
Preface

Fibrosis or scar, defined pathologically as inappropriate repair by connective tissue, is increasingly recognized as an important feature of many chronic diseases (Table 1), and as such, represents an enormous health burden. The United States government estimates that 45% of deaths in the United States can be attributed to fibrosing disorders. Fibrosis can affect virtually every tissue and organ system. Traditionally, fibrosis has been viewed as the irreversible, end-stage sequel to a multitude of diverse disease processes (Table 2). Excessive scarring following physical, thermal, metabolic, ischemic, infectious, inflammatory, or immunological injury can occur in any part of the body, and can cause destruction of the affected structures. Fibrotic tissue is characterized by a loss of normal architecture, paucity of stromal cells, and replacement of blood vessels and other essential parenchymal structures by dense, homogeneous, and increasingly stable extracellular matrix. The scar is composed primarily of type I collagen, but types III and IV collagens, proteoglycans, fibronectin, glycoproteins, and matricellular proteins are also prominent. The process leads to progressive distortion of tissue architecture with consequent dysfunction and ultimate failure of fibrotic organs. Many of the key morphological features of fibrosis are common to scarring affecting the lungs, the liver, the kidneys, the heart, or the skin.

Fibrosis is the “dark side” of normal tissue repair. Following injury, a complex and tightly orchestrated repertoire of cellular responses is called into play, and normally the wound is rapidly and efficiently repaired. This process is spatially and temporally self-limited. In contrast, under some conditions repair is excessive, resulting in pathological scar formation. The pathogenesis of fibrosis remains poorly understood. Fibrosis involves a “fibrogenic cascade” integrating multiple molecular pathways and cellular targets. Historically, the link between inflammation and fibrosis has been emphasized, providing the rationale for anti-inflammatory or immunosuppressive therapies for fibrosis. It has become increasingly evident that these types of interventions are generally ineffective. The lack of effective treatments, and the high mortality and increasing morbidity attributed to chronic fibrotic diseases, has stimulated an explosion of research into the cellular, molecular, and genetic basis of fibrosis.

Recent research progress has led to new and evolving concepts regarding the mechanisms that drive the process of fibrosis. Novel insights include the identification and characterization of an increasingly large panel of molecules

Table 1**Selected Diseases Where Fibrosis Is a Major Cause of Morbidity or Mortality***Organ-specific*

Lung fibrosis (idiopathic and drug-induced pulmonary fibrosis, asthma, sarcoidosis, COPD)

Liver fibrosis (alcoholic cirrhosis, posthepatitis C cirrhosis, schistosomiasis, primary biliary cirrhosis, sclerosing cholangitis)

Kidney fibrosis (diabetic nephropathy, lupus glomerulonephritis)

Heart fibrosis (post-MI scar)

Vascular fibrosis (postangioplasty arterial restenosis, atherosclerosis)

Skin fibrosis (burn scar, hypertrophic scar, keloid, nephrogenic fibrosing dermatopathy)

Eye fibrosis (retro-orbital fibrosis, postcataract surgery, proliferative vitreoretinopathy)

Bone marrow (idiopathic and drug-induced myelofibrosis)

Other (Peyronie's disease, thyroid, Dupuytren's contracture, dermatomyositis)

Systemic

Systemic sclerosis

Chronic graft vs host disease

Table 2**Association of Fibrosis With Types of Injury: Representative Examples**

Burn	Skin and soft tissue scarring and contraction
Physical	Hypertrophic scar, keloid, postangioplasty vascular restenosis
Radiation	Pulmonary fibrosis; skin fibrosis; myelofibrosis
Surgery	Adhesions of visceral organs
Metabolic	Alcoholic cirrhosis, diabetic nephropathy, dialysis-related fibrosing dermatopathy
Ischemic	Post-MI cardiac fibrosis, post-stroke brain scarring
Autoimmune	Systemic sclerosis, myositis, lupus nephritis, chronic graft vs host disease
Inflammatory	Retroperitoneal fibrosis, asthma, sarcoidosis, atherosclerosis, primary biliary cirrhosis
Infectious	Hepatitis C-associated cirrhosis, schistosomal cirrhosis, HIV-associated lymphoid fibrosis
Malignant	Myelofibrosis

