U54 Urology O'Brien Center Summaries

NIH-NIDDK CAIRIBU

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

CAIRIBU George M. O'Brien U54 Urology Cooperative Research Centers Program

University of Wisconsin-Madison George M. O'Brien Center

2014-2019 and 2019-2024 - PI, Will Ricke, PhD

Cellular and Molecular Mediators of Fibrosis in the Development of Urinary Tract Dysfunction

PROJECT SUMMARY. The overarching goal of the UW-Madison O'Brien Center for Benign Urology Research is to identify mechanisms that result in lower urinary tract dysfunction (LUTD) that result in benign prostatic hyperplasia (BPH) related lower urinary tract symptoms (LUTS). Criteria for successful completion are defined by the RFA 18-029 and include performing and disseminating outstanding benign urologic research, provide highly needed resources for the field, and provide outstanding educational enrichment while promoting the next generation of benign urology researchers. The Center targets new and exciting mechanisms of LUTD namely prostate fibrosis and translates it to clinically relevant therapeutics and biomarkers for patient stratification. BPH/LUTS can be life-threatening, affect quality of life, and is a costly disease, which NIDDK wants eradicate.

Will Ricke, PhD, PI

No consequential advances in medical treatment of prostate-related lower urinary tract symptoms (LUTS) have emerged in decades. Existing medical therapies improve LUTS but robustness of these effects are marginal. Not all men respond to existing therapies, some respond with adverse effects requiring discontinuation of therapy, and most experience a progressive worsening of symptoms pursuant to initial relief. Multiple mechanisms drive development and progression of prostate-related LUTS. The overarching goal of the O'Brien Center for Benign Urology Research is to identify mechanisms that result in lower urinary tract dysfunction and prostate-related LUTS. The overarching hypothesis of the center is that fibrosis is a cause of male LUTS. In contrast to benign prostatic enlargement and smooth muscle dysfunction, prostatic fibrosis remains untargeted by existing therapies. In order to advance the scientific understanding and medical management of prostatic fibrosis, it will be necessary to: (1) identify cellular and molecular mediators of fibrosis and therapeutically- susceptible pathways using clinical specimens, (2) develop and validate preclinical mouse models of prostatic fibrosis and strategies for granular assessment of voiding function, (3) test new therapies in these preclinical models with the long term goal of treating fibrosis in men, and (4) develop new non-invasive radiologic imaging strategies with the long-term goal of diagnosing prostatic fibrosis in men. Two additional goals will advance the urologic research community: (1) develop and publicly disseminate resources to increase research efficiency, reproducibility, and rigor, and (2) cultivate an outstanding educational enrichment program to attract and retain young basic- and physician-scientists into the benign urologic research field. The Center will apply state of the art molecular and histological methods to visualize and characterize fibrosis in a range of human and animal prostatic tissues and examine how prostatic fibrosis develops, progresses, and responds to treatment. Interactions and engagement with the O'Brien Centers' Interaction Core, the UW O'Brien Centers Website, and GUDMAP will accelerate the dissemination of data, software, methods, and tissue resources to the greater biomedical community. The leadership and experience within the Center will allow for the promotion of interactions among Center Projects, the Biomedical Research Core, and other Centers (U54, P20, K12) through communication, collaboration, and coordination. The larger vision is that O'Brien Centers will be a nidus for ideas, research, resources, training, and a unified voice across the urologic research community. To realize this vision, the Centers must become more than the sum of their parts. The UW O'Brien Center and its affiliates

will contribute to this synergism by leveraging existing Center assets and relationships, conducting rigorous investigation, fostering teaching and learning, and through vigorous pursuit of innovation. With the solid financial support and "buy-in" from UW and its affiliates, Core A will lead this vision for this O'Brien Center.

