in Schizophrenia

Principal Investigator: Rakesh Karmacharya, MD, PhD, Massachusetts General Hospital

Co-Investigators: Shaunna Berkovitch, PhD, Massachusetts General Hospital
Doug Richardson, PhD, Harvard Center for Biological Imaging

Pathological examinations of postmortem schizophrenia brain tissue show well-replicated but subtle differences in specific neuronal populations in the brain's pyramidal neurons in cortical layer III, but not in other cortical layers, show decreased soma size, shorter dendritic length, and decreased spine density. While these morphological differences are hypothesized to be related to disease biology, postmortem studies are confounded by exposure to antipsychotic medications as well as the chronic effects of the disease on the brain. Investigations of schizophrenia at the cellular level have been hindered by the inability to study disease-related cellular deficits in vitro. Recent advances that enable reprogramming of human-induced pluripotent stem cells (iPSCs) provide a way to generate patient-derived neuronal cells and develop in vitro cellular disease models. We are reprogramming iPSCs from schizophrenia patients and matched controls and are differentiating iPSCs to specific neuronal subtypes in order to delineate disease-specific features in in vitro assays. However, dendrites and dendritic spines are too small to resolve with a standard light microscope. Hence, to detect differences between schizophrenia and control neurons on a nanometer scale, we propose to use super-resolution light microscopy. We believe that these studies will help develop image-based cellular assays using patient-derived cells that can be used to develop clinically-relevant disease biomarkers. These studies will also will lay the groundwork for us to identify disease "signatures" that can subsequently be used for high-throughput screens to discover small molecules and that modulate disease-specific features.

Super-resolution Microscopy as a Novel Diagnostic Approach in Renal Pathology

Principal Investigator: Astrid Weins, MD, PhD, Brigham and Women's Hospital

In contrast to most other pathology subspecialties, in which light microscopic studies form the basis of a diagnostic work-up, ultrastructural examination by electron microscopy and immunofluorescence microscopy are indispensable tools in kidney pathology. Particularly injury to the nanometric structure of the kidney filter, the morphologic hallmark of proteinuria, to date can only be definitively confirmed using electron microscopy. We identified B7-1, a T cell costimulatory molecule, as a novel biomarker in the glomeruli of a subgroup of patients with nephrotic syndrome. B7-1 is targeted by a specific inhibitor, Abatacept, which reduced proteinuria in patients suffering from therapy-resistant or recurrent nephrotic syndrome. This breakthrough finding poses new diagnostic challenges to the field. In a subset of human biopsies from patients with proteinuric kidney disease, we observe disparate B7-1 staining patterns in the glomerulus, which vary by disease. This suggests differences in localization of B7-1 within the kidney filter as well as different pathogenic pathways, which cannot be resolved using conventional immunofluorescence microscopy methods. Therefore, this exposes a need

for high-resolution immunofluorescence imaging. This proposal outlines the use of super-resolution microscopy on fresh frozen human kidney biopsies positive for B7-1, aimed at discerning the exact localization of B7-1 in the glomerulus. Results from this study will refine our understanding of the role of B7-1 in mediating proteinuria and contribute to a new diagnostic classification of proteinuric kidney diseases, with the ultimate goal to streamline our therapeutic efforts in patients suffering from resistant and thus difficult-to-treat kidney diseases.

Visualizing Hematopoietic Stem Cell Engraftment in its Endogenous Niche

Principal Investigator: Leonard Zon, MD, Boston Children's Hospital

Co-Investigators: Bradley Blaser, MD, PhD, Dana-Farber Cancer Institute
Friedrich Kapp, MD, Harvard Faculty of Arts and Sciences, Harvard University
Owen Tamplin, PhD, Boston Children's Hospital

The entire blood system is supported throughout life by a small number of cells called Hematopoietic Stem Cells (HSC). These blood stem cells are only produced during a brief window of development in the embryo before they populate the bone marrow and persist throughout adulthood. Hematologic malignancies, such as leukemia, are caused by malignant transformation of HSC. A curative treatment for these blood diseases is HSC transplantation (HSCT). This involves elimination of patients' bone marrow by chemotherapy or radiotherapy, followed by replacement transfusion with healthy HSC or bone marrow. Although clinical HSCT has improved significantly, the majority of patients are not cured by this treatment. After transfusion HSC enter patients' circulation and bone marrow, which is essentially a "black box" where they cannot be observed. To improve on current HSCT protocols, there is a need to directly track and image HSC as they engraft in a new site of hematopoiesis or "niche". We propose developing such a system using the zebrafish — a well-established model organism with HSC that are very similar to humans. In fact, one potential drug for improving HSCT, prostaglandin E2, was already identified using the zebrafish and has rapidly moved into clinical trials. We will use transparent zebrafish, and the advanced microscopes at the Harvard Center for Biological Imaging, to directly observe the interactions between HSC and their niche during engraftment. We believe this project will increase our understanding of HSC engraftment and have potential to improve clinical HSCT.