

# METHODS IN MOLECULAR BIOLOGY

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

For further volumes:  
<http://www.springer.com/series/7651>



# **The Tumor Microenvironment**

## **Methods and Protocols**


Edited by

**Josie Ursini-Siegel**

*Lady Davis Institute for Medical Research, Department of Oncology  
McGill University, Montreal, QC, Canada*

**Nicole Beauchemin**

*Goodman Cancer Research Centre, McGill University, Montreal, QC, Canada*

 **Humana Press**

*Editors*

Josie Ursini-Siegel  
Lady Davis Institute for Medical Research  
Department of Oncology  
McGill University  
Montreal, QC, Canada

Nicole Beauchemin  
Goodman Cancer Research Centre  
McGill University  
Montreal, QC, Canada

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-3799-8              ISBN 978-1-4939-3801-8 (eBook)  
DOI 10.1007/978-1-4939-3801-8

Library of Congress Control Number: 2016945043

© Springer Science+Business Media New York 2016

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature  
The registered company is Springer Science+Business Media LLC New York

---

## **Preface**

The tumor microenvironment includes the cellular and noncellular constituents that surround and support tumor cells. This includes fibroblasts, blood vessels, innate and adaptive immune cells along with secreted signaling molecules, extracellular vesicles, and components of the extracellular matrix. Indeed, extensive research has firmly established an instrumental role for the tumor microenvironment in supporting cancer initiation, dormancy, progression, and metastatic spread.

This book covers core and emerging in vitro and in vivo protocols that are used to study how various components of the tumor microenvironment are established and subsequently interact with tumor cells to facilitate carcinogenesis. In addition, the book displays research topics including cellular and molecular biology approaches, in vivo genetic approaches, various “omics”-based strategies, therapeutic strategies to target the microenvironment, and, finally, advanced techniques in the fields of tissue engineering and nanotechnology. Written and validated in the laboratories of a number of trusted collaborating authors, these protocols should facilitate further studies in this exciting field.

Thus, this protocol book constitutes a compendium of techniques now available to a broad audience including basic and clinician scientists, systems biologists, and biological engineers.

*Montreal, QC, Canada*

*Josie Ursini-Siegel  
Nicole Beauchemin*



---

# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
1 Methods of Immunohistochemistry and Immunofluorescence: Converting Invisible to Visible . . . . .	1
<i>Hidetoshi Mori and Robert D. Cardiff</i>	
2 Laser Capture Microdissection as a Tool to Study Tumor Stroma . . . . .	13
<i>Nicholas R. Bertos and Morag Park</i>	
3 Quantitative Analysis of Human Cancer Cell Extravasation Using Intravital Imaging . . . . .	27
<i>Lian Willetts, David Bond, Konstantin Stoletov, and John D. Lewis</i>	
4 Studies on the Tumor Vasculature and Coagulant Microenvironment . . . . .	39
<i>Esterina D'Asti, Brian Meehan, and Janusz Rak</i>	
5 A Microfluidic Method to Mimic Luminal Structures in the Tumor Microenvironment . . . . .	59
<i>José A. Jiménez-Torres, David J. Beebe, and Kyung E. Sung</i>	
6 Measuring Vascular Permeability In Vivo . . . . .	71
<i>Eelco F.J. Meijer, James W. Baish, Timothy P. Padera, and Dai Fukumura</i>	
7 Hydroxylation-Dependent Interaction of Substrates to the Von Hippel-Lindau Tumor Suppressor Protein (VHL) . . . . .	87
<i>Pardeep Heir and Michael Ohh</i>	
8 Analyzing the Tumor Microenvironment by Flow Cytometry . . . . .	95
<i>Yoon Kow Young, Alicia M. Bolt, Ryuhjin Ahn, and Koren K. Mann</i>	
9 Detecting Secreted Analytes from Immune Cells: An Overview of Technologies . . . . .	111
<i>Kelly A. Pike, Caitlyn Hui, and Connie M. Krawczyk</i>	
10 Purification of Immune Cell Populations from Freshly Isolated Murine Tumors and Organs by Consecutive Magnetic Cell Sorting and Multi-parameter Flow Cytometry-Based Sorting . . . . .	125
<i>Camilla Salvagno and Karin E. de Visser</i>	
11 Viral Engineering of Chimeric Antigen Receptor Expression on Murine and Human T Lymphocytes . . . . .	137
<i>Joanne A. Hammill, Arya Afsahi, Jonathan L. Bramson, and Christopher W. Helsen</i>	
12 Methods to Evaluate the Antitumor Activity of Immune Checkpoint Inhibitors in Preclinical Studies . . . . .	159
<i>Bertrand Allard, David Allard, and John Stagg</i>	