PROJECT 1: ESTROGENS STIMULATE PROSTATIC COLLAGEN SYNTHESIS TO DRIVE FIBROSIS AND LUTD. Healthcare costs for lower urinary tract symptoms (LUTS) ascribed to benign prostatic hyperplasia (BPH) are in the billions of dollars annually. Therapies for BPH/LUTS target smooth muscle contractility with α-blockers or hyperplasia with 5α-reductase inhibitors. Although these therapies can be medicinal, they are not effective/durable for all; this leaves millions of men in the US needing more effective therapies. The standard of medical care for BPH/LUTS currently over-treats this patient population, in part due to a poor understanding of etiology and progression. There is an apparent need to define what BPH represents in patient populations as well as to identify the true anatomic, cellular, and molecular causes of the disease. This may elucidate the true causes in development and progression of the disease as well as institute effective therapies. The overarching goal of the O'Brien Center for Benign Urology Research is to identify the mechanisms that result in lower urinary tract dysfunction and prostate-related LUTS. Previous studies have demonstrated prostatic collagen deposition coincident with prostate stiffness, LUTS, and failed medical treatment supporting the concept that BPH/LUTS is, in part, a fibrotic disease. However, this brings up a translational challenge because treatment of prostatic fibrosis cannot occur until cellular and molecular pathways have been identified. As such, the goal is to identify the anatomical, cellular, and molecular origins of prostate fibrosis in men with BPH/LUTS. Recently, estrogens, specifically signaling through estrogen receptor (ER)α, was discovered to be necessary for the development of prostatic fibrosis and LUTD in mice. Although, multiple stromal and epithelial cells express ERα, a subpopulation of ERα positive prostatic fibroblasts and/or smooth muscle cells could be responsible for increased collagen deposition. These cells are sensitive to estrogens and produce large amounts of collagen in vitro and in vivo. Aim 1 will address the ER molecular mechanism of action in the transcription of Col1a1 by determining if classical or non-classical ER signaling is necessary. Next, collagen accumulation has been linked with BPH/LUTS, but it is uncertain if collagen/fibrosis acts independently or in collaboration with prostate hyperplasia; Aim 2 will test the hypothesis that gain of collagen function promotes LUTD independent of prostate hyperplasia. Clinical translation of our findings is a goal of the center; Aim 3 will test the hypothesis that clinically relevant antifibrotics are effective in the treatment of prostatic fibrosis. Lastly, stratification of men with fibrotic prostates is imperative to increase treatment efficacy. To address this challenge, advanced and novel collagen MR imaging techniques will be used to assess whether prostatic fibrosis can be identified in preclinical models. By establishing cellular and molecular mechanistic connections between fibrosis and BPH/LUTS as well as preclinical testing and patient stratification our collaborative and synergistic research, Project 1 will lay the groundwork for impactful discoveries that elucidate an important etiology of BPH/LUTS and may ultimately translate into the clinic.

PROJECT 2: CTGF DRIVES VOIDING DYSFUNCTION THROUGH EXPRESSION OF COLLAGEN IN PERIURETHRAL SRD5A2+ FIBROBLASTS. Lower urinary tract symptoms cost more than $4 billion annually. Though current medical therapies reduce prostate volume and relax smooth muscle to address symptoms, existing therapies are not curative. Three things are clear: (1) male lower urinary tract symptoms derive from multiple underlying pathologies, not just prostatic enlargement or muscle dysfunction (2) current therapies do not effectively target pathologies outside of benign enlargement and smooth muscle dysfunction, and (3) there is a need to identify additional mechanisms underlying lower urinary tract symptom etiology to formulate therapies that are more effective. The overarching goal of the O'Brien Center for Benign Urology Research is to identify mechanisms that result in lower urinary tract dysfunction and prostate-related lower urinary tract symptoms (LUTS). Prostatic collagen accumulation (fibrosis) has been identified as a cause of male lower urinary tract symptoms. Prostatic collagen accumulation has been linked to prostatic stiffness, lower urinary tract symptoms, and failed medical treatment. It will not be possible to treat prostatic fibrosis and associated voiding

dysfunction until prostatic collagen-producing cells are identified. The goal of this project is to tackle this challenge by pinpointing the cellular and molecular origins of pathological collagen production in the prostate. A subpopulation of human prostatic fibroblasts residing in close proximity to the urethra and expressing steroid five alpha reductase type II (SRD5A2) has been identified, supporting the central hypothesis that inflammation causes these fibroblasts to proliferate, synthesize connective tissue growth factor (CTGF) and produce collagen. Collagen accumulation in turn leads to urethral stiffening, bladder outlet obstruction, urinary retention, and voiding dysfunction. The proposed studies offers essential insight into the pathogenesis of prostatic fibrosis, a mechanism of lower urinary tract symptom medical therapy failure. Aim 1 will test the hypothesis that inflammation increases human prostatic SRD5A2+ fibroblast frequency and drives CTGF and COL1A2 expression. Aim 2 tests whether inflammation increases frequency of mouse prostatic Srd5a2+ fibroblasts and whether depletion of these fibroblasts resolves inflammation-mediated collagen accumulation and voiding dysfunction. Aim 3 will test whether CTGF overexpression is sufficient to drive mouse prostatic collagen accumulation and voiding dysfunction and whether an investigational new CTGF blocking drug resolves the problems. The proposed studies will pinpoint CTGF expression in SRD5A2+ fibroblasts as a therapeutic target for treating lower urinary tract symptoms. By establishing mechanistic connections between inflammation, CTGF and COL1A2 abundance, and urinary dysfunction, the studies launch an original line of research into a disease process that is yet-to-be leveraged as a target for medical therapies addressing lower urinary tract symptoms.

