NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (NIDDK)
NATIONAL INSTITUTES OF HEALTH (NIH)

Targeting Fibrosis in Kidney, Bone Marrow, and Urological Diseases

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Bethesda, MD

Summary Report

OPENING REMARKS AND OBJECTIVES

Griffin P. Rodgers, M.D., M.A.C.P., Director, NIDDK, NIH, Bethesda, MD

Dr. Rodgers welcomed the meeting attendees and thanked them for attending. He introduced the topic of the meeting as the exploration of mechanisms leading to the development of pathological fibrosis, which is a critical subject because the pathway leading to organ failure often is characterized by irreversible fibrosis. During pathological fibrosis, normal structures are replaced by scar tissue, which is characterized by excess synthesis and deposition of interstitial matrix and basement membrane structural proteins. Deposition occurs faster than removal, leading to accumulation of these proteins. Fibrosis has been considered irreversible, but a growing body of knowledge indicates that there may be hope for functional restoration. Researchers' understanding of the underlying mechanisms of fibrosis and its turnover in diseased tissue has increased in recent years and will lead to development of new treatments that might restore the function of underlying organs. Notably, prevention of inflammation is critical to prevent fibrotic scarring.

In planning for this meeting, the organizers considered several main topics: detection, measurement, and animal models. Three NIDDK organ systems received particular attention during the meeting: the kidney, lower urinary tract (LUT), and bone marrow. The agenda was designed to focus on the detection and measurement of pathologic fibrosis, and address the following important questions:

1) Can we differentiate between pathological fibrosis and that of normal aging?

2) Does fibrosis correlate with organ dysfunction?

3) Does imaging of fibrosis correlate with organ dysfunction, recovery, and regression?

4) What novel biomarkers or technological advances should be pursued to detect and measure pathologic fibrosis?

Dr. Rodgers reviewed the meeting agenda and commented on the breadth, depth, and importance of the scientific topics to be explored. He expressed the expectation that studies developing from this scientific meeting will illuminate broader principles that can be applied to other organ systems. The NIDDK currently is requesting applications for research on the detection and measurement of fibrosis, which will spur the development of new imaging methods and biomarkers (Novel Methods for Detection and Measurement of Organ Fibrosis in Kidney, Bone Marrow, and Urological Diseases [RFA-DK-13-026]). Dr. Rodgers emphasized the truism that "if you can't measure it, you can't manage it"; accordingly, novel and accurate methods to measure fibrosis are needed urgently. Dr. Rodgers thanked Drs. Michael Flessner and Robert Star, as well as the Planning Committee, for stimulating the important discussion on targeting fibrosis.

STATE-OF-THE-ART LECTURES

Moderator: *Deborah Hoshizaki, Ph.D., NIDDK, NIH, Bethesda, MD*

Mechanisms of Fibrosis

Thomas Wynn, Ph.D., National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD

Dr. Wynn explained that fibrosis represents an excessive wound-healing response. In many situations, fibrosis is not pathological and is, in fact, necessary for wound repair. Damaged tissues activate immune responses and drive fibroblast recruitment. Upon an acute tissue-damaging event, the development of fibrosis is the body's attempt to restore tissue architecture. With chronic injuries, the fibrotic response becomes dysregulated and causes extra deposition of extracellular matrix (ECM). The pathological causes of fibrosis include: hepatitis, schistosomiasis, inherited genetic disorders, Duchene's muscular dystrophy, tumorigenesis, autoimmune inflammation, chronic alcoholism, exposure to toxins, and treatment with bleomycin (a cancer drug that is found to cause pulmonary fibrosis as a side effect). Allergens and asthma cause epithelial fibrosis of the lung; asthma becomes more difficult to treat as the lungs develop fibrosis. Fibrosis also is a major problem in organ transplantation, as almost 50 percent of heart and lung transplants terminate because of development of severe fibrosis.

In the case of liver fibrosis, scarring in the liver can lead to blood vessel growth, making patients prone to bleeding, which in many cases is the proximate cause of death. Dr. Wynn's group studies schistosomiasis and liver fibrosis in response to the parasitic infection. Patients get infected with parasites when they wade into freshwater lakes and streams. The parasites penetrate the skin, migrate to the heart and lung, and ultimately find their way to the gut where they produce eggs. The eggs pass through the wall of the intestine and are excreted into the feces to complete the organisms' life cycle. Some of the eggs remain in the bloodstream, are deposited in the liver, and cause the development of hepatic fibrosis.

Dr. Wynn's group discovered an important role for macrophages in the pathogenesis of fibrosis. Macrophages are found in close proximity to collagen-producing myofibroblasts and produce growth factors (e.g., transforming growth factor beta [TGF-β], insulin-like growth factor [IGF], interleukins [ILs]) that can activate fibroblasts. The cytokine interleukin 13 (IL-13) plays a key role in driving fibrosis. Dr. Wynn's laboratory identified three main pathways that inhibit IL-13 mediated progression of fibrosis. The pathways are partly redundant, and inhibition of one pathway is compensated by upregulation of the remaining pathways. Because of this, the group genetically engineered a triple knockout mouse (i.e., deficient in interleukin 10 [IL-10], the p40 subunit of interleukin12 [IL-12p40], and IL receptor subunit alpha-2 [IL-13Rα2]) as a model for studying fibrosis. The triple knockout mouse develops fibrosis rapidly (five- to 10-fold increase over controls) and thus allows faster testing of novel antifibrotic drugs. When infected with parasites, the triple knockout mouse develops cirrhotic complications, splenomegaly, decreased red blood cell and platelet counts, and increased white blood cell counts, eventually succumbing to the infection after 10 weeks. Notably, infected animals treated with anti-IL-13 are more resistant to the infection.

There is great interest in the upstream mechanisms governing epithelial-derived cytokine mediators that may emerge as potential targets for antifibrotic drug development. One such pathway is driven by IL-13, which activates TGF-β and drives collagen deposition. Dr. Wynn described the various cellular signaling pathways and subsets of macrophages that play a role in the initiation, maintenance, and resolution of fibrosis, several of which were discovered by treating the triple knockout mice with various drugs. Of the multiple, interacting mechanisms that lead to the development of fibrosis, the Wynn laboratory currently is focusing on the regulation of TH1 responses to identify mechanisms that might slow the progression of fibrosis.