that mediate abnormal deposition of extracellular matrix, as well as the cellular constituents that produce and/or react to these molecules. Significant unanswered issues involve basic cellular and molecular mechanisms related to cell proliferation, motility, differentiation, and gene expression. For instance, recent discoveries on the heterogeneity of fibroblast populations in fibrotic lesions have relevance to understanding cellular differentiation and program of gene expression that determines cell phenotype. The demonstration of epithelial–mesenchymal transdifferentiation as a key element in the pathogenesis of renal fibrosis illustrates the need for further research into basic mechanisms to advance the field. The importance of the myofibroblastic phenotype requires improvement in current understanding of how activation and/or transdifferentiation is regulated in the context of progressive fibrosis. Elucidation of mechanisms involved in the regulation of extracellular matrix production and degradation is critical for understanding the basis for the abnormal deposition of connective tissue elements that is the hallmark of fibrosis. The advent of powerful techniques for global assessment of gene expression in cells and tissues should prove an impetus for rapid progress in this area. At the tissue and organismal level, improvement in methods for evaluating the extent, severity, and activity of fibrosis is essential for more precise and less invasive diagnostic and prognostic assessment, as well as for monitoring of therapeutic efficacy.

It is now widely appreciated that the diverse fibroproliferative diseases, be they immunological, metabolic, inflammatory, genetic, or iatrogenic in nature, all have important pathogenetic features in common. That is to say, despite their differing anatomic distribution and clinical manifestations, etiology and natural history, the pathogenesis of these disorders appears to be related. Thus, regardless of whether fibrosis involves the lung parenchyma or airways, the renal glomerulus or interstitium, the parenchyma or biliary tree of the liver, the gastrointestinal tract, the heart, the dermis or tendons, or the eye, in each case secretion and activation of profibrotic cytokines, particularly transforming growth factor (TGF)- β , expansion and activation of mesenchymal cell populations, and extracellular matrix synthesis and organization similarly result in progressive destruction of normal tissue (Table 3).

Fibrosis research is an emerging field. The challenges facing investigators engaged in fibrosis research are many: to describe and annotate the full repertoire of fibrotic mediators and effector cells involved in the fibrotic response, and characterize their deregulation in pathological fibrosis; to identify genes and their variants linked to fibrosis, and define their role in pathogenesis; to recognize shared pathogenetic themes that are common to distinct forms of fibrosis; and ultimately, to translate these insights into the development of specific

Table 3**Underlying Pathophysiologic Processes Common to Different Fibrotic Diseases***Genetic susceptibility**Inflammation*

T-cell activation, Th2 polarization; macrophage activation; eosinophil and mast cell chemotaxis; cytokine and chemokine production
Vascular injury, endothelial adhesion molecule upregulation

Mesenchymal cell activation

Increased synthesis of extracellular matrix
Secretion and activation of TGF- β , other fibrotic cytokines, autocrine stimulation and amplification
Migration

Cell differentiation

Myofibroblast transdifferentiation
Epithelial–mesenchymal transition
Bone marrow-derived mesenchymal progenitor cell influx and differentiation

Scar formation and persistence; tissue destruction

Connective tissue contraction
Blood vessel obliteration, defective vasculogenesis, tissue hypoxia
Matrix stabilization, collagen crosslinking
Defective matrix remodeling and degradation

and sensitive diagnostic tools and safe and effective therapies to slow, prevent, and reverse organ fibrosis. For this undertaking to succeed, interactions between laboratory researchers and clinical investigators and clinicians from many different specialities is a must, since fibrosis impacts all fields of Medicine. The 22 chapters of *Fibrosis Research: Methods and Protocols* highlight the interdisciplinary nature of fibrosis research, and emphasize the broad applicability of these experimental methodologies to all areas of fibrosis research.

The book is divided into four parts. Part 1 features a brief introduction to the problem of fibrosis, with clinical overviews of pulmonary and renal fibrosis as examples illustrative of the many fibrosing disorders listed in Table 1, and a bird's-eye view of TGF- β as a paradigm profibrotic mediator. Part 2 focuses on widely used research methodologies utilizing cultured cells to model various aspects of the fibrotic response *in vitro*. The isolation, characterization, and propagation of mesenchymal cells are described, highlighting similarities and differences between methods that are appropriate for different types of

fibroblasts. Chapters 7 to 10 discuss approaches for studying collagen gene regulation and TGF- β production. Part 3 focuses on experimental methodologies utilizing animal models to study the pathogenesis of fibrosis, discussing the advantages and limitations of each. Part 4 covers the evolving genetic approaches to identifying “fibrosis genes” or allelic polymorphisms in human populations, microarray studies for describing global patterns of gene expression associated with fibrosis, and proteomic approaches to the same.

As editors of this new and innovative book, *Fibrosis Research: Methods and Protocols*, we hope that it will reach a broad audience, and prove to be helpful to investigators studying any aspect of pathological fibrosis. It is our further hope that the book will stimulate discussion and promote interdisciplinary research collaborations, with the goal of understanding the daunting problem of fibrosis.

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