PROJECT 3: PERSISTENCE OF AN IL-4/IL-13 AUTOCRINE LOOP PROMOTES FIBROSIS-MEDIATED URINARY VOIDING DYSFUNCTION. Lower urinary tract symptoms (LUTS) are a costly and potentially critical medical problem for millions of aging men. This spectrum disorder encompasses symptoms such as weak stream, nocturia, incomplete emptying and intermittent urination, all of which are indicative of lower urinary tract dysfunction (LUTD). Surgical ablation of prostate tissue and medical approaches may improve urinary flow, but such treatments are not effective for all men, can produce adverse effects that result in discontinuation of the therapeutic regimen, and do not abrogate the risk for disease progression. If left untreated or treated ineffectively, LUTD can progress to bladder dysfunction, which can lead to urinary retention, recurrent UTI, bladder calculi, and, eventually, renal impairment. Work accomplished by the Macoska laboratory and this Center have shown that collagen accumulation around the prostatic urethra consistent with tissue fibrosis is an untreated pathobiology contributing to LUTD. The overarching goal of the O'Brien Center for Benign Urology Research is to identify mechanisms that result in lower urinary tract dysfunction and prostate-related lower urinary tract symptoms (LUTS). New evidence (presented in this application) indicates heterogeneity among peri-urethral collagen-producing cells as well as inflammatory cells within the prostatic microenvironment. Inflammatory cells secrete a medley of pro-fibrotic proteins into the prostatic microenvironment. Among these proteins, IL-4 and IL-13 are of particular interest because they share a common signaling axis which, as shown here for the first time, establishes and perpetuates an autocrine loop that activates JAK/STAT signaling to promote the expression and maintenance of IL-4, IL-13, their cognate receptors, regulatory transcription factors, and ECM components, even in the absence of inflammatory cells. Based on preliminary data presented here we hypothesize that some peri-urethral stromal cell populations establish an IL-4/IL-13 axis that self-perpetuates, induces myofibroblast phenoconversion and survival, and upregulates collagen accumulation, thereby promoting consequent urinary voiding dysfunction. To test this hypothesis, Project 3 will: 1) Determine whether signaling through the IL-4 receptor creates a STAT6- and GATA-3-mediated positive feedback loop; 2) Elucidate the mechanisms through which the IL-4/IL-13 axis promotes myofibroblast phenoconversion and concordant collagen expression; 3) Determine whether IL-4 pathologically represses myofibroblast apoptosis and thereby promotes myofibroblast survival, and 4) Test whether the IL-4/IL-13 signaling axis promotes lower urinary tract fibrosis and urinary voiding dysfunction in vivo. The results of these preclinical studies will elucidate previously unknown interleukin-mediated molecular and cellular mechanisms that promote lower urinary tract fibrosis and dysfunction. Using JAK/STAT

inhibitors to therapeutically target this potentially self-perpetuating mechanism may 'break the cycle' and successfully treat recalcitrant peri-urethral prostatic fibrosis contributing to LUTD development and progression.

ADMINISTRATIVE CORE. Core A will achieve these goals by providing outstanding leadership, vision, and efficiency in the overarching administrative duties. The organization structure and leadership of Core A includes two outstanding investigators with recognized and complementary abilities in leading research groups and training programs. **Dr. Ricke** continues to serve as Core A director and will assume primary responsibility for day to day management and oversight of Core A. He will also be responsible for obtaining and managing the Opportunity Pool and maintain extensive interactions within the biomedical community. **Dr. Vezina** will serve as Associate Director for Core A and will direct the Educational Enrichment Program. Core A will interact with members of its external advisory board (EAB) and internal advisory board (IAB) on a semi-annual basis. All members or associated members of the center will be invited to partake in center functions including seminars, retreats, business meetings, and other special events. Drs. Ricke and Vezina meet with the NIDDK Executive Steering committee (ESC) and External Expert Panel (EEP) at NIDDK's annual reverse site visit (see letter of reference Mark Nelson, PhD, ESC Chair). Their leadership and experience will allow us to promote interactions between our Center Projects, Core B, as well as other centers (U54, P20, K12) through: communication, collaboration, and coordination. Further interactions and data dissemination will occur in conjunction with the NIDDK's O'Brien Center Interaction core, NIDDK program officials, American Urological Association Office of Research, scientific societies, and other venues. As directed by NIDDK, the benign urology research community has a viable focal point--The O'Brien Centers--in which to centralize ideas, research, resources, training, provide consensus, and offer a unified voice. The O'Brien Centers are more than the sum of parts, rather they provide leadership, synergize with researchers, and provide to the urology community above and beyond serving one's own Center. Core A will lead this NIDDK shared vision.