Discussion

Dr. Jill Macoska, University of Massachusetts, asked whether any of the described signaling pathways could be promoted to reverse fibrosis in addition to stopping the process. Dr. Wynn answered that reversal of established disease is not yet possible. It is necessary to understand the beneficial aspects of the inflammatory response, including the specific subset of macrophages that produce ECM-degrading enzymes that allows normal repair to happen. Only then will it be possible to understand pathological aspects to fibrosis and mechanisms that allow fibrosis to be reversed.

Dr. Sun-sang Sung, University of Virginia, queried the requirement for inducible nitric oxide synthase (iNOS) signaling. Dr. Wynn commented that blocking NOS2 inhibits the antifibrotic effect of macrophages, but the mechanism remains to be elucidated.

Dr. Paul Kimmel, NIDDK, noted the "fantastic complexity" of the mechanisms involved in the fibrotic response and asked whether the responses are tissue-specific (i.e., whether the mechanisms of liver fibrosis can be extrapolated to the kidney). Dr. Wynn answered that the schistosomiasis model of fibrosis had little in common with the acute bleomycin model; different cytokines play different roles depending on the context.

Given the incredible complexity in the very large number of potential therapeutic targets, Dr. Simeon Taylor, University of Maryland, asked how much therapeutic benefit will be derived from targeting a single molecule or pathway. He also questioned the degree to which an animal model is representative of human disease (e.g., Is the genetically engineered triple knockout mouse that developed fibrosis representative of fibrosis in human disease?) Dr. Wynn answered that treatment of fibrosis will likely require targeting multiple pathways. Whether mouse models are translatable is a good question that remains controversial.

Dr. Star emphasized the importance of detecting fibrosis early using noninvasive techniques, and Dr. Wynn commented that serum biomarkers would represent an ideal development. Periostin might be a good predictor of fibrosis because levels rise with inflammation and then become level. He also reiterated the importance of developing imaging technologies to quantify and assess progression of fibrosis.

The Myeloproliferative Neoplasms as an Opportunity to Understand the Origins of Pathological Tissue Fibrosis

Kenneth Kaushansky, M.D., Stony Brook University School of Medicine, Stony Brook, NY

Marrow fibrosis (myelofibrosis) presents an opportunity to understand the origins of pathological tissue fibrosis. A myriad of conditions result in myelofibrosis, including chronic infections (tuberculosis, histoplasmosis), marrow-invasive cancer and radiation therapy. In these states the mechanisms that generate fibrosis are similar to reactive fibrosis in other organs. However, myelofibrosis associated with one of the myeloproliferative neoplasms (MPNs) is an important new paradigm for understanding the origins of myelofibrosis, as much is now understood of the mechanisms that cause myeloproliferation in patients with MPNs. The MPNs include polycythemia vera, essential thrombocythemia and primary myelofibrosis, and are chronic marrow disorders characterized by unregulated growth of one or more blood cell lineage that may extend to extramedullary sites, most commonly the spleen or liver. They arise in a single hematopoietic stem cell (HSC), and the symptoms/signs of the disorders are related to excess blood cells and/or fibrosis. As myelofibrosis disorders marrow architecture, the laboratory signs are abnormal size and shape of blood cells. such as tear-dropped shaped red cells. Essential thrombocythemia is characterized by high platelet counts, and the platelets are abnormal in size and shape.

Hematopoietic stem cells are normally dependent on growth factors for survival, proliferation and differentiation, including stem cell factor (SCF) and thrombopoietin. SCF plays a role very early in the hematopoietic lineage progression, stimulating maturation of cells. Thrombopoietin (TPO) is best known for effects on platelet production, but like that for SCF, HSC numbers plummet if TPO or its receptor is absent in animals and humans. Both of these growth factors stimulate cells to activate the signaling kinase Jak2, initiating the pro-survival and proliferation effects of SCF and TPO. However, the survival and proliferation of HSCs and their progeny are not dependent on growth factors in the MPNs, like in other malignancies, a hallmark of these disorders. In contrast, fibroblasts are not part of the malignant clone in the MPNs; they are "bystanders" that are driven to inflict collagen fibrosis by the MPN microenvironment, by the cytokines, interleukins, and growth factors elaborated by the malignant cells.

In 2005, several groups simultaneously demonstrated that a single, acquired, activating missense mutation in the JAK2 kinase, valine 617 converted to phenylalanine, was present in a majority of patients with MPNs. Subsequently, activating mutations in the TPO receptor and other Jak2 mutations were also found to contribute to the origins of MPNs. One mechanism by which mutant Jak2 contributes to myelofibrosis is through megakaryocyte-derived transforming growth factor (TGF)-β. Additional work in our laboratory has revealed that for mutant Jak2 to contribute to the MPN, it must bind to the TPO receptor. Thus, the origins of myelofibrosis in the MPNs are increasingly understood in molecular terms, and should lead to new therapeutics to interfere with the excessive myeloproliferation and its sequelae, marrow fibrosis.

Discussion

Dr. Benjamin Humphreys, Brigham and Women's Hospital, asked about the source of the fibroblasts in the marrow that are reacting to the growth factors. Dr. Kaushansky replied that there are many cytokines, and the signaling pathways between cytokines and fibroblasts are not well understood. The source of the fibroblasts is unknown.

Dr. Star asked about opportunities to detect and measure fibrosis in the marrow at the tissue level. Dr. Kaushansky responded that it is difficult and there are no good answers. In hematology, access to tissue is relatively easy, but there still are too few methods or biomarkers of fibrosis.

Dr. Edward Macarak, Jefferson University, inquired about the type of collagen found in myelofibrosis. He commented that fibrosis appears to exhibit type III collagen, whereas fibrosis of the lung and liver are primarily type I collagen. This distinction might be important for understanding the different pathways leading to the development of disease.

Dr. Robert Toto, University of Texas Southwestern Medical Center at Dallas, asked whether there are suitable biomarkers for the initiation of the disease process (e.g., a critical point where the signal first begins to attract fibroblasts). Dr. Kaushansky replied that unfortunately, it is difficult to catch the disease in its early stages and most patients are identified only when the disease has progressed. Better biomarkers and methods of early detection are needed.

***In Absentia*: Advances in Liver Fibrosis Research**

Scott L. Friedman, M.D., Icahn School of Medicine at Mount Sinai, New York, NY

Ongoing progress in defining critical events underlying fibrosis pathogenesis continues to yield new targets for therapy. Activation of hepatic stellate cells remains the central feature of liver injury, as described in several recent reviews. However, other advances also include:

(1) The sources of fibrogenic cells in different types of liver injury have been clarified.

(2) Progressive changes in the inflammatory milieu, including chemokines, may contribute to either progression or regression of fibrosis.

