METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

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Fibrosis

Methods and Protocols

Edited by

Laure Rittié

Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI, USA; GlaxoSmithKline, Dermatology Therapeutic Area, Collegeville, PA, USA

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Editor
Laure Rittié
Department of Dermatology
University of Michigan Medical School
Ann Arbor, MI, USA

Dermatology Therapeutic Area
GlaxoSmithKline
Collegeville, PA, USA

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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, TGFβ, 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*

Contents

Contributors

Johann Bauersachs • *Department of Cardiology and Angiology, Medical School Hannover, Hannover, Germany*

George Bou-Gharios • *Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK*

Scott N. Byrne • *Cellular Photoimmunology Group, Discipline of Infectious Diseases and Immunology, Sydney Medical School at The Charles Perkins Centre, University of Sydney, Sydney, Australia*

Héctor Capella-Monsonís • *Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway (NUI Galway), Galway, Ireland; Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway (NUI Galway), Galway, Ireland*

Grace Chu • *Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK*

Riccardo Cicchi • *National Institute of Optics, National Research Council (INO-CNR), University of Florence, Sesto Fiorentino, Italy; European Laboratory for Non-linear Spectroscopy (LENS), University of Florence, Sesto Fiorentino, Italy*

João Quintas Coentro • *Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway, Galway, Ireland; Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Galway, Ireland*

Benoit de Crombrugghe • *MD Anderson Cancer Center, The University of Texas, Houston, TX, USA*

Elaine C. Davis • *Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada*

Rodney J. Dilley • *Ear Science Institute Australia, Perth, Australia; School of Surgery, and Center for Cell Therapy and Regenerative Medicine, University of Western Australia, Perth, Australia*

Jie Ding • *Wound Healing Research Group, Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Alberta, Edmonton, AB, Canada*

Cole R. Drifka • *Laboratory for Optical and Computational Instrumentation, University of Wisconsin at Madison, Madison, WI, USA; Department of Biomedical Engineering, University of Wisconsin at Madison, Madison, WI, USA; Morgridge Institute for Research, Madison, WI, USA*

Beate Eckes • *Department of Dermatology, University of Cologne, Cologne, Germany*

Kevin W. Eliceiri • *Laboratory for Optical and Computational Instrumentation, University of Wisconsin at Madison, Madison, WI, USA; Department of Biomedical Engineering, University of Wisconsin at Madison, Madison, WI, USA; Morgridge Institute for Research, Madison, WI, USA*

Farzad Fereidouni • *Department of Pathology and Laboratory Medicine, University of California-Davis Medical Center, Sacramento, CA, USA*
Gary J. Fisher • *Department of Dermatology, Medical School, University of Michigan, Ann Arbor, MI, USA*
Daniela Fraccarollo • *Department of Cardiology and Angiology, Medical School Hannover, Hannover, Germany*
Paolo Galuppo • *Department of Cardiology and Angiology, Medical School Hannover, Hannover, Germany*
Aziz Ghahary • *BC Professional Firefighters' Burn and Wound Healing Laboratory, Department of Surgery, Division of Plastic Surgery, University of British Columbia, Vancouver, BC, Canada*
Vasiliki Gkretsi • *Cancer Biophysics Laboratory, Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus*
Valeria Graceffa • *Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway, Galway, Ireland; Science Foundation Ireland Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Galway, Ireland*
Yang Guo • *Department of Chemistry, University of Michigan, Ann Arbor, MI, USA*
Zachary T. Harmany • *Department of Pathology and Laboratory Medicine, University of California-Davis Medical Center, Sacramento, CA, USA*
J. Robin Harris • *Institute of Zoology, University of Mainz, Mainz, Germany*
Jamar Hawkins • *Department of Mechanical and Industrial Engineering, University of Massachusetts, Amherst, MA, USA*
Julia Hoffmann • *Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria*
Amy L. Horwell • *Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK*
Michael S. Hu • *Hagey Laboratory for Pediatric Regenerative Medicine, Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine, Stanford, CA, USA; Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA; Department of Surgery, John A. Burns School of Medicine, University of Hawai'i, Honolulu, HI, USA*
Audrey E.K. Hutcheon • *Schepens Eye Research Institute/MEE and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
Gisli Jenkins • *Division of Respiratory Medicine, School of Medicine, University of Nottingham, Nottingham, UK*
Sergio A. Jimenez • *The Joan and Joel Rosenbloom Center for Fibrotic Diseases and The Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA*
Alison E. John • *Division of Respiratory Medicine, School of Medicine, University of Nottingham, Nottingham, UK*
Adib Keikhosravi • *Laboratory for Optical and Computational Instrumentation, University of Wisconsin at Madison, Madison, WI, USA; Department of Biomedical Engineering, University of Wisconsin at Madison, Madison, WI, USA*
Sabine Klein • *Laboratory for Liver Fibrosis and Portal Hypertension, Department of Internal Medicine I, University Clinic Bonn, Bonn, Germany*
Grazyna Kwapiszewska • *Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria; Institute of Physiology, Medical University of Graz, Graz, Austria*
Tripp Leavitt • *Hagey Laboratory for Pediatric Regenerative Medicine, Division of Plastic and Reconstructive Surgery, Department of Surgery, School of Medicine, Stanford University, Stanford, CA, USA; School of Medicine, Boston University, Boston, MA, USA*