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Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

CAIRIBU George M. O'Brien U54 Urology Cooperative Research Centers Program

Columbia University George M. O'Brien Center

2014-2019 and 2020-2025 – Co-PIs, Cathy Mendelsohn, PhD; Ali Gharavi, MD; Jonathan Barasch, MD, PhD; and Anne-Catrin Uhlemann, MD, PhD

Investigating the Genetic, Cellular, and Metabolic Events Important for Urothelial Homeostasis and Response to Injury

Cathy Mendelsohn, PhD

Ali Gharavi, MD

PROJECT SUMMARY. The Columbia University George M. O'Brien Urology Cooperative Research Center is made up of Columbia's leading Urologists, Microbiologists, Geneticists, Developmental and Cell Biologists dedicated to solving the central problems of Benign Urology. Our focus builds on the work conducted in prior cycles and proposes to identify solutions to clinical diseases at the juncture between clinical urology, epithelial biology and microbiology. Our faculty include the leading clinical and scientific minds at Columbia, including Chairs and Professors of Urology, Nephrology, Pathology & Cell Biology, Genetics & Development and Medicine. Together our faculty provide the expertise to identify the root causes of urologic disability in three scientific groups. **Dr. Gharavi** has identified major risk loci for vesiculoureteral reflux and is now correlating developmental phenotypes with both changes in the urinary microbiome and lower urinary tract symptoms. Dr. Gharavi's tools include large datasets such as the Lower Urinary Tract Symptoms Research Network (LURN), the UK Biobank and the eMERGE consortium. This effort establishes the field of personalized genomics in benign urology. **Dr. Mendelsohn** evaluates signaling pathways downstream of Pparg, a nuclear receptor that turns out to be a major regulator of cell type specific differentiation in the urothelium, as well as the inflammatory response to injury and infection. The work identifies an off-the-shelf drug that can be a potential treatment for urothelial repair. **Dr. Barasch** focuses on epithelial metabolism identifying a central mechanism of defense against UTI called "nutritional immunity". He discovered NGAL a protein that blocks iron capture by bacteria, and now has identified a highly active pathway of heme metabolism that produce CO gas. His tools include novel methods of RNA isolation from small amounts of cells, novel probes and chelators of CO, of heme and iron and reporter bacteria and mice. **Dr. Uhlemann** focuses on the evolutionary basis of drug-resistant microorganisms deciphering their molecular mechanisms of virulence. The Microbial Genomics Biomedical Core not only directs all microbiological studies in the O'Brien but also serves as a national resource for microbiome and metagenomic analyses and as a biorepository for drug- resistant UTI isolates. The excitement of our group is encapsulated in the interactions of each component of research from gene discovery to therapeutic applications including both human and mouse models. In this new cycle, we will continue to contribute to urological sciences by generating new genomics datasets (to be shared on dbGAP and GEO), gene lists, animal models (deposited at JAX), bacterial gene editing plasmids and reagents that can be shared with the urology community. We will continue to collaborate with urology experts in Wisconsin, Missouri, Maryland. We will fund new Opportunity Pool recipients, building on the roster of 6 new investigators already funded; and continue to train the next generation of investigators, building on the success of the nearly 90 students who have trained and who have published, received national awards and who are now Urologists and medical doctors.