(3) Stellate cell activation is driven by autophagy, a pathway that has evolved to maintain energy homeostasis. Studies from Dr. Friedman's laboratory using genetic models in which autophagy deficiency could be restricted to hepatic stellate cells demonstrated that animals with liver injury had reduced fibrosis. While these findings are exciting, global inhibition of autophagy is unrealistic because of its important contribution to homeostasis of hepatocytes and other resident liver cells. Therefore, Dr. Friedman and his colleagues currently are seeking alternative targets within the autophagy pathway that might represent more cell-specific targets for antifibrotic therapies. Among these, mediators of endoplasmic reticulum (ER) stress and reactive oxygen species injury are appealing.

(4) MicroRNAs regulate hepatic stellate cell biology and are released into the circulation. In particular, downregulation of miR-29b accompanies stellate cell activation and could represent a new biomarker of disease activity.

(5) The inflammasome, an intracellular scaffold of cytokine activation, also contributes to stellate cell activation as well as to steatohepatitis pathogenesis. Studies in animal models of fatty liver disease have identified a critical role not only for the microbiome, but also signaling through toll-like receptors on parenchymal and nonparenchymal cells in liver in mediating the risk of fibrosis and cancer.

(6) During resolution of fibrosis, not only do some activated stellate cells undergo apoptosis, but also a sizable fraction instead reverts to a more quiescent phenotype, albeit with a heightened capacity to reactivate upon recurrent injury.

(7) Significant progress is being made in linking the pathogenesis of fibrosis to the accelerated risk of hepatocellular carcinoma in patients with advanced liver disease. A number of critical pathways have emerged beyond the scope of this review, but changes in the stromal environment, as well as specific signaling by Hedgehog, angiogenic mediators, and innate immunity pathways all increasingly are implicated in the pathogenesis of liver cancer associated with fibrosis. This is an especially critical issue in liver because cancer almost always arises on a substrate of moderate to severe fibrosis, strongly implicating the fibrotic milieu as a driver of carcinogenesis.

Recently genome-wide association studies have indicated that fibrosis progression is driven not only by the extent of injury from either hepatitis viruses, alcohol, or metabolic derangements, but also from genetic factors. Specifically, a cirrhosis risk score comprised of seven single nucleotide polymorphisms has been identified in patients with hepatitis C and validated in three separate prospective studies. Interestingly, the gene variants that emerged as predictive of fibrosis within this score are largely within genes not previously linked to fibrosis pathogenesis. These include Azin 1, aquaporin 2, as well as Toll-like receptor 4 and several others. Thus, studies of this type have led to new directions of research to understand how these variants change fibrogenic signaling and stellate cell activation. Another key implication of these findings is the possibility that fibrosis also is regulated genetically in other tissues apart from liver. For example, patients with a high cirrhosis risk score might also be more susceptible to fibrosis in lung, kidney, or other tissues if the primary insult is to these organs. Future studies will hopefully address the genetic determinants of fibrosis across tissues to define a broad "fibrogenic phenotype."

Ample evidence demonstrates that hepatic fibrosis is reversible in human liver diseases. This important observation has become clear only as specific therapies for liver disease have been developed, especially effective antivirals. Reversibility seems especially likely in patients in whom HBV therapy suppresses viral replication; cirrhosis reversion has also been reported, however, in some HCV patients following sustained viral remission. Moreover, when reversal occurs in HCV, it leads to improved clinical outcomes and reduced portal pressure.

At what point cirrhosis becomes irreversible is uncertain, but irreversibility becomes more likely as the scar thickens, becomes more acellular and is chemically cross-linked, reinforcing the importance of matrix cross-linking to the disease. More recent evidence suggests that this enzyme may have other activities unrelated to extracellular matrix that may contribute to its antifibrotic activity. Ongoing trials of a monoclonal antibody to this enzyme are underway in patients with fibrotic liver disease, and new data are anticipated.

Points of attack for emerging therapies include: (a) eliminate the cause of injury and its mediators; (b) reduce inflammation and the immune response; (c) target specific signaling—receptor-ligand interaction, intracellular signaling; (d) reduce fibrogenesis, inhibit matrix synthesis; and (e) resolve fibrosis by increasing scar matrix degradation, stimulating apoptosis of stellate cells or bone marrow or cell transplantation.

A key challenge limiting progress in the testing of antifibrotic drugs is the lack of sufficient endpoints that are noninvasive yet correlate well with clinical outcomes. Currently, all clinical trials of antifibrotic drugs require a liver biopsy to assess fibrosis before and after treatment. This requirement imposes several limitations on clinical trial design, including the invasive nature of biopsy, and therefore, the limited access to tissues. Current efforts are aggressively seeking surrogate markers or noninvasive determinants that can supplant biopsy. For example, noninvasive imaging using MR technologies or elastography may emerge as indicative of drug response. Better yet, serum markers of fibrogenic activity would be ideal, in that they could be sampled regularly and might even indicate response to therapy before the biopsy is likely to change. An alternative and or complementary strategy would be the use of functional tasks that assess underlying liver functional liver reserve. These are especially appealing because the functional reserve capacity of the liver is likely to correlate closely to risk of clinical events and long- and short-term outcomes.

An additional consideration is whether antifibrotic targets in one organ are likely to be preserved across organs. A useful paradigm is to distinguish between core and regulatory pathways in fibrosis pathogenesis. Core pathways are those conserved across organs and species, whereas regulatory pathways are likely to be restricted to specific cell types or organs. Antagonism of core pathways is appealing in identifying antifibrotics that are potentially active across many organs, but they risk the concern of greater off-target effects because the molecule being antagonized is not tissue-restricted. On the other hand, antagonism of regulatory pathways may be more specific and less prone to off-target effects, but their relative utility, and therefore the relative market size, may be more limited.

There are several antifibrotic trials already underway in liver, and should any one of these prove effective, it will likely have a catalyzing effect on the field. Progress will be incremental and iterative, as it has been in antiviral therapy drug development, with continued refinements that improve trial design and outcomes. Given the capacity of the liver to resorb scar tissue based on antiviral trials, however, emergence of effective drugs that ameliorate fibrosis is approaching and will transform the outlook for patients with chronic fibrotic liver disease.