Richard M. Levenson • *Department of Pathology and Laboratory Medicine, University of California-Davis Medical Center, Sacramento, CA, USA*
Ian M.H. Li • *Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK*
Ling Li • *Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada*
Lawrence J. Liew • *Ear Science Institute Australia, Perth, Australia; School of Surgery, University of Western Australia, Perth, Australia*
Tianju Liu • *Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA*
Yuming Liu • *Laboratory for Optical and Computational Instrumentation, University of Wisconsin at Madison, Madison, WI, USA*
Michael T. Longaker • *Hagey Laboratory for Pediatric Regenerative Medicine, Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine, Stanford, CA, USA; Institute for Stem Cell Biology and regenerative Medicine, Stanford University, Stanford, CA, USA*
Edward Macarak • *The Joan and Joel Rosenbloom Center for Fibrotic Diseases and The Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA*
Guneet S. Mehta • *Laboratory for Optical and Computational Instrumentation, University of Wisconsin at Madison, Madison, WI, USA; Department of Biomedical Engineering, University of Wisconsin at Madison, Madison, WI, USA*
Anne Maria Mullen • *Teagasc Research Centre, Ashtown, Dublin, Ireland*
Layla Nabai • *BC Professional Firefighters' Burn and Wound Healing Laboratory, Department of Surgery, Division of Plastic Surgery, University of British Columbia, Vancouver, BC, Canada*
Giulia Nobile • *Unit of Biochemistry, Department of Molecular Medicine, University of Pavia, Pavia, Italy*
Huan Ting Ong • *Ear Science Institute Australia, Perth, Australia*
Costas S. Patrickios • *Department of Chemistry, University of Cyprus, Nicosia, Cyprus*
Francesco S. Pavone • *National Institute of Optics, National Research Council (INO-CNR), European Laboratory for Non-linear Spectroscopy (LENS), University of Florence, Sesto Fiorentino, Italy; Department of Physics, University of Florence, Sesto Fiorentino, Italy*
Sem H. Phan • *Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA*
Sonsoles Piera-Velazquez • *The Joan and Joel Rosenbloom Center for Fibrotic Diseases and The Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA*
Giampiero Pietrocola • *Unit of Biochemistry, Department of Molecular Medicine, University of Pavia, Pavia, Italy*
Bartosz Pilecki • *Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark*
Joanne Porte • *Division of Respiratory Medicine, School of Medicine, University of Nottingham, Nottingham, UK*
Nahum Puebla-Osorio • *Department of Lymphoma/Myeloma, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA*
Michael Raghunath • *Centre for Cell Biology and Tissue Engineering, Competence Centre Tissue Engineering for Drug Development (TEDD), Department Life Sciences and Facility Management, Institute for Chemistry and Biotechnology (ICBT), Zürich University of Applied Sciences, Wädenswil, Switzerland*