PROJECT 1: HUMAN GENETIC APPROACHES TO LOWER URINARY TRACT PHENOTYPES. Genomic technologies such as exome sequencing and GWAS have not been systematically applied for most benign urological phenotypes prior to our work at the Columbia O'Brien Urology Research Center. We have already successfully applied genome-wide association study (GWAS) and rare copy-number variant analysis to identify novel genes and loci associated with vesicoureteral reflux (VUR). Here we propose to perform exome sequence of VUR patients to identify diagnostic rare single nucleotide variants and perform an exploratory VUR whole exome association study. In collaboration with the Microbial Genomics Biomedical Core we will conduct 16S rRNA sequencing from urine samples of a set of these patients, with both VUR and urinary tract infections (UTI) to generate urinary microbiota profiles. We will then and analyze microbiota profile associations with phenotypic variants and outcomes and with rare genetic variants, and perform a microbiota-common variant genome-wide association study (mGWAS). To extend these genomic studies to other important benign urology phenotypes we will perform a GWAS of common lower urinary tract symptoms (LUTS) phenotypes, followed by phenome-wide association study to uncover risk factors and comorbidities with common genetic etiology. Our proposed studies will leverage existing data and biospecimens from four NIDDK-funded national cohorts, the RIVUR (UTI with VUR), CUTIE (UTI without VUR), CKiD (pediatric chronic kidney disease, including reflux VUR) and LURN (LUTS) study cohorts; as well as two large population cohorts that combine electronic medical records and genomics, from the UK Biobank and the NHGRI-funded eMERGE network. The proposed studies will provide new insight into the pathogenesis of disorders of high relevance to benign urology and also facilitate introduction of genetic testing into the practice of Urology.


Jonathan Barasch, MD, PhD

PROJECT 2: A NOVEL METABOLIC PATHWAY REGULATES URINARY TRACT INFECTIONS IN THE BLADDER. Urinary tract infections are the most common urogenital abnormality worldwide affecting multiple organs of the urinary tract including the urethra, bladder, prostate, ureter, and kidney. To establish a urinary tract infection, bacteria must obtain nutrients in order to undergo continuous replication. These nutrients derive from the urine and the epithelia of the urinary tract. Iron is a "precious metal" for bacteria because metabolic processes including energy production and cell division require ~100,000 atoms of iron per bacterium. Yet it is the most difficult nutrient to obtain because the common form, called ferric iron, is insoluble in water (Ksp=10-12M). Gram-negative organisms have devised methods to obtain iron even with these vanishing low concentrations. They do this by the production of a series of small molecules known as siderophores, each with astronomical affinity for iron. One type of siderophore, called Enterochelin is the most prevalent. Yet, the epithelial cells of the urogenital tract recognize this threat and rapidly produce a protein called NGAL (Lcn2) in great abundance. NGAL captures Ent:Fe and prevents its iron from reaching bacteria. We discovered that the urothelium and the tracts up into the kidney expresses NGAL. While most of our studies have focused on NGAL:Ent:Fe, there must be alternative nutrient pathways and alternative mammalian defenses. We propose that bacterial heme transporters also steal our iron and that epithelia in turn capture and metabolize heme. By adapting a novel method to analyze "snapshots" of nascent RNA, we found that bladder urothelia and collecting ducts express heme capture, heme metabolism, and iron sequestration and transport proteins, which compete with bacteria for heme. Most intriguing is our finding that upon infection, the urothelial "heme machine" is activated and releases a byproduct of heme metabolism, called Carbon Monoxide, a bacteriostatic agent. Moreover, the heme machine is the core complex of the Circadian Clock, which is regulated by CO. Here we test the basic tenets of our hypothesis. We carefully document heme and iron transport and metabolism in the urothelium to test the notion of that the urothelium and bacteria compete for heme. We suggest that these mechanisms are re-purposed for "nutritional immune defense" from the daily defense against the lysis of RBC

that traverse the bladder each day. To investigate these mechanisms, we have invented novel methods of RNA isolation, imaging tools to detect bacterial responses, a method to detect and capture CO in vivo, novel mouse ko's, and bacteria isolated from our patients with UTI's carrying mutations in siderophore and heme pathways. We are working with a leading microbiologist (Uhlemann), animal geneticist (Mendelsohn), UTI specialist (Mysorekar) and the leading scientist in heme biology (Hamza). Taken together, our studies demonstrate that rather than a passive barrier, the urothelium is a metabolically active cell layer that uses iron biology to detoxify hematuria and defend itself from UTI.