DETECTION (IMAGING) OF PATHOLOGIC FIBROSIS

Moderator: *Maren Laughlin, Ph.D., NIDDK, NIH, Bethesda, MD*

Molecular Imaging of Collagen to Diagnose and Stage Fibrosis

Bryan Fuchs, Ph.D., Massachusetts General Hospital, Boston, MA

Dr. Fuchs explained that fibrosis is an excessive wound-healing response to tissue injury and results in the deposition of scar tissue. If fibrosis progresses, it can disrupt normal tissue architecture and eventually lead to organ failure. Some therapeutic interventions can reverse early stage fibrosis, but current imaging techniques can only detect late stage disease. There is a major unmet medical need for non-invasive tools to detect and monitor fibrosis.

Dr. Fuchs described several probes that are currently being used, including EP-3533, a probe whose preclinical development is being pursued by a startup company called Collagen Medical. Another probe, Gd-Hyd, detects cross-linked collagen fibers and the activity of cross-linking enzymes, thus allowing the detection of active areas of fibrosis formation. EP-2104R is another probe that detects fibrin and the activity of thrombin, an enzyme that has important roles in the development of fibrosis.

Dr. Fuchs's team focuses on magnetic resonance imaging (MRI) of the liver, which presents several advantages, including soft tissue contrast, complete coverage of the entire liver, deep tissue penetration, and lack of ionizing radiation. One disadvantage is that MRI is not at as sensitive as positron emission tomography (PET) imaging, but that can be overcome by using collagen (which is abundant) as the probe target. EP-3533 is useful for detecting and even staging liver fibrosis in mice by measuring the change in the contrast -to-noise ratio between the probe signal in the liver and that in the muscle. Gd-Hyd is a probe that detects collagen cross-links as fibrosis progresses. Unlike EP-3533, Gd-Hyd does not exhibit long-term retention in the kidney; therefore, it would be a suitable probe to study renal fibrosis.

Discussion

Dr. Brian Garra, U.S. Food and Drug Administration (FDA), asked whether Dr. Fuchs had investigated the dose dependency or molar dose of the peptide probes. Dr. Garra suggested that better results could be obtained by decreasing the affinity of the probe for its targets and increasing the dose of the probe. Dr. Fuchs agreed that this might be possible, but also noted that his group had not had a chance yet to examine the effects of changing the affinity of the probe for its target.

Dr. Darren Yuen, Li Ka Shing Knowledge Institute, asked the following two questions: (1) For the Gd-Hyd probe, is there any risk for renal disease patients? and (2) Is Gd-Hyd a useful probe for detecting early stages of fibrosis? In response to the first question, Dr. Fuchs answered that Gd-Hyd is rapidly excreted from body; therefore, it is safer than EP-3533 for studying renal fibrosis. It remains to be seen whether Gd-Hyd is a useful probe for detecting early stages of fibrosis.

Dr. Wynn asked whether the signals of the fibrosis probes are dependent on how much of the collagen is accessible. For example, in a fibrotic liver, much of the collagen may be inaccessible. Dr. Fuchs responded that it was an excellent point. He commented that EP-3533 imaging is similar in both diethylnitrosamine (DEN)-induced fibrotic livers and DEN-induced severely cirrhotic livers and this could be because some collagen becomes in accessible during cirrhosis.

In response to a question, Dr. Fuchs commented that elastography is very difficult in mice, but his team will be starting those studies in several months. He noted that elastography in conjunction with collagen-targeted imaging might better distinguish early from late fibrosis.

Dr. Fuchs clarified that the labels are injected via tail veins in the mice. Dr. William Ricke, University of Wisconsin, commented that delivering the label to the bladder (by allowing the label to diffuse across the urothelium) could be used to study bladder fibrosis.

Novel Tools for Measuring and Understanding Pathologic Fibrosis
Benjamin Humphreys, M.D., Ph.D., Brigham and Women's Hospital, Boston, MA

Dr. Humphreys introduced his study system for imaging pathologic fibrosis in the kidney. Using a technique called fluorescence microangiography (FMA), his group can image capillary dynamics in response to acute kidney injury and also in the context of chronic kidney disease. Dr. Humphreys is interested in developing a molecular description of the kidney myofibroblasts during fibrosis. The clinical problem investigated by the laboratory is the mechanism by which acute kidney injury (AKI) substantially increases the risk for developing long-term chronic kidney disease (CKD). Repair of the epithelium in response to AKI causes permanent loss of peritubular capillaries, which results in chronic hypoxia in the kidney tubule. That, in turn, leads recurring cycles of injury, fibrosis and CKD.

Dr. Humphreys described the preparation of animal tissue for FMA imaging of endothelial cells. Using a mouse model of moderate and severe ischemia, he demonstrated that areas with particularly severe fibrosis also are severely depleted of peritubular capillaries. The capillary area and perimeter are reduced by 40 percent. Changes in capillary size and perfusion correlate with both acute injury and future kidney disease. The FMA tool allows new investigations of the mechanisms by which the damaged peritubular capillaries lead to zones of hypoxia and therefore to fibrosis.

The second project in the laboratory investigates the transcriptional profile of myofibroblasts during kidney fibrosis. This approach enables the lab to isolate myofibroblast-specific mRNAs in a single-step procedure from whole kidney lysate. Using this novel polysome immune-precipitation method, the group was able to extract polysomal RNA from pericytes and myofibroblasts during kidney fibrosis using a transgenic model developed by that group. Then, the transcriptional profile of myofibroblasts was compared to that of tissue from the rest of the kidney. This investigation identified the earliest transcriptional changes in myofibroblasts of fibrotic kidneys and may lead to the identification of novel biomarkers for kidney fibrosis.

Discussion

A participant noted that using collagen alpha 1 (COL1A1) as the marker to identify myofibroblasts might comprise circular logic, and suggested alternative approaches to identifying the cells. Dr. Humphreys answered that the cells can be readily tracked because the COL1A1 transgenic allele drives expression of enhanced green fluorescent protein (EGFP) transgene. He added that it is important to know the correct hierarchy of cells and identify the molecular differences between cell types.

Dr. Toto asked whether the attenuation of capillaries is due to the ischemic event itself, or whether it is due to subsequent tissue scarring. Dr. Humphreys commented that it is difficult to answer that question directly, but he suggested that it is probably due to acute damage to the capillaries and failure to repair.

Dr. Kimmel asked whether the imaging methods are able to distinguish the underlying cause of the reduction in capillary size. In other words, are the remaining small capillaries small because the large ones have been eliminated, or because the lumen of the large capillaries has decreased in size?
Dr. Humphreys replied that it was a great question, and the answer is currently unknown. He noted that careful time-course experiments could produce strong correlative data to elucidate this mechanism.