Simonetta Rindi • *Unit of Biochemistry, Department of Molecular Medicine, University of Pavia, Pavia, Italy*
Laure Rittié • *Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI, USA; Dermatology Therapeutic Area, GlaxoSmithKline, Collegeville, PA, USA*
Joel Rosenbloom • *The Joan and Joel Rosenbloom Center for Fibrotic Diseases and The Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA*
Francina Gonzalez De Los Santos • *Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA*
Seri N.E. Sarchio • *Cellular Photoimmunology Group, Discipline of Infectious Diseases and Immunology, Sydney Medical School at The Charles Perkins Centre, University of Sydney, Sydney, Australia; Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Sibille Sauer-Lehnen • *Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry, RWTH University Hospital Aachen, Aachen, Germany*
Gabriele Scherr • *Department of Dermatology, University of Cologne, Cologne, Germany*
Robert Schierwagen • *Laboratory for Liver Fibrosis and Portal Hypertension, Department of Internal Medicine I, University Clinic Bonn, Bonn, Germany*
Grith Lykke Sørensen • *Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark*
Pietro Speziale • *Unit of Biochemistry, Department of Molecular Medicine, University of Pavia, Pavia, Italy*
Triantafyllos Stylianopoulos • *Cancer Biophysics Laboratory, Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus*
Andreas Stylianou • *Cancer Biophysics Laboratory, Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus*
Yubing Sun • *Department of Mechanical and Industrial Engineering, University of Massachusetts, Amherst, MA, USA*
Frank Tacke • *Department of Internal Medicine III, RWTH University Hospital Aachen, Aachen, Germany*
Carmen G. Tag • *Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry, RWTH University Hospital Aachen, Aachen, Germany*
Amanda L. Tatler • *Division of Respiratory Medicine, School of Medicine, University of Nottingham, Nottingham, UK*
Richard Townsend • *Biotechnology, Dearborn, MI, USA*
Jonel Trebicka • *Laboratory for Liver Fibrosis and Portal Hypertension, Department of Internal Medicine I, University Clinic Bonn, Bonn, Germany*
Edward E. Tredget • *Wound Healing Research Group, Division of Plastic and Reconstructive Surgery, and Division of Critical Care Medicine, Department of Surgery, University of Alberta, Edmonton, AB, Canada*
Lam C. Tsoi • *Department of Dermatology, Medical School, University of Michigan, Ann Arbor, MI, USA; Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA; Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA*
Franziska E. Uhl • *Department of Med-Pulmonary, College of Medicine, University of Vermont, Burlington, VT, USA*
Stephen E. Ullrich • *Department of Immunology and the Center for Cancer Immunology Research, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA*

Frank Erhard Uschner • *Laboratory for Liver Fibrosis and Portal Hypertension, Department of Internal Medicine I, University Clinic Bonn, Bonn, Germany*
Darcy E. Wagner • *Comprehensive Pneumology Center, Lung Repair and Regeneration, Helmholtz Zentrum München and University Hospital of the Ludwig Maximilians Universität, Member of the German Center for Lung Research (DZL), Munich, Germany*
Fang Wang • *Department of Dermatology, University of Cologne, Cologne, Germany*
Ralf Weiskirchen • *Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry, RWTH University Hospital Aachen, Aachen, Germany*
Sabine Weiskirchen • *Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry, RWTH University Hospital Aachen, Aachen, Germany*
Daniel J. Weiss • *Department of Med-Pulmonary, College of Medicine, University of Vermont, Burlington, VT, USA*
Jochen Wilhelm • *Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany; Universities of Giessen and Marburg Lung Center (UGMLC), Giessen, Germany*
Christal A. Worthen • *Department of Dermatology, Medical School, University of Michigan, Ann Arbor, MI, USA*
Zhuning Wu • *Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway, Galway, Ireland; Science Foundation Ireland Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Galway, Ireland*
Tianfa Xie • *Department of Mechanical and Industrial Engineering, University of Massachusetts, Amherst, MA, USA*
Toshiyuki Yamamoto • *Department of Dermatology, Fukushima Medical University, Fukushima, Japan*
Dimitrios I. Zeugolis • *Regenerative, Modular and Developmental Engineering Laboratory (REMODEL), National University of Ireland Galway, Galway, Ireland; Science Foundation Ireland Centre for Research in Medical Devices (CÚRAM), National University of Ireland Galway, Galway, Ireland*
James D. Zieske • *Schepens Eye Research Institute/MEE and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
Paola Zigrino • *Department of Dermatology, University of Cologne, Cologne, Germany*

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