Characterization of Fibrillar Collagens and Extracellular Matrix of Glandular Benign Prostatic Hyperplasia Nodules

Wei Huang, MD
Department of Pathology and Laboratory Medicine, University of Wisconsin—Madison

OBJECTIVE: Recent studies have associated lower urinary tract symptoms (LUTS) in men with prostatic fibrosis, but a definitive link between collagen deposition and LUTS has yet to be demonstrated. The objective of this study was to evaluate ECM and collagen content within normal glandular prostate tissue and glandular BPH, and to evaluate the association of clinical parameters of LUTS with collagen content. METHODS: Fibrillar collagen and ECM content was assessed in normal prostate (48 patients) and glandular BPH nodules (24 patients) using Masson's trichrome stain and Picrosirius red stain. Second harmonic generation (SHG) imaging was used to evaluate collagen content. Additional BPH tissues (n = 47) were stained with Picrosirius red and the association between clinical parameters of BPH/LUTS and collagen content was assessed. RESULTS: ECM was similar in normal prostate and BPH (p = 0.44). Total collagen content between normal prostate and glandular BPH was similar (p = 0.27), but a significant increase in thicker collagen bundles was observed in BPH (p = 0.045). Using SHG imaging, collagen content in BPH (mean intensity = 62.52; SEM = 2.74) was significantly higher than in normal prostate (51.77+/-3.49; p = 0.02). Total collagen content was not associated with treatment with finasteride (p = 0.47) or alpha-blockers (p = 0.52), pre-TURP AUA symptom index (p = 0.90), prostate-specific antigen (p = 0.86), post-void residual (PVR; p = 0.32), prostate size (p = 0.21), or post-TURP PVR (p = 0.51). Collagen content was not associated with patient age in patients with BPH, however as men aged normal prostatic tissue had a decreased proportion of thick collagen bundles. CONCLUSIONS: The proportion of larger bundles of collagen, but not total collagen, is increased in BPH nodules, suggesting that these large fibers may play a role in BPH/LUTS. Total collagen content is independent of clinical parameters of BPH and LUTS. If fibrosis and overall ECM deposition are associated with BPH/LUTS, this relationship likely exists in regions of the prostate other than glandular hyperplasia.

Resident Perivascular Cells and Organ Fibrosis

Benjamin D. Humphreys, MD, PhD
Brigham and Women's Hospital, Boston, MA

Tissue fibrosis, or scar formation, is the common final pathway of virtually all progressive diseases and it accounts for up to 45% of all deaths in the industrialized world. Fibrosis inflicts damage in every major solid organ including kidney, heart, lung and liver as well as soft tissues such as blood vessels, skin, and skeletal muscle. Scar tissue can form after an acute insult, or more slowly as a result of years of chronic injury/agitation from a separate underlying malady. Fibrotic matrix may initially aid in the tissue repair process, and even subside in cases of mild injury as functional tissue regenerates. However, during chronic or repetitive injury, fibrotic matrix deposition goes unchecked, slowly disrupting tissue architecture and choking blood supply, preventing

normal function, and inhibiting repair ultimately leading to organ failure. Tissue scarring destroys the kidneys of diabetic or hypertensive patients, the liver of hepatitis C patients, the heart of patients with hypertension or cardiomyopathy, and the lungs of patients with idiopathic pulmonary fibrosis.

Novel and targeted therapies are urgently needed to treat the vast and growing patient population suffering from organ fibrosis. In every organ and tissue fibrosis is driven by cells called myofibroblasts; contractile cells that secrete matrix proteins and multiply during fibrotic injury. The source of progenitor cells that can differentiate into myofibroblasts is highly controversial and debated in all disciplines and organs. A major limiting factor is that no one has identified a common protein that identifies the majority of these cells in fibrosis across organs. We have now identified a previously unrecognized kidney resident cell type defined by expression of the transcription factor Gli1 and our experimental evidence suggests these are the critical myofibroblast progenitor population, and that they are mesenchymal stem cells (MSC). Using solid organ FACS isolation we have determined that these cells are CD31-, F4/80-, CD45-, PDGFR□+, Sca1+, CD29+, CD105+, a consensus MSC surface profile. They represent a very small subset of the total PDGFR□+ pericyte/fibroblast pool, yet they expand by 20-fold in kidney fibrosis models. Critically, we have proof-of-principle evidence that these cells are therapeutic targets because their genetic ablation ameliorates fibrosis in the unilateral ureteral obstruction fibrosis model. These observations suggest this new cell type is the long sought true myofibroblast progenitor. Intriguingly, these cells are also present in heart, where they play important roles in myocardial hypertrophy and cardiomayopathy in heart failure models.

This finding should enable a more precise understanding of the biology of organ fibrosis, and illuminate therapeutic pathways that might be targeted to prevent organ failure in progressive fibrotic disease. I will provide preliminary evidence that drugs targeting Gli1+ cells have strong potential as anti-fibrotic drugs in chronic kidney disease.

Molecular changes of fibrosis during cancer pathogenesis

Patricia J Keely, PhD
Department of Cell and Regenerative Biology, University of Wisconsin—Madison

Long before carcinomas develop, there are early changes in cells and tissue structure. Moreover, conditions of wounding and inflammation often precede tumorigenesis. We seek to understand the changes in collagen structure and organization that accompany and may contribute to tumor progression, and how cells respond to those changes. We utilize animal and culture models and state-of-the-art multiphoton, SHG, and fluorescent lifetime imaging approaches to assist in our goals. We have helped to define collagen reorganization at the tumor/stromal boundary, demonstrating that aligned collagen facilitates invasion. Moreover, we find that changes in local collagen structure affect the expression of genes related to proliferation, cell migration, and cellular metabolism. We

also note that fibrosis surrounding tumors contributes to, and results from, the infiltration of inflammatory cells.

Macrophage Subsets in Fibrosis

Thomas A. Wynn, PhD

Program in Tissue Immunity and Repair, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Macrophages are found in close proximity with collagen-producing myofibroblasts and play key roles in the pathogenesis of fibrosis. They produce growth factors and pro-fibrotic mediators that directly activate fibroblasts, including transforming growth factor beta, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived growth factor. They also regulate extracellular matrix turnover by influencing the balance of various matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. Macrophages also regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells and by producing various inflammatory and anti-inflammatory cytokines. With their potential to act in both a pro- and anti-fibrotic capacity at distinct stages of the wound healing response, macrophages and the factors they express are integrated into all stages of the fibrotic process. These various and sometimes opposing functions are performed by distinct macrophage subpopulations. In this presentation I will describe our recent studies that have focused on elucidating the regulatory role of macrophages and specific macrophage subpopulations in the pathogenesis of fibrosis.