Dr. Star asked whether there exist less toxic probes and probe delivery mechanisms that could be used in humans. The agar used in Dr. Humphreys' probe preparation does not work well in humans. Dr. Humphreys suggested that the radiologists might be able to answer that question. The objective is to develop a tracer that focuses on the microvasculature to image early stages of the AKI-CKD transition.

Fibrosis and Prostate Disease

William A. Ricke, Ph.D., University of Wisconsin, Madison, WI

Dr. Ricke presented the results of his research on prostatic fibrosis in normal developing and mature prostates, as well as in normal and benign prostatic hyperplasia (BPH) samples. BPH, defined histologically by nodules of proliferation and clinically by lower urinary tract symptoms (LUTS), has a high incidence; is costly; and has unknown etiologies, although hormones are thought to play a role, and BPH is associated with aging. Dr. Ricke combines animal modeling and patient samples with molecular and histological methods , including gene expression-and image-analyses, to measure fibrosis, with the goal of differentiating between pathologic fibrosis and normal aging. Although there are differences between the mouse and human prostate, the mouse prostate is sufficiently similar to serve as a model of the aging process in human tissues. Dr. Ricke presented data on collagen deposition in mouse prostatic tissue during development and the aging process. In men, he found no correlation between aging and fibrosis in glandular prostatic tissues. In normal men and mice, prostatic fibrosis/collagen deposition occurred in prostatic tissue during neonatal development, but did not appear to be associated with aging in the adult.

A limited number of studies have implicated fibrosis in lower urinary tract dysfunction (LUTD), but the role of fibrosis in BPH and LUTS remains enigmatic. Gene expression analyses have shown upregulation of genes that are linked to fibrosis. Although BPH has been defined as the growth of prostatic nodules which may lead to obstructive urinary flow, some men with LUTS have normal-sized prostates, apparently independent of significant nodular growth. Dr. Ricke and his colleagues showed that relative to normal prostates, men with BPH had increased epithelial surface area and cell numbers, as well as increased numbers of stromal cells, but no significant increase in stromal tissue within nodular specimens. In men, increased fibrosis was not observed in glandular BPH nodules when compared to normal prostate tissue.

Dr. Ricke explored whether fibrosis occurs in the prostatic urethra, a possible cause of LUTS in men without enlarged prostates. Male mice with surgical implants of testosterone and estradiol-17β were used to model the hormonal environment of men during aging, providing a mouse model of LUTD. Hormones induced LUTD in mice, including hypertrophy in the bladder, increased prostate mass, and abnormal urination (i.e., increased frequency and lower volume). Fibrosis was enhanced in the periurethral prostatic tissue relative to untreated controls. Overall urethral collagen and bundle size increased in hormone-treated mice. A three-dimensional (3-D) reconstruction of the mouse prostatic urethra when compared with fibrosis suggested increased urethral rigidity, as did decreased staining for urethral elasticity. To determine the mechanism by which fibrotic tissue might be obstructing urethral expansion, Dr. Ricke is using multiphoton microscopy/second harmonic generation to assess collagen alignment in the prostatic urethra. In addition, he indicated that there is a range of collagen deposition in the non-nodular human prostate, suggesting possible changes in the periurethral climate that may be independent of nodules. Future work includes using new techniques for imaging urethral fibrosis, as well as development of proteomic and metabolomic urinary biomarkers in human and mouse models.

Discussion

Dr. Macoska asked whether voiding function was correlated with BPH in humans and periurethral fibrosis in mice. Dr. Ricke responded that this is something that he plans to study, but it requires samples

of normal human prostates. He and his team are in the process of obtaining tissue from patients to generate data.

In light of increased use of testosterone replacement therapy, Dr. Wynn asked whether there was epidemiological evidence showing increased fibrosis. Dr. Ricke replied that no evidence exists to his knowledge, but he is in the process of investigating whether exogenous testosterone is harmful or helpful, particularly as it may relate to testosterone metabolism into estradiol-17β..

In light of increased use of testosterone replacement therapy, Dr. Wynn asked whether there was epidemiological evidence showing increased fibrosis. Dr. Ricke replied that no evidence exists to his knowledge, but he is in the process of investigating whether exogenous testosterone is harmful or helpful.

PATHOLOGIC FIBROSIS AND ORGAN DYSFUNCTION

Moderator: *Terry Bishop, Ph.D., NIDDK, NIH, Bethesda, MD*

Urinary Obstruction and Fibrosis

Dr. Jill Macoska, University of Massachusetts, Boston

Dr. Macoska's laboratory investigates the role of fibrosis in lower urinary tract dysfunction (LUTD). LUTD is correlated with aging, which also is correlated with prostate enlargement. Importantly, tissue inflammation resulting from aging, infection, or inflammatory disease processes (including type 2 diabetes) is associated epidemiologically with the subsequent development of tissue fibrosis in multiple organ systems. Using *in vitro* assays of fibroblasts derived from young and aged prostates, Dr. Macoska's group showed that with age, the prostatic stroma loses the ability to regulate epithelial and stromal cell proliferation. Older prostate fibroblasts secrete growth factors that encourage the proliferation of epithelial cells. Several gene transcripts, including CXC-type chemokines, also are upregulated in older prostate fibroblasts. Consequent to aging, senescent fibroblasts secrete growth factors and inflammatory proteins, such as ILs and CC- and CXC-type chemokines.

In collaboration with mechanical engineer Dr. Ellen Arruda, Dr. Macoska's group explored whether changes in prostatic tissue architecture were associated with LUTD by measuring prostate tissue stiffness and collagen content. In older, fibrotic prostate, tissues become stiff and may cause obstructive symptoms when urine flows through a fibrotic urethra. Using a tensile tester, they determined that prostate tissue stiffness correlates with higher American Urological Association Symptom Index (AUASI) scores, higher collagen content of the tissue, and increased inflammation.

Further experiments showed that the same inflammatory proteins that promote prostate growth, particularly the CXC-type chemokines, can also promote myofibroblast phenotypic conversion and fibrosis. Furthermore, inflammation caused by obesity and diabetes was found to promote fibrosis of the lower urinary tract in a SAMP6 mouse model of accelerated senescence, mimicking the phenomena observed in diabetic or obese humans. The complexity of the fibrotic response to inflammation and ageing requires continued investigation.

Discussion

Dr. Wynn asked a question about the SAMP6 mouse model. Dr. Macoska clarified that the mouse is not a knockout mouse, but a senescence-accelerated mouse in which a high-fat diet induces obesity, diabetes, and impaired urinary function. Dr. Wynn commented that it is challenging to identify fibrotic transitions in normal mice that are fed a high-fat diet.

