

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>

Mammary Stem Cells

Methods and Protocols

Edited by

Maria del Mar Vivanco

CIC bioGUNE, Technological Park of Bizkaia, Derio, Spain

 **Humana Press**

Editor

Maria del Mar Vivanco
CIC bioGUNE
Technological Park of Bizkaia
Derio, Spain

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-2518-6 ISBN 978-1-4939-2519-3 (eBook)
DOI 10.1007/978-1-4939-2519-3

Library of Congress Control Number: 2015939081

Springer New York Heidelberg Dordrecht London
© Springer Science+Business Media New York 2015

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

Humana Press is a brand of Springer
Springer Science+Business Media LLC New York is part of Springer Science+Business Media (www.springer.com)

Preface

The concept of stem cells was first postulated more than a century ago, and over the last few years it has become a focus of interest and discussion even in nonscientific circles. The fact that this book is dedicated to Mammary Stem Cells reflects the attention that these cells have recently received and, as a consequence, many of the variety of approaches that researchers are using to investigate their properties and function are discussed here. Studying stem cells from the healthy mammary gland may not appear as relevant as the intriguing work presently being carried out with adult stem cells from other tissues, such as the heart or the nervous system, which epitomizes the great hope and potential for regenerative medicine in the future. However, understanding the role that stem cells play during development and in the physiology of the mammary gland is not only interesting in its own right but also essential to understand the molecular mechanisms underlying the alterations that lead to breast cancer, particularly when considering the hypothesis that the molecular basis of tumorigenesis is a stem/progenitor cell gone awry. Thus, the identification of normal and breast cancer stem cells more than a decade ago has offered a new vision of this heterogeneous disease and new hopes for its prognosis and treatment.

The collection of chapters in this book aims to guide the researcher interested in mammary stem cells, to isolate them and characterize them, at various different levels. Max Wicha, one of the protagonists in the field, opens the book with an extensive introduction to the subject that takes us from the nineteenth century to the current state of the art and a look into the future implications of targeting these cells in the clinic. Mammary cell lineage specification occurs during embryogenesis and thus Chapter 2 presents a protocol that allows the study of progenitor cells during embryonic mammary development *ex vivo*. The following two chapters provide guidance and experimental details that should help a researcher to start isolating stem cells using fluorescent-activated cell sorting. Once the stem/progenitor cells have been identified and isolated, there is a wide variety of methods to study them. Omics techniques are quickly becoming very informative tools, and there are three chapters that discuss DNA and mRNA sequencing, as well as proteomics techniques to assist in profiling cells in a more efficient manner. Chapter 8 focuses on lentiviral cell transduction, which provides an invaluable method to stably modify gene expression and facilitate functional studies. Transplantation studies have been considered the gold standard to demonstrate normal and cancer stem cell potential, and there are two chapters that provide detailed protocols to investigate mammary gland development and tumorigenesis by taking advantage of the mammary fat pads of mice. A more recent addition to the experimental alternatives to study stem cells is *in vivo* lineage tracing, which allows the researcher to track cell fate *in situ*, and this is discussed in detail in Chapter 11. Cancer stem cells not only function as tumor initiating cells but also can be implicated in resistance to therapy and disease dissemination. The finding that these cells display properties that allow them to establish themselves in a different environment is investigated in two chapters, which aim to follow stem cells from their initial niche to the new microenvironment at their metastasis site, to study their metastatic re-initiation capabilities and to establish an experimental system that allows monitoring of the survival, dormancy, and proliferation of

disseminated cancer cells. Finally, the last chapters are striking examples of how scientists are reaching out into other areas, from biology to physics and mathematics and back to biomedicine.

I hope that this book, by including some of the most basic techniques and exciting new developments, will help members of the scientific community new to the field to explore the behavior of stem cells and to learn how to tackle them. This knowledge should guide the design of new and complimentary strategies to be applied in the clinic with the final aim of fighting breast cancer.

