METHODS IN MOLECULAR BIOLOGY™

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

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

In Vivo Cellular Imaging Using Fluorescent Proteins

Methods and Protocols

Edited by

Robert M. Hoffman

AntiCancer Inc., Department of Surgery, University of California, San Diego, CA, USA

🔆 Humana Press

Editor Robert M. Hoffman AntiCancer Inc., Department of Surgery University of California San Diego, CA, USA

ISSN 1064-3745 ISSN 1940-6029 (electronic) ISBN 978-1-61779-796-5 ISBN 978-1-61779-797-2 (eBook) DOI 10.1007/978-1-61779-797-2 Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2012936123

© Springer Science+Business Media New York 2012

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. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

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.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Dedication

This volume is dedicated to Charlene M. Cooper who has devoted 16 years of way-beyond the call-of-duty to AntiCancer Inc. Without Charlene's devotion, superb administration, and thoughtfulness, this volume could not have been written.

Preface

The discovery and genetic engineering of fluorescent proteins has revolutionized cell biology. What was previously invisible in the cell often can be made visible with the use of fluorescent proteins. This volume presents state-of-the-art research contributing to the revolution fluorescent proteins brought the visualization of biological processes in the live animal. This is the first volume in the new field of in vivo cell biology. The chapters in this volume are highlighted below.

Chapter 1 describes the use of the chick CAM model to visualize cancer cell migration and metastasis in a physiologically-relevant, but simple, in vivo setting using fluorescent proteins and other fluorescent probes.

Chapter 2 describes intravital fluorescent imaging of the real-time behavior of the individual cells of mammary tumors labeled with fluorescent proteins using multiphoton microscopy.

Chapter 3 describes the use of window chambers for cellular and subcellular imaging of cancer cells in mice.

Chapter 4 describes imaging of tumor-host interaction between pancreatic cancer cells and host-derived stroma and vasculature in which cancer cells and the host mice are colorcoded with fluorescent proteins.

Chapter 5 describes stable transformation of cancer cells with fluorescent protein genes, using lentiviral vectors, which can be used for whole-body imaging on essentially any organ in mice.

Chapter 6 describes an in vivo imaging system consisting of mouse-implanted fluorescent protein-tagged metastatic cancer cell lines and a hand-held detection device for external, noninvasive and real-time monitoring of the therapeutic effects of drugs.

Chapter 7 describes three-dimensional imaging of tumors in mice expressing red fluorescent protein.

Chapter 8 describes real-time high-resolution imaging of angiogenesis and vascular response to anticancer and antiangiogenic therapy in live mice with orthotopic breast cancer labeled with fluorescent proteins.

Chapter 9 describes a tumor-specific, replication-competent, telomerase-dependent, GFP-expressing adenovirus to label tumors and metastasis with GFP in mice for detection and surgical navigation.

Chapter 10 describes a replication-competent, tumor-specific herpes simplex virus expressing GFP to label cancer cells in mice for visualization by endoscopy and in vivo microscopy.

Chapter 11 describes tumor-targeting GFP-expressing vaccinia viruses and bacteria to label tumors in mice for high-resolution imaging.

Chapter 12 describes genetic engineering of rats, rabbits, and pigs to express GFP which can be used for cell therapy and transplantation.

Chapter 13 describes the matching of exogenous fluorophores and endogenous fluorescent proteins in cancer cells to develop sensitive and specific cancer-targeting probes.

Chapter 14 describes embryo culture and fluorescent proteins to image developing vasculature and hemodynamics.

Chapter 15 describes new fluorescent proteins, with a wide range of spectral colors, including those that switch colors and kindle, isolated from coral reefs.

Chapter 16 describes how new improved far-red and infrared fluorescent proteins can be designed.

Chapter 17 describes imaging the effects of siRNA and microRNA in vivo.

Chapter 18 describes the use of different color fluorescent proteins to image the nuclearcytoplasmic dynamics of cancer cells in vivo.