Dr. Ricke asked about the tensile strain assays and whether nodule tissue had been measured. Dr. Macoska replied that they only had investigated periurethral tissue, but it would be useful to investigate mechanical properties of other tissues within the prostate.

Dr. Trinity Bivalacqua, Johns Hopkins Hospital, expressed interest in the mechanisms by which inflammation affects periurethral tissue. Dr. Macoska offered to share the original peri-urethral prostate tissues reported in the J. Urol. paper with Dr. Bivalacqua to test antibodies.

Biology of Fibrosis and Mechanisms for Reversal Therapy
Raghu Kalluri, M.D., Ph.D., The University of Texas MD Anderson Cancer Center, Houston, TX

Dr. Kalluri explained that a continued interest in his laboratory was to understand mediators of fibrosis. Understanding fibrosis is critical because irrespective of any disease etiology, the common feature that leads to organ death is fibrosis. Additionally, in the context of cancer progressions, fibrotic tissue drives tumor progression and metastasis.

The key events leading to fibrosis can be divided into five pathways: parenchymal damage/apoptosis, inflammation/immunity, capillary rarefaction/focal hypoxia, activation/accumulation of myofibroblasts, and deposition of ECM. Protecting a functional parenchyma can circumvent the other pathways that lead to damage in fibrosis. Perpetual parenchyma damage and apoptosis can lead to a continuous stress response and repair, which can cause cells to experience phenotypic changes. Under stress, it is common for the epithelium to gain mesenchymal-like characteristics. Dr. Kalluri described several mouse models developed to understand the programmatic changes that occur during epithelial to mesenchymal transitions (EMT). Protecting epithelial cells is critical to protect and prolong organ function. Mouse models also demonstrated the importance of bone morphogenic protein 7 (BMP7) and activin-like kinase 3 (ALK3) in fibrotic development.

The activation and accumulation of myofibroblasts is another area of active research in Dr. Kalluri's laboratory. He described an ambitious project to create 37 transgenic mice labeled with specific tags for each compartment, all of which would be available to the scientific community. His research group also is performing experiments to eliminate proliferating cells, which will help to elucidate fibrotic mechanisms. Bone marrow transplantation is another technique to identify the source of proliferating myofibroblasts.

Epigenetic mechanisms might contribute to fibrosis. The hypermethylation of RAS protein activator like-1 (*RASAL1*) can induce proliferation and collagen deposition, and attenuating the RAS pathway might help to inhibit fibrosis. Dr. Kalluri is investigating whether patients progress faster depending on the state of hypermethylation indicated in core biopsies.

Discussion

A participant noted that his experiments show smooth muscle actin (SMA) staining co-localizing with TGF-β labeling. Dr. Kalluri explained that his TGF-β reporter mice do not exhibit the same co-localization, and he commented that the reason might be antibody-specific. Antibodies behave differently in diverse systems. Dr. Kalluri indicated that lineage tracing would be a defining experiment.

Fibrosis in Hematologic Disorders: Monitoring Dysfunction and Recovery following Hematopoietic cell Transplantation

H. Joachim Deeg, M.D., Fred Hutchinson Cancer Research Center, Seattle, WA

Dr. Deeg focused on the impact of hematopoietic cell transplantation (HCT) on marrow fibrosis. He pointed out that the signals that lead to fibrosis development in the marrow of patients with hematologic disorders (myeloproliferative neoploasms [MPN]) are derived from the clonal cells that constitute the disease. Therefore, in MPNs (e.g., primary myelofibrosis, polycythemia vera, essential thrombocythemia), effective eliminating of clonal cells will lead to regression of the fibrosis. Although not included in any of the classifications schemes for MPN, osteosclerosis, often developing in patients with longstanding fibrosis, is associated with a poorer prognosis. Marrow fibrosis also occurs with other disorders, however, such as autoimmune diseases and infections (e.g., tuberculosis), in which case the underlying diagnosis needs to be treated.

Dr. Deeg indicated that particularly with primary myelofibrosis, fibrosis occurs at extramedullary sites, and likely develops in a time-dependent manner. Dr. Deeg presented data on fibrosis in extramedullary sites from patient biopsies, including sinusoidal fibrosis in the liver, and extramedullary hematopoiesis and fibrosis in the lung.

There is ample evidence that fibrosis is associated with cytokine dysfunction. Data from a study at the Mayo Clinic showed dysregulation of circulating cytokines, including interleukin 8 (IL-8), which was associated with reduced leukemia-free survival, and cytokines that were associated with the presence of the JAK2 mutation; there was no correlation, however, with fibrosis. Recent studies by Dr. Ronald Hoffman at Mount Sinai Hospital in New York showed also showed altered tissue cytokine expression, including upregulation of CXCL12, in patients with MPN, involving marrow stromal cells, endothelial cells, and osteoblasts, in a pattern quite different from that observed in the spleen. Based on these results, Dr. Hoffman has hypothesized that enhanced production of cytokines attracts clonal cells preferentially to the marrow cavity, creating a "cancer-activated niche," which might favor propagation of clonal cells by enhanced production of cytokines with fibrogenic potential. Such a hypothesis would be analogous to a model proposed for myelodysplastic syndromes (MDS). In addition,studies have shown striking increases in TGF-β, implying that treatments blocking TGF-β -dependent signaling might be beneficial. To date, however, all studies using molecules targeting TGF-β have been negative for patients with MPNs. Additional findings on *in situ* expression of cytokines show that fibroblasts from spleens of patients with primary myelofibrosis express high levels of alpha smooth muscle actin and vimentin, and that these fibroblasts facilitate proliferation of clonal CD34+ cells at the expense of nonclonal precursors. Dr. Deeg presented a general model in which clonal hematopoietic stem cells affect the microenvironment, leading to production of fibrotic extracellular components that modify the microenvironment in a direction that favors that the clone(s).

The current, most widely used schemes for risk classification for nontransplanted patients, are the Dynamic International Prognostic Scoring System (DIPPS) and the DIPPS plus, which do not include fibrosis as a risk factor for prognosis. However, several studies have shown that the DIPPS (plus) risk categories correlate with life expectancy in nontransplanted patients. The main causes of death in nontransplanted patients are infections, marrow failure, leukemic transformation, and emaciation. Currently, one JAK2 inhibitor (ruxolitinib) has been approved to treat patients with primary myelofibrosis, and several others are under investigation.