13	Isolation and Characterization of Low- vs. High-Density Neutrophils in Cancer . . . . .	179
	<i>Jitka Y. Sagiv, Sandra Voels, and Zvi Granot</i>	
14	Analysis of Extracellular Vesicles in the Tumor Microenvironment . . . . .	195
	<i>Khalid Al-Nedawi and Jolene Read</i>	
15	Visualizing the Tumor Microenvironment of Liver Metastasis by Spinning Disk Confocal Microscopy . . . . .	203
	<i>Liane Babes and Paul Kubes</i>	
16	Intravital Microscopy for Imaging the Tumor Microenvironment in Live Mice . . . . .	217
	<i>Victor Naumenko, Craig Jenne, and Douglas J. Mahoney</i>	
17	Development of a Patient-Derived Xenograft Model Using Brain Tumor Stem Cell Systems to Study Cancer . . . . .	231
	<i>Chirayu Chokshi, Manvir Dhillon, Nicole McFarlane, Chitra Venugopal, and Sheila K. Singh</i>	
18	Modeling Breast Tumor Development with a Humanized Mouse Model. . . . .	247
	<i>Lisa M. Arendt</i>	
19	CRISPR/Cas9 Genome Editing as a Strategy to Study the Tumor Microenvironment in Transgenic Mice . . . . .	261
	<i>Yojiro Yamanaka</i>	
20	Metabolomics Analyses of Cancer Cells in Controlled Microenvironments. . . . .	273
	<i>Simon-Pierre Gravel, Daina Avizonis, and Julie St-Pierre</i>	
21	Analysis of the Tumor Microenvironment Transcriptome via NanoString mRNA and miRNA Expression Profiling. . . . .	291
	<i>Marie-Noël M'Boutchou and Léon C. van Kempen</i>	
22	RNA-Seq as a Tool to Study the Tumor Microenvironment. . . . .	311
	<i>Pudchalaluck Panichnantakul, Mathieu Bourgey, Alexandre Montpetit, Guillaume Bourque, and Yasser Riazalhosseini</i>	
23	Sample Preparation for Mass Spectrometry Analysis of Protein–Protein Interactions in Cancer Cell Lines and Tissues . . . . .	339
	<i>Alice Beigbeder, Lauriane Vélot, D. Andrew James, and Nicolas Bisson</i>	
	<i>Index</i> . . . . .	349



