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Fibrosis

Methods and Protocols

Edited by

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Preface

The term “fibrosis” designates the formation of excess fibrous connective tissue that can affect a plethora of tissues and organs. Extracellular matrix (ECM) deposition is typically a normal reaction to injury that allows tissue repair and restoration of tissue strength. However, when the response turns awry, ECM deposition becomes pathogenic and results in thickened extracellular environment that hampers tissue properties and may lead to organ failure.

Fibrosis’ prevalence within so many organs makes it quite complex to study. Researchers thereby rely on multiple in vitro, ex vivo, and in vivo models aimed at recapitulating one of several aspects of the fibrotic reaction. *Fibrosis: Methods and Protocols* was crafted with the objective of creating a “bench manual” for scientists concentrating on fibrosis research. This volume compiles a collection of state-of-the-art protocols that will serve not only as recipes for bench scientists but also as accepted methods for the field. For each chapter, experts in their respective field present their routinely used method to study one aspect of fibrosis and, maybe most importantly, their most helpful tips, organized in “Notes.” They also detail, in the “Introduction” section, the advantages and limitations of their method. Lastly, most chapters are illustrated with examples of experimental settings, screenshots, or typical results, meant to ensure a detailed comprehension of the method for accurate execution and replication.

In this volume, the reader will first find a thorough perspective encompassing the clinical scope of fibrosis, an up-to-date review of the molecular mechanisms leading to the development of tissue fibrosis, and an overview of the current challenges of fibrosis research. This perspective will serve as the introduction of *Fibrosis: Methods and Protocols*, which is organized in four parts. Part I focuses on animal models of fibrosis. Nine chapters will provide detailed protocols on how to mimic fibrosis in the lung, the skin, the liver, the cornea, and the heart, and how to generate transgenic mouse models overexpressing a gene of interest in fibroblasts, predominant mesenchymal cells activated during fibrosis.

Part II focuses on cell culture systems. Four chapters provide detailed methods for studying the cell types that are increasingly viewed as important for the development of fibrosis, i.e., hepatic stellate cells, adipose-derived stromal cells, dermal cell populations, and peripheral tissues’ mast cells. Four additional chapters will concentrate on cell culture models aimed at studying the biomechanical influence that the ECM exerts on cells: the cell-populated collagen lattice model, the deformable microposts model, the hydrogels, and the decellularized lung matrices model.

Part III focuses on the purification, quantification, and analysis of the ultimate architects of fibrosis: the ECM proteins. The reader will find protocols to isolate type I collagen and fibronectin for in vitro experimentation purposes, to isolate and quantify transcripts from laser capture-microdissected tissue, to quantify collagen I, TGF β , and elastin proteins in tissues, to study collagen assembly in vitro, and to specifically identify fibrillar collagens in tissue samples by picro-Sirius red staining or by second harmonic generation (SHG) imaging (a stain- and dye-free imaging technique specific for collagen).

Lastly, Part IV focuses on the more modern optical and computational methods. The reader will find tremendously useful protocols describing computer-assisted methods for quantifying fibrillar collagen alignment, exploring the nano-surface of collagen with atomic force microscopy (AFM), for enhancing the quality of multiplex staining using spectral unmixing, and for interrogating the increasingly abundant deposited gene expression datasets commonly seen as intimidating for the non-bioinformatician, but no longer intimidating after reading this chapter.

In all, *Fibrosis: Methods and Protocols* was crafted by 94 scientists and physicians (40% female) dispersed on thirteen countries and four continents. I wish to thank each of them for their tremendous contribution: be rewarded in that each chapter will undoubtedly be very useful to many investigators around the world. It has been my pleasure to work with you and bring our readers a volume that I believe will make a difference in ascertaining quality and repeatability of research experiments in the fibrosis field. Readers, please share your appreciation by citing the chapter(s) you used for your experiments. I would also like to thank the Series editor, John Walker, for his guidance during the process of compiling this volume and acknowledge the executive staff at Springer for their logistical help and support. Nurturing this book has been a great journey. I learned a lot, and I encourage scientists at all levels to do the same and jump in the adventure of becoming an ad hoc editor.

Ann Arbor, MI, USA

Laure Rittié

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