Allogeneic hematopoietic cell transplantation is at present the only definitive therapy for primary myelofibrosis (and other MPNs), but only between 5 and 15 percent of patients are actually undergoing transplantation. Dr. Deeg suggested that multiple factors that are not included in the MPN prognostic classifications might be important for transplant success, including severity of marrow fibrosis and

fibrosis in other organs, spleen size, duration of the disease, and mutations. A study of post-transplantation survival of 170 patients showed differences in outcome depending on DIPPS classification. Surprisingly, these variations in survival resulted primarily from nonrelapse mortality, possible related to extramedullary fibrosis and the associated complications in nonhematopoietic organs. Dr. Deeg presented data showing regression of fibrosis and osteosclerosis and rebuilding of normal marrow architecture following transplantation.

Discussion

Dr. Joel Rosenbloom, Jefferson Medical College, asked which drugs had been used in attempts to block TGF-β signaling. Dr. Deeg responded that he did not have specific information, but monoclonal antibodies have been use..

FDA PROCEDURES FOR QUALIFICATION

Moderator: *Michael Flessner, M.D., PhD, NIDDK, NIH, Bethesda, MD*

Dr. Flessner encouraged the meeting participants to apply for the NIDDK's Request for Applications (RFA) DK-13-026: Novel Methods for Detection and Measurement of Organ Fibrosis in Kidney, Bone Marrow, and Urological Diseases. He stated that one element required for the grant proposal is an understanding of FDA procedures to qualify a new biomarker and/or device. The presentations by FDA staff were designed to inform the participants how to approach this component of the RFA.

Perspectives on Biomarker Test Validation From the Office of *In Vitro* Diagnostics and Radiological Health/Center for Devices and Radiological Health (OIR/CDRH) at the FDA

Kellie Kelm, Ph.D., OIR, FDA, Silver Spring, MD

Dr. Kelm stated that the CDRH regulates investigational clinical tests that may be developed for marketing purposes. Dr. Kelm outlined the following questions to be addressed in her presentation: What are biomarkers? What are biomarker tests? What are they used for? How are they regulated? What is required for biomarker validation?

Dr. Kelm specified that a biomarker is not a test, it is a characteristic; a biomarker test is a method to measure the biomarker. Biomarker tests are used for a variety of purposes, including screening, diagnosis, and monitoring of progression or treatment response. Biomarker tests must demonstrate clinical and analytical validity for their intended use. Regulation depends on the intended use of the device, and tests are classified by the intended use including the risk of incorrect results (i.e., the consequences of a false result). Biomarker tests are employed in drug discovery; preclinical research; and clinical settings, including patient management and research. As diagnostic markers, they can identify and/or classify diseases; screen populations, often those that are asymptomatic; monitor for recurrence; and select responders to specific drugs. As prognostic markers, they are used to stratify disease by severity and or risk; predict disease development and progress; and identify the patients who are at risk for an adverse drug reaction. Biomarker tests are sought as surrogate endpoints for diagnosis and prognosis, but validation for surrogate endpoints is difficult and requires special attention when validating decision points.

The risk of an incorrect result is evaluated prior to granting regulatory approval, and experiments must be conducted to understand test sensitivity, specificity, positive predictive value, and negative predictive value. Evaluating effectiveness involves considering the sensitivity and specificity of the test. Sensitivity—measured by a test's positive predictive value—indicates how likely it is for a patient with a

positive test result to have the disease, whereas specificity—measured by a test's negative predictive value—indicates the likelihood that a patient with a negative test result does not have the disease. Predictive value depends on disease prevalence; if prevalence is very low, even a highly sensitive and specific test will result in many false positives. Biomarker tests therefore must be validated in the intended use population .

Dr. Kelm presented several examples of relevant FDA submissions and potential challenges. She emphasized that study design is important for unbiased evaluation of effectiveness and reliability.

Discussion

Dr. Taylor asked about the level of evidence that is necessary and sufficient to approve a biomarker from a regulatory perspective. Dr. Kelm answered that the tests must be validated properly. FDA is concerned when relevant parts of study populations are excluded during biomarker test studies. .

Dr. Garra asked whether there is an advantage in registering a biomarker if a pharmaceutical company wants to prove the efficacy of a drug. Dr. Kelm responded that there will be several additional talks about biomarker qualification and imaging, and those speakers will be better at answering this question. FDA provides three pathways for marketing devices and regulatory approval of biomarkers for drug development.

Biomarker Qualification at the Center for Drug Evaluation and Research (CDER), FDA
Shashi Amur, Ph.D., Office of Translational Sciences, CDER, FDA, Silver Spring, MD

Dr. Amur presented an overview of the role of biomarkers in drug development and the process of biomarker qualification. The Critical Path Initiative at the CDER is FDA's effort to stimulate and facilitate a national effort to modernize the scientific process by which a potential human drug, biological product, or medical device is transformed from discovery or "proof-of-concept" into a medical product. In 2004, a white paper was published about how to remedy gaps, addressing the questions of why the drug development pipeline is so slow, and why investment in research and development is not translating into clinical products. This was followed by a white paper, Critical Path Opportunities List and Report,,about different therapeutic areas that require the development of newdrugs/biologics. Suggested improvements to the drug development pipeline include developing better evaluation tools, streamlining clinical trials, using biomarkers in drug development, and expediting drug development pathways.

The NIH defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or biological responses to a therapeutic intervention." Biomarkers can be applied for different purposes during drug development, from understanding disease heterogeneity and safety assessment in nonclinical studies and clinical trials, to development of a companion diagnostic needed for clinical decision making before prescribing the therapeutic. The goals of biomarker qualification are to provide a framework for scientific development and regulatory acceptance of biomarkers for use in drug development, facilitate integration of biomarkers in the regulatory review process, and encourage the identification of new and emergent biomarkers for evaluation and utilization in the regulatory review process.

The biomarker qualification process consists of the following three main stages: initiation, consultation and advice, and review. Currently, the CDER has four types of biomarkers (i.e., preclinical safety, clinical safety, patient selection, and activity/efficacy response biomarkers) at various stages of the qualification process. Retrospective data from completed clinical trials and studies can be used for the qualification process using a prospective statistical analysis plan; new data need not be generated for the qualification.

Ninety percent of submissions to the biomarker qualification process are from consortia and groups due to the significant resources necessary for biomarker development.

Discussion

Dr. Star asked where a biomarker submission should be sent if it is not useful in drug development. Dr. Amur stated that the FDA will ensure that the applications/submissions are sent to the correct centers/programs; for example, device/assay submissions to be used in clinical practice are cleared/approved by CDRH (center for devices and radiological health).