San Diego, CA, USA

Robert M. Hoffman

Contents

	face ntributors	vii xi
1	Assessing Cancer Cell Migration and Metastatic Growth In Vivo	
1	in the Chick Embryo Using Fluorescence Intravital Imaging	1
2	The Use of Fluorescent Proteins for Intravital Imaging of Cancer Cell Invasion James Hulit, Dmitriy Kedrin, Bojana Gligorijevic, David Entenberg, Jeffrey Wyckoff, John Condeelis, and Jeffrey E. Segall	15
3	High-Resolution In Vivo Imaging of Fluorescent Proteins Using Window Chamber Models Gregory M. Palmer, Andrew N. Fontanella, Siqing Shan, and Mark W. Dewhite	31 rst
4	In Vivo Imaging of Pancreatic Cancer with Fluorescent Proteins in Mouse Models	51
5	Lentivirus-Based DsRed-2-Transfected Pancreatic Cancer Cells for Deep In Vivo Imaging of Metastatic Disease	69
6	Noninvasive and Real-Time Fluorescence Imaging of Peritoneal Metastasis in Nude Mice	85
7	Three-Dimensional In Vivo Imaging of Tumors Expressing Red Fluorescent Proteins	97
8	Real-Time Visualization and Characterization of Tumor Angiogenesisand Vascular Response to Anticancer TherapiesH. Rosie Xing and Qingbei Zhang	115
9	In Vivo Imaging of Human Cancer with Telomerase-Specific Replication-Selective Adenovirus <i>Toshiyoshi Fujiwara</i>	129
10	Real-Time Fluorescence Imaging of Abdominal, Pleural, and Lymphatic Metastases Susanne Carpenter and Yuman Fong	141

х	Contents	
11	Real-Time Imaging of Tumors Using Replication-Competent Light-Emitting Microorganisms	159
12	GFP-Transgenic Animals for In Vivo Imaging: Rats, Rabbits, and Pigs <i>Takashi Murakami and Eiji Kobayashi</i>	177
13	The Use of Fluorescent Proteins for Developing Cancer-Specific Target Imaging Probes	191
14	In Vivo Imaging of the Developing Mouse Embryonic Vasculature	205
15	Screening Reef Corals for Novel GFP-Type Fluorescent Proteins by Confocal Imaging	217
16	What Does It Take to Improve Existing Fluorescent Proteins for In Vivo Imaging Applications? Marc Zimmer	235
17	In Vivo Imaging of Oligonucleotide Delivery Fumitaka Takeshita, Ryou-u Takahashi, Jun Onodera, and Takahiro Ochiya	243
18	Subcellular Imaging In Vivo: The Next GFP Revolution	255
Index		

Contributors

- LYAYSAN R. ARSLANBAEVA Laboratory of Physical Biochemistry, A.N. Bach Institute of Biochemistry of Russian Academy of Sciences, Moscow, Russia
- ANATOLY YU BARYSHNIKOV Institute of Experimental Diagnostics and Therapy of Tumors, N.N. Blokhin Russian Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russia
- MICHAEL BOUVET GI Cancer Unit, Moores Cancer Center, University of California San Diego, La Jolla, CA, USA
- OLGA S. BUROVA Institute of Experimental Diagnostics and Therapy of Tumors, N.N. Blokhin Russian Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russia
- SUSANNE CARPENTER Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
- ANN F. CHAMBERS The London Regional Cancer Center, London, ON, Canada
- PETER L. CHOYKE Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
- JOHN CONDEELIS Department of Anatomy and Structural Biology, Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA
- MARK W. DEWHIRST Department of Radiation Oncology, Duke University, Durham, NC, USA; Department of Biomedical Engineering, Duke University, Durham, NC, USA
- MARY E. DICKINSON Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX, USA
- DAVID ENTENBERG Department of Anatomy and Structural Biology, Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA
- ILYA I. FIKS Biophotonics Laboratory, Institute of Applied Physics of Russian Academy of Sciences, Nizhny Novgorod, Russia
- YUMAN FONG Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
- ANDREW N. FONTANELLA Department of Biomedical Engineering, Duke University, Durham, NC, USA
- TOSHIYOSHI FUJIWARA Center for Gene and Cell Therapy, Division of Surgical Oncology, Department of Surgery, Okayama University Graduate School of Medicine & Dentistry, Okayama, Japan
- BOJANA GLIGORIJEVIC Department of Anatomy and Structural Biology, Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA
- ROBERT M. HOFFMAN AntiCancer, Inc., Department of Surgery, University of California San Diego, CA, USA
- JAMES HULIT Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA; Centre for Tumour Biology, Barts and the London Queen Mary's Medical and Dental School, London, UK
- SEIJI ITO Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan

- YUICHI ITO Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan
- DMITRIY KEDRIN Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA
- MICHAEL S. KLESHNIN Biophotonics Laboratory, Institute of Applied Physics of Russian Academy of Sciences, Nizhny Novgorod, Russia
- EIJI KOBAYASHI Division of Development of Advanced Treatment, Center for Development of Advanced Medical Technology, Jichi Medical University, Tochigi, Japan
- HISATAKA KOBAYASHI Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
- YASUHIRO KODERA Department of Surgery II, Nagoya University School of Medicine, Nagoya, Japan
- NOBUYUKI KOSAKA Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
- IRINA V. LARINA Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX, USA
- HON SING LEONG The London Regional Cancer Center, London, ON, Canada
- JOHN D. LEWIS University of Alberta, Edmonton, AB, Canada
- MAKOTO MATSUI Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan
- THOMAS E. MCCANN Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institute of Health, Bethesda, MD, USA
- IRINA G. MEEROVICH Laboratory of Physical Biochemistry, A.N. Bach Institute of Biochemistry of Russian Academy of Sciences, Moscow, Russia
- KAZUNARI MISAWA Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan
- TAKASHI MURAKAMI Laboratory of Tumor Biology, Takasaki University of Health and Welfare, Gunma, Japan
- HAYAO NAKANISHI Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan
- TAKAHIRO OCHIYA Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan
- JUN ONODERA Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan
- ANNA G. ORLOVA Biophotonics Laboratory, Institute of Applied Physics of Russian Academy of Sciences, Nizhny Novgorod, Russia
- GREGORY M. PALMER Department of Radiation Oncology, Duke University, Durham, NC, USA
- ANYA SALIH Confocal Bio-Imaging Facility (CBIF), School of Science and Health, University of Western Sydney, Sydney, NSW, Australia
- ALEXANDER P. SAVITSKY A.N. Bach Institute of Biochemistry of Russian Academy of Sciences, Moscow, Russia
- JEFFREY E. SEGALL Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA
- ALEXANDER M. SERGEEV Institute of Applied Physics of Russian Academy of Sciences, Nizhny Novgorod, Russia
- SIQING SHAN Department of Radiation Oncology, Duke University, Durham, NC, USA

- DARINA V. SOKOLOVA Institute of Experimental Diagnostics and Therapy of Tumors, N.N. Blokhin Russian Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russia
- ALADAR A. SZALAY Rudolf-Virchow-Center, DFG-Research Center for Experimental Biomedicine, University of Wuerzburg, Wuerzburg, Germany; Genelux Corporation, San Diego Science Center, San Diego, CA, USA
- RYOU-U TAKAHASHI Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan
- FUMITAKA TAKESHITA Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, Tokyo, Japan
- ELENA M. TRESHCHALINA Institute of Experimental Diagnostics and Therapy of Tumors, N.N. Blokhin Russian Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russia
- ILYA V. TURCHIN Biophotonics Laboratory, Institute of Applied Physics of Russian Academy of Sciences, Nizhny Novgorod, Russia
- STEPHANIE WEIBEL Department of Biochemistry, Biocenter, University of Wuezburg, Wuezburg, Germany
- JEFFREY WYCKOFF Department of Anatomy and Structural Biology, Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA
- H. ROSIE XING Department of Pathology & Radiation Oncology, University of Chicago, Chicago, IL, USA
- YONG A. YU Genelux Corporation, San Diego Science Center, San Diego, CA, USA
- ZEQIAN YU Department of General Surgery, Zhongda Hospital, Southeast University, Nan Jing City, Jiang Su Province, China
- QINGBEI ZHANG Department of Pathology, Cellular and Radiation Oncology, University of Chicago, Chicago, IL, USA
- VICTORIA V. ZHERDEVA Laboratory of Physical Biochemistry, A.N. Bach Institute of Biochemistry of Russian Academy of Sciences, Moscow, Russia
- JIAHUA ZHOU Department of General Surgery, Zhongda Hospital, Southeast University, Nan Jing City, Jiang Su Province, China
- MARC ZIMMER Hale Laboratory, Connecticut College, New London, CT, USA