Anne-Catrin Uhlemann, MD, PhD

MICROBIAL GENOMICS BIOMEDICAL CORE. The mission of the Columbia University O'Brien Urology Research Center is to identify genetic factors and congenital malformations, such as vesicoureteral reflux, that predispose individuals to urinary tract infections (UTI) and to explore host-bacterial metabolic processes, such as heme pathways, that enable or prevent infection. UTI represent one of the most common infections, affecting 150 million people per year worldwide. In support of the goals of the Columbia O'Brien Urology Research Center the Microbial Genomics and Biomedical Core (MGBC) will provide high quality services for biobanking, extended bacterial culture, microbial genomics (microbiome analyses, comparative genomics), microbial genetics, consultation for study design and outreach for novel investigations into the pathogenesis of urinary tract infections. The MGBC will enable Columbia O'Brien Center investigators to have access to specialized genomic technologies and coordinated expert consultations for improved rigor and reproducibility in genomics and precision medicine. In recognition of the increasing impact of antimicrobial resistance in urinary tract infections the MGBC has established a biorepository of multi-drug resistant UTI isolates. The services and biorepository are specifically designed to meet the needs of two of the Research Projects of the Columbia O'Brien Center and Opportunity Pool Awardees and will be available to the wider urology research community. The MGBC will accomplish these goals through the following aims: 1) Expand a biorepository of multi-drug resistant UTI isolate to multiple sites across New York City, establish a urine biorepository from clinically well-defined patients with different UTI symptoms and perform extended urine culture for organism identification; 2) Provide Microbiome and metagenome analyses and Hi-C/MetaPore-C sequencing; 3) Facilitate whole genome sequencing of dominant UTI pathogenic clones to elucidate bacterial determinants of UTI symptomatology; 4) Generate bacterial mutants in multi-drug resistant clinical UTI isolates leveraging a highly efficient CRISPR-Cas system; and 6) Provide consultations and support of study design for the Research Projects. Importantly, the MGBC will serve as a national resource for members of other George M. O'Brien Cooperative Research Centers and other NIDDK Urologic Research Programs. In close collaboration with the Administrative Core the MGBC will ensure adherence to all fiscal, administrative, and resource sharing policies. The MGBC will also contribute to the goals of the Educational Enrichment and Opportunity Pool Programs through lectures, student mentorship, and guidance on experimental design related to microbial genomics for Opportunity Pool awardees. Taken together, the Columbia O'Brien U54 MGBC will enable state- of-the-art characterization of microbes in UTIs, with a focus on evolving multi-drug resistance, by providing a complementary set of tools harnessing molecular biology, genetics, and 'omics technologies.

ADMINISTRATIVE CORE. The mission of the Columbia University George M. O'Brien Urology Cooperative Research Center is to advance the understanding of benign genitourinary diseases/disorders. The Center consists of an Administrative (Admin) Core, a Microbial Genomics Biomedical Research Core, and three Research Projects that include preclinical and clinical studies. Our focus includes: (1) the intersection of the host genome and the urinary microbiome; (2) the transcriptional regulation of urothelial differentiation during homeostasis and repair in response to UTI; and (3) how the regulation of urinary chemistry and urothelial iron metabolism ("iron-heme machine") is a component of the urothelial response to UTI. We anticipate that the

proposed studies will provide new insight into the pathogenesis of disorders of high relevance to benign urology and also facilitate introduction of genetic testing into the practice of Urology and even suggest new treatments. We will examine the interactions between host and invading bacteria, and the role of nutrient iron in shaping the outcome of UT infections; we will undertake a new GWAS analysis to search for mutations that lead to LUTD, including incontinence, obstruction and pelvic floor prolapse, we will analyze the urinary microbiome of patients with lower urinary tract dysfunction (LUTD), and we propose to identify pathways that program urothelial cell types that may be used to treat benign urothelial abnormalities associated with disease. The most important goal of this center is to utilize the expertise and experience of the urological community to increase the scope of our studies and to help direct the kinds of questions we will address. We have directly integrated members of the P20/U54 community as well as clinicians and scientists in the benign urological community. Co-investigators on our projects include **Indira Mysorekar, PhD** (P20), an expert in UTI and urothelial biology who will work with Dr. Mendelsohn and Dr. Barasch, **Chad Vezina, PhD** (Wisconsin U54) and collaborating scientists and clinicians from the benign urological community, (**Lori Birder, PhD, Gerry Apodaca, PhD, Doug Strand, PhD**). In addition, we have invited outside experts to join our work, for example, the heme biologist, **Iqbal Hamza, PhD** (University of Maryland). We are also highly focused on education and outreach: Our summer program has trained 73 students since our center has been established, including undergraduates, fourth year medical students and urology residents. we have provided opportunity pool funds to **Dr. Catherine Putonti** (Loyola University) and **Dr. Catherine Brownstein**, (Harvard University). We held a Symposium in October of 2019 that was extremely successful "The problem of UTI: The microbiome of the Urogenital Tract and its immune Defense" bringing together a dozen experts with >138 members of the Urological community.