Dr. Seth Porter, FibroGen, Inc., discussed the findings of a clinical trial that he is involved in to use imaging methods for correlating fibrosis with pulmonary function. He asked whether it would be appropriate to go through the process of establishing a biomarker with the FDA. Dr. Amur replied that with sufficient data to support the biomarker, it would make sense to apply for the qualification. She suggested writing a letter of intent to the Biomarker Qualification Program. In the scenario where a biomarker needs to be integrated in the development of a NME (New Molecular Entity),Dr. Amur suggested that they discuss the possibility with the review division in the context of an IND/NDA/BLA.

Device/Technique Qualification
Brian Garra, M.D., Office of Science and Engineering Laboratories, FDA, Silver Spring, MD

Dr. Garra discussed current efforts in fibrosis imaging and quantification, which have focused on the liver. Established methods (i.e., computed tomography [CT], ultrasound, and MRI) are useful only for changes in organ morphology because of sensitivity issues. More recent methods (ultrasound backscatter analysis and elastography) are potentially more sensitive and quantitative but are not ready for clinical use. Ultrasound backscatter analysis, introduced in the 1980s, produces images by ultrasound or radiofrequency (RF) signal analysis. The methodological problems of ultrasound backscatter analysis were found to outweigh its ability to classify liver fibrosis correctly, however, and it was not adapted in the clinical setting.

In the early 1990s, elastography, which detects fibrosis by increased organ stiffness, was introduced. The two major types are strain elastography, which compares data pre- and postcompression of the organ, and shear wave elastography, which estimates the velocity of shear waves induced in tissues by applying a rapid compression. Dr. Garra provided example elastogram images of a localized breast tumor and a diffuse breast carcinoma, as well as an Acoustic Radiation Force Impulse (ARFI) elastographic image of thyroid nodules. Elastogram quantification for strain elastography depends on comparing the strain of the area of interest to that of "normal" tissue, whereas in shear wave elastography, it is estimated from the shear wave velocity and tissue density. In one study, shear wave elastography with quantification was able to discriminate between adjacent benign and malignant breast lesions. In MR elastography, shear waves of known frequency are transmitted into tissue, and the shear wavelength is correlated with tissue stiffness. Dr. Garra showed high-quality MR elastographic images that distinguish between a fibrotic and healthy liver. In addition to the liver, elastography is applicable to almost every other organ except the lung and bone marrow. Elastographic images are highly equipment-dependent, however, and confounded by biological factors such as inflammation and the presence of overlying fluid or soft tissue.

Dr. Garra described two qualification efforts for fibrosis estimation: the Radiological Society of North America (RSNA) Quantitative Imaging Biomarker Alliance (QIBA) and FDA's biomarker qualification program. The QIBA assesses candidate biomarkers and qualifies selected biomarkers for particular clinical purposes; QIBA selected shear wave speed (SWS) for liver fibrosis. Dr. Garra reported that the QIBA technical committee progress on SWS qualification has proceeded through completion of a draft QIBA profile of expected ranges of results from particular instruments and settings. The FDA has a two-

stage process for imaging biomarker approval: clearance or approval of the device used and qualification of the biomarker or surrogate for use in drug evaluation. The FDA considers performance and labeling requirements for new measurements, including a training program, which is part of the QIBA process. FDA's biomarker qualification program supports group efforts by multiple sponsors to establish biomarkers, as is the case for SWS. Dr. Garra indicated that FDA approval is an iterative process that can take several years and is best begun early in development. Biomarker qualification is for a specific "context of use" for application in decision making. Biomarker targets include confirming diagnoses, treatment selection, and monitoring treatment response. Dr. Garra noted that meeting the requirements for reimbursement of insurers and the Center for Medicare and Medicaid Services (CMS), which requires well-designed clinical trials, generally is a much longer and more stringent process than FDA approval.

Discussion

A participant asked a general question about toxicity and safety studies for biomarkers and whether any biomarkers were noninjectables. Dr. Garra noted that QIBA performs safety studies and indicated that the FDA's decision on whether a biomarker is to be considered a device or a drug is made internally. Dr. Amur clarified that ingested agents are considered to be drugs.

A participant stated that given the high annual cost of dialysis treatment, he was surprised that biomarker efforts are not underway for kidney disease. Dr. Garra responded that one of QIBA's selection criteria for biomarkers is that qualification is likely within 3 years.

SUMMARY

Robert A. Star, M.D., NIDDK, NIH, Bethesda, MD
Michael Flessner, M.D., Ph.D., NIDDK, NIH, Bethesda, MD

Dr. Star expressed appreciation to the meeting participants for braving the cold weather to attend the excellent meeting. He emphasized that fibrosis is pervasive across all organ systems relevant to the NIDDK's mission. Dr. Star commented on the important topics discussed at the meeting, including the identification of disagreements in the field related to identifying the cells that are important in the development of fibrosis, identifying the signaling molecules that mediate the fibrotic response, and selecting the best tools to use to better understand the underlying mechanisms. He explained that there is a funding mechanism within NIDDK's Division of Kidney, Urologic, and Hematologic Diseases (KUH) to resolve any major discrepancies in the field (e.g., to identify the origin of fibrosis-producing cells). The laboratories involved in the scientific dispute need to develop a single protocol, agree to perform the same experiments in both laboratories, and publish the results together.

Dr. Star reminded participants about the RFA, due March 2014, which will address how to translate ideas into methods of detecting and measuring fibrosis in KUH organs. Applications for minimally invasive or noninvasive animal or human studies are being solicited because of the great need to capture fibrotic changes early. Future studies might investigate the existence of different types of collagen, detection of organ burden, or hysteresis of fibrotic changes to better measure and understand fibrotic pathology. Methods to identify cell types, investigate the signaling mediators (many of which were mentioned during the meeting, including IL-10 and IL-13), or measure using urinalysis are welcome. Dr. Star noted the many potential intervention targets identified during the meeting, as well as the description of state-of-the-art contrast methods (e.g., MRI, CT).

Dr. Star thanked the attendees for coming together to share ideas and contribute feedback about fibrosis across the organ systems. He commented that even understanding schistosomiasis, for example, can help researchers understand what might be occurring in the kidney. Dr. Star expressed his appreciation to the speakers, participants, and planning committee for their efforts, especially the planning committee

members who essentially had planned the meeting twice due to the government shutdown-related cancellation of the fall meeting. Drs. Flessner and Star adjourned the meeting and wished the participants safe travels.