---

## Contributors

- ARYA AFSAHI • *Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada*
- RYUHJIN AHN • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada*
- BERTRAND ALLARD • *Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Institut du Cancer de Montréal, Montréal, QC, Canada; Faculté de Pharmacie, Université de Montréal, Montréal, QC, Canada*
- DAVID ALLARD • *Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Institut du Cancer de Montréal, Montréal, QC, Canada; Faculté de Pharmacie, Université de Montréal, Montréal, QC, Canada*
- KHALID AL-NEDAWI • *Division of Nephrology, Department of Medicine, McMaster University, Hamilton, ON, Canada; Hamilton Centre for Kidney Research (HCKR), St. Joseph's Hospital, Hamilton, ON, Canada*
- LISA M. ARENDT • *Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA*
- DAINA AVIZONIS • *Metabolomics Core Facility, Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- LIANE BABES • *Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada*
- JAMES W. BAISH • *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; Department of Mechanical and Biomedical Engineering, Bucknell University, Lewisburg, PA, USA*
- DAVID J. BEEBE • *Division of Cellular and Gene Therapies, Office of Cellular, Tissue, and Gene Therapies, Center for Biologics Evaluation and Research, The US Food and Drug Administration, Silver Spring, MD, USA*
- ALICE BEIGBEDER • *Division of Oncology, Centre de Recherche du CHU de Québec, Québec, QC, Canada; Cancer Research Centre, Université Laval, Québec, QC, Canada*
- NICHOLAS R. BERTOS • *Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- NICOLAS BISSON • *Division of Oncology, Centre de Recherche du CHU de Québec, Québec, QC, Canada; Cancer Research Centre, Université Laval, Québec, QC, Canada; Quebec Network for Research on Protein Function, Engineering, and Applications (PROTEO), Université Laval, Québec, QC, Canada; Department of Molecular Biology, Medical Biochemistry and Pathology, Université Laval, Québec, QC, Canada*
- ALICIA M. BOLT • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada*
- DAVID BOND • *Department of Oncology, University of Alberta, Edmonton, AB, Canada*
- MATHIEU BOURGEY • *McGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada*

- GUILLAUME BOURQUE • *Department of Human Genetics, McGill University, Montréal, QC, Canada; McGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada*
- JONATHAN L. BRAMSON • *Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada*
- ROBERT D. CARDIFF • *Center of Comparative Medicine, University of California, Davis, CA, USA*
- CHIRAYU CHOKSHI • *McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada*
- ESTERINA D'ASTI • *Montréal Children's Hospital, The Research Institute of the McGill University Health Centre, Montréal, QC, Canada*
- MANVIR DHILLON • *McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada*
- DAI FUKUMURA • *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
- ZVI GRANOT • *Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel Canada, Hebrew University Medical School, Jerusalem, Israel*
- SIMON-PIERRE GRAVEL • *Department of Biochemistry, McGill University, Montréal, QC, Canada; Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- JOANNE A. HAMMILL • *Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada*
- PARDEEP HEIR • *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada*
- CHRISTOPHER W. HELSEN • *Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada*
- CAITLYN HUI • *Department of Microbiology and Immunology, Goodman Cancer Research Center, McGill University, Montréal, QC, Canada; Department of Physiology, Goodman Cancer Research Center, McGill University, Montréal, QC, Canada*
- D. ANDREW JAMES • *Department of Chemistry, York University, Toronto, ON, Canada*
- CRAIG JENNE • *Snyder Institute for Chronic Diseases, Calgary, AB, Canada; Department of Microbiology, Immunology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, AB, Canada*
- JOSÉ A. JIMÉNEZ-TORRES • *Division of Cellular and Gene Therapies, Office of Cellular, Tissue, and Gene Therapies, Center for Biologics Evaluation and Research, The US Food and Drug Administration, Silver Spring, MD, USA*
- LÉON C. VAN KEMPEN • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada; Department of Pathology, Jewish General Hospital, McGill University, Montréal, QC, Canada*
- CONNIE M. KRAWCZYK • *Department of Microbiology and Immunology, Goodman Cancer Research Center, McGill University, Montréal, QC, Canada; Department of Physiology, Goodman Cancer Research Center, McGill University, Montréal, QC, Canada*
- PAUL KUBES • *Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada*
- JOHN D. LEWIS • *Department of Oncology, University of Alberta, Edmonton, AB, Canada*
- MARIE-NOËL M'BOUTCHOU • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada*