Stanford University George M. O'Brien Center

2021-2026 – PI, James Brooks, MD

Defining the Molecular, Cellular, Microenvironmental, Histological, and Macrosopic Dimensions of Human Benign Prostatic Hyperplasia


James Brooks, MD, PI

PROJECT SUMMARY. Benign prostatic hyperplasia (BPH) is the most common cause of urinary symptoms in older men, yet we understand little about its origins, drivers of growth, and how it causes lower urinary tract symptoms. Since BPH-caused Lower Urinary Tracts Symptoms (LUTS) appears unique to man, we propose a highly integrated project to create an atlas encompassing the molecular, cellular, microenvironmental, histological and macroscopic dimensions of human BPH. Definition of the features responsible for growth and progression of BPH could ultimately lead to new therapeutic approaches to treat or prevent BPH. Our overall goal is to expand research in benign urology to improve our understanding and treatment of urological diseases. The components of the Stanford O'Brien Urology Research Center include: The Administrative Core is based in the Department of Urology and directed by **Dr. James Brooks**, an experienced clinician and translational scientist in prostate disease who will administer the Center to ensure the scientific and training goals are realized and interface with the NIDDK and Urology Research Consortia. He will be advised by an Internal and External Advisory Board, to ensure progress is made and to provide scientific advice to ensure success. He will meet with the Investigator Committee to formulate plans, integrate findings between projects and allocate Project and Core resources to ensure projects succeed. The Biospecimen/Bioimaging Core, directed by **Dr. Robert West** provides critical support to projects of the Center by providing human BPH tissues with deidentified data, generates histological images and manages these and the MRI images and provides Multiplexed Ion Beam Imaging (MIBI) and data analysis for Projects 1, 2 & 3. The Core also provides this service to the O'Brien Urology Centers and Urology Disease Centers. Project 1 seeks to define the role of fibroblast subtypes in the development and progression of BPH. Project 2 characterizes the immune microenvironment and investigates how it is shaped by the stromal cells and how it influences the stromal and epithelial compartments of BPH. Project 3 uses MR Images with associated International Prostate Symptom Scores (IPSS) and Bothersome Indices (BI) to construct 3D models of BPH overlayed with histology. These models serve as an atlas for integrating stromal and immune microenvironment data and gene expression subtypes and will provide a means to test how molecular, cellular, microenvironment, histological and radiologic features and their heterogeneity relate BPH to LUTS. These projects serve as the nucleus for training of undergraduate, graduate, and post graduate students to become the next generation of leaders in urological science.

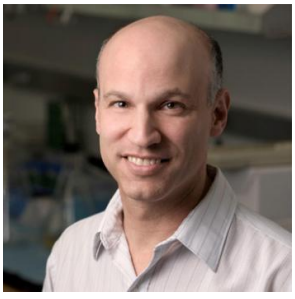
PROJECT 1: FIBROBLAST SUBSETS IN BPH PATHOGENESIS. Benign Prostatic Hyperplasia (BPH) is the benign enlargement of the prostate gland that occurs in older men, obstructing bladder outflow. The resultant lower urinary tract symptoms, such as urgency, frequency and incomplete emptying, have considerable morbidity, and carry annual healthcare costs in the billions. Current BPH treatments are not very effective because the drugs target normal prostate physiology but not BPH pathophysiology, which is still poorly understood. New disease-targeted therapies will require a more detailed knowledge of BPH pathogenesis. In genomic studies of

BPH clinical samples, we discovered a stromal gene signature that correlated with BPH symptoms, and an enrichment of fibroblasts overexpressing signaling proteins BMP5 and CXCL13. Fibroblast BMP5 enhanced prostate epithelial proliferation and drove gene expression profiles to mimic BPH tissue. From these data, we hypothesize that BPH is driven (at least in part) by pathogenic fibroblast cell subset(s), where defining those subsets will provide important new opportunities for disease targeted therapies. Towards that goal, the proposed studies aim to define the fibroblast subsets in BPH versus normal prostate; determine the key interactions between BPH fibroblast subsets and prostate epithelium that drive prostate enlargement; and distinguish between BPH origins in embryonic re-awakening versus injury response. Study findings will provide new understanding of the contribution of prostate stroma to BPH pathogenesis, and identify new strategies for targeted treatment.