- DOUGLAS J. MAHONEY • *Alberta Children's Hospital Research Institute, Calgary, AB, Canada; Arnie Charbonneau Cancer Institute, Calgary, AB, Canada; Department of Microbiology, Immunology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, AB, Canada*
- KOREN K. MANN • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada*
- NICOLE MCFARLANE • *McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada*
- BRIAN MEEHAN • *Montréal Children's Hospital, The Research Institute of the McGill University Health Centre, Montréal, QC, Canada*
- EELCO F.J. MEIJER • *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
- ALEXANDRE MONTPETIT • *McGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada*
- HIDETOSHI MORI • *Center of Comparative Medicine, University of California, Davis, CA, USA*
- VICTOR NAUMENKO • *Alberta Children's Hospital Research Institute, Calgary, AB, Canada; Arnie Charbonneau Cancer Institute, Calgary, AB, Canada; Snyder Institute for Chronic Diseases, Calgary, AB, Canada; Department of Microbiology, Immunology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, AB, Canada*
- MICHAEL OHH • *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; Department of Biochemistry, University of Toronto, Toronto, ON, Canada*
- TIMOTHY P. PADERA • *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
- PUDCHALALUCK PANICHNANTAKUL • *Department of Human Genetics, McGill University, Montréal, QC, Canada; McGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada*
- MORAG PARK • *Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada; Department of Experimental Medicine, McGill University, Montréal, QC, Canada; Department of Biochemistry, McGill University, Montréal, QC, Canada; Department of Oncology, McGill University, Montréal, QC, Canada; Department of Pathology, McGill University, Montréal, QC, Canada*
- KELLY A. PIKE • *Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- JANUSZ RAK • *Montréal Children's Hospital, The Research Institute of the McGill University Health Centre, Montréal, QC, Canada*
- JOLENE READ • *Division of Nephrology, Department of Medicine, McMaster University, Hamilton, ON, Canada; Hamilton Centre for Kidney Research (HCKR), St. Joseph's Hospital, Hamilton, ON, Canada*
- YASSER RIAZALHOSSEINI • *Department of Human Genetics, McGill University, Montréal, QC, Canada; McGill University and Genome Quebec Innovation Centre, Montréal, QC, Canada*
- JITKA Y. SAGIV • *Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel Canada, Hebrew University Medical School, Jerusalem, Israel*
- CAMILLA SALVAGNO • *Division of Immunology, Netherlands Cancer Institute, Amsterdam, The Netherlands*

- SHEILA K. SINGH • *McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada; Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada; Department of Surgery, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada*
- JOHN STAGG • *Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Institut du Cancer de Montréal, Montréal, QC, Canada; Faculté de Pharmacie, Université de Montréal, Montréal, QC, Canada*
- KONSTANTIN STOLETOV • *Department of Oncology, University of Alberta, Edmonton, AB, Canada*
- JULIE ST-PIERRE • *Department of Biochemistry, McGill University, Montréal, QC, Canada; Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- KYUNG E. SUNG • *Division of Cellular and Gene Therapies, Office of Cellular, Tissue, and Gene Therapies, Center for Biologics Evaluation and Research, The US Food and Drug Administration, Silver Spring, MD, USA*
- LAURIANE VÉLOT • *Division of Oncology, Centre de Recherche du CHU de Québec, Québec, QC, Canada; Cancer Research Centre, Université Laval, Québec, QC, Canada*
- CHITRA VENUGOPAL • *McMaster Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada*
- KARIN E. DE VISSER • *Division of Immunology, Netherlands Cancer Institute, Amsterdam, The Netherlands*
- SANDRA VOELS • *Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel Canada, Hebrew University Medical School, Jerusalem, Israel*
- LIAN WILLETTS • *Department of Oncology, University of Alberta, Edmonton, AB, Canada*
- YOJIRO YAMANAKA • *GCRC Transgenic Facility, Department of Human Genetics, Goodman Cancer Research Centre, McGill University, Montréal, QC, Canada*
- YOON KOW YOUNG • *Lady Davis Institute for Medical Research, McGill University, Montréal, QC, Canada*