PROJECT 2: IMMUNE MICROENVIRONMENT IN BPH PATHOGENESIS. Benign prostatic hyperplasia affects the majority of men in later life and leads to considerable dysfunction. Current treatments do not address the pathophysiology of the disease but rather target the overall prostate physiology. The goal of our proposal is to identify BPH (Benign Prostatic Hyperplasia) specific treatments by uncovering its pathophysiology. BPH is marked by proliferation of both epithelial and stromal cells in the transition zone of the prostate. This expansion of tissue surrounding the prostatic urethra presumably gives rise to many of the lower urinary tract symptoms (LUTS) associated with BPH. A number of biologic pathways have been proposed to drive BPH including epithelial or stromal senescence, inflammation possibly related to infection, and aberrant activation of developmental pathways. We have recently performed gene expression profiling of BPH and identified at least two subtypes of BPH with possible therapeutic implications. One of the two most significantly differentially expressed genes in our study is CXCL13 which modulates the immune response. The CXCL13 finding represents an important lead in understanding how the immune response is established in BPH. We hypothesize that immune-related pathways play a significant role in BPH pathophysiology and that pathways associated with CXCL13 are drivers. The Aims of this proposal undertake to identify differences in immune response between BPH and normal prostate and to uncover important pathophysiology relationships that arise from these differences. In Aim 1, we will profile immune cell types in BPH and normal prostate using MIBI-TOF (Multiplexed Ion Beam Imaging by Time-Of-Flight) to measure 14 different immune cells and understand their spatial relationships with the epithelium and stroma. In Aim 2, we will identify spatial relationships and interactions between immune and other cell types. We will do this by combining RNA profiling of the stromal and epithelial compartments with multiplex IHC and computational modeling. In Aim 3, we will determine whether CXCL13 expression and accompanying immune cells is part of an immune response or a senescence response. We will examine whether senescent cell accumulation correlates with CXCL13 expression and BPH subtypes. We will also look for the presence of B and/or T cell clonality.

ADMINISTRATIVE CORE. The Administrative Core will be an essential resource in the success or the Stanford O'Brien Center. The goals of the Administrative Core will encompass external engagement, including integration with the O'Brien Center Collaborative Network, interfacing with other programs in urological research, coordinating training activities between research centers, and coordinating activities with NIDDK leadership. The Administrative Core also directs the Research Center's Educational Enrichment and Opportunity Pool Programs. The overarching goal of the Administrative Core is to provide the operational support necessary to

Stanford University O'Brien Center Project Leaders:



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successfully achieve the goals we have laid out for each project and program as a whole. The Administrative Core will be responsible for managing, coordinating, and monitoring overall progress, and supervising the entire range of the program's activities. Moreover, the Administrative Core will distribute results to scientific audiences and the general public. We will maximize efficiency, cost-effectiveness, and productivity by centralizing resources used by each of the projects and core. The Administrative Core has the following specific aims: (1) provide operational oversight and integration of projects and cores, (2) promotion of effective collaboration, communication, and interactions within and outside the Center, (3) provide an effective, centralized unit to manage finances and resources, (4) broadly disseminate results and report Center activity, and (5) develop and execute the Center's Educational Enrichment and Opportunity Pool Programs. The organization of the Administrative Core will have clear lines of authority and will leverage both an Investigator Committee and Internal and External Advisory Committees to ensure effective use of resources, scientific guidance and evaluation, and clear decision- making processes. Overall the Administrative Core will provide fully integrated administrative services for the management, communications, coordination, and financial administration of the Stanford O'Brien Center.

BIOSPECIMEN/BIOIMAGING CORE. The Biospecimen/Bioimaging Core leverages infrastructure of the Stanford Urology and Pathology departments, but augments services to benefit O'Brien Center investigators. Led by a qualified team, the centralized resource provides qualitative advantages and efficiencies of scale. The Core procures and provides Center investigators with needed biospecimens, including fresh, freshly-frozen, and formalin-fixed paraffin- embedded (FFPE) prostate issues. The Core also performs less visible but equally important activities, including patient consent, quality control/assurance, database management, clinicopathologic annotation, and regulatory compliance. In addition, the Core provides tissue characterization services, including histology, tissue microarrays, laser microdissection, and multiplex immunohistochemistry (IHC) using MIBI-TOF (Multiplexed Ion Beam Imaging by Time of Flight). MIBI-TOF is a powerful and robust technology that harnesses metal-tagged antibodies for the simultaneous quantification of 40 or more antibody targets, with superior spatial resolution and dynamic range. The Core also stores and provides portal access to images including prostate MRIs, whole-mount histology and IHC, and MIBI-TOF data, as well as an integrated multi- scale prostate Atlas spanning MRI to histology, cells and molecules. The Biospecimen/Bioimaging Core provides essential tissue procurement, characterization and imaging services to support the three Center Projects, the Education Enrichment and Opportunity Pool programs, as well as outside O'Brien Centers, P20 grant programs, and the broader benign urology community.