Derio, Spain

Maria del Mar Vivanco

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>ix</i>
1 Breast Cancer Stem Cells: Current Advances and Clinical Implications <i>Ming Luo, Shawn G. Clouthier, Yadwinder Deol, Suling Liu, Sunitha Nagrath, Ebrahim Azizi, and Max S. Wicha</i>	1
2 A Protocol for Studying Embryonic Mammary Progenitor Cells During Mouse Mammary Primordial Development in Explant Culture <i>Naoko Kogata and Beatrice A. Howard</i>	51
3 FACS Sorting Mammary Stem Cells <i>Oihana Iriondo, Miriam Rábano, and María d.M. Vivanco</i>	63
4 Side Population. <i>Fariba Bebbod and María d.M. Vivanco</i>	73
5 Single-Cell Genome and Transcriptome Processing Prior to High-Throughput Sequencing <i>Ana M. Aransay, Laura Barcena, Aintzane Gonzalez-Lahera, and Nuria Macias-Camara</i>	83
6 Shotgun Proteomics on Tissue Specimens Extracted with Acid Guanidinium-Thiocyanate-Phenol-Chloroform <i>René B.H. Braakman, Anieta M. Sieuwerts, and Arzu Umar</i>	115
7 Antibody-Based Capture of Target Peptides in Multiple Reaction Monitoring Experiments <i>Tommaso De Marchi, Eric Kuhn, Steven A. Carr, and Arzu Umar</i>	123
8 Lentiviral Transduction of Mammary Epithelial Cells. <i>Richard Iggo and Elodie Richard</i>	137
9 The Transplantation of Mouse Mammary Epithelial Cells into Cleared Mammary Fat Pads <i>Marisa M. Faraldo, Marina A. Glukhova, and Marie-Ange Deugnier</i>	161
10 Humanization of the Mouse Mammary Gland <i>A. Wronski, L.M. Arendt, and Charlotte Kuperwasser</i>	173
11 Lineage Tracing in the Mammary Gland Using Cre/lox Technology and Fluorescent Reporter Alleles <i>Renée van Amerongen</i>	187
12 Modeling the Breast Cancer Bone Metastatic Niche in Complex Three-Dimensional Cocultures <i>Rebecca Marlow and Gabriela Dontu</i>	213

13 Mammary Cancer Stem Cells Reinitiation Assessment
at the Metastatic Niche: The Lung and Bone. 221
*Marc Guin, Enrique J. Arenas, Sylwia Gawrzak, Milica Pavlovic,
and Roger R. Gomis*

14 Nanomechanical Characterization of Living Mammary Tissues
by Atomic Force Microscopy. 231
Marija Plodinec and Roderick Y.H. Lim

15 Mathematical Modeling as a Tool to Understand Cell Self-Renewal
and Differentiation 247
Philipp Getto and Anna Marciniak-Czochra

16 Mammary Stem Cells: A Clinician’s View 267
José Schneider

Index. 273

Contributors

- RENÉE VAN AMERONGEN • *Section of Molecular Cytology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands*
- ANA M. ARANSAY • *Genome Analysis Platform, CIC bioGUNE & CIBERehd, Derio, Spain*
- ENRIQUE J. ARENAS • *Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona), Barcelona, Spain*
- L.M. ARENDT • *Developmental, Molecular, and Chemical Biology Department, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA; Raymond and Beverly Sackler Laboratory for the Convergence of Biomedical, Physical and Engineering Sciences, Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA, USA; Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA*
- EBRAHIM AZIZI • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*
- LAURA BARCENA • *Genome Analysis Platform, CIC bioGUNE, Derio, Spain*
- FARIBA BEHBOD • *Pathology and Laboratory Medicine, University of Kansas Medical Center, Lawrence, Kansas City, USA*
- RENÉ B.H. BRAAKMAN • *Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands; Postgraduate School of Molecular Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; Center for Translational Molecular Medicine, Eindhoven, The Netherlands*
- STEVEN A. CARR • *Broad Institute of MIT and Harvard, Cambridge, MA, USA*
- SHAWN G. CLOUTHIER • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*
- YADWINDER DEOL • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*
- MARIE-ANGE DEUGNIER • *Centre de Recherche, UMR144, CNRS, Institut Curie, PSL Research University, Paris, France*
- GABRIELA DONTU • *Stem Cell Group, Guy's Hospital, King's College London School of Medicine, London, UK; Research Oncology, Division of Cancer Studies, Guy's Hospital, King's College London, London, UK*
- MARISA M. FARALDO • *Centre de Recherche, UMR144, CNRS, Institut Curie, PSL Research University, Paris, France*
- SYLWIA GAWRZAK • *Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona), Barcelona, Spain*
- PHILIPP GETTO • *Fachrichtung Mathematik, Institut für Analysis, TU Dresden, Dresden, Germany; Basque Center for Applied Mathematics (BCAM), Bilbao, Spain*
- MARINA A. GLUKHOVA • *Centre de Recherche, UMR144, CNRS, Institut Curie, PSL Research University, Paris, France*
- ROGER R. GOMIS • *Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona), Barcelona, Spain; Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain*
- AINTZANE GONZALEZ-LAHERA • *Genome Analysis Platform, CIC bioGUNE & CIBERehd, Derio, Spain*

- MARC GUIU • *Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona), Barcelona, Spain*
- BEATRICE A. HOWARD • *Division of Breast Cancer Research, Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK*
- RICHARD IGGO • *Bergonié Cancer Institute, University of Bordeaux, Bordeaux, France*
- OIHANA IRIONDO • *CIC bioGUNE, Technological Park of Bizkaia, Derio, Spain*
- NAOKO KOGATA • *Division of Breast Cancer Research, Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK*
- ERIC KUHN • *Broad Institute of MIT and Harvard, Cambridge, MA, USA*
- CHARLOTTE KUPERWASSER • *Developmental, Molecular, and Chemical Biology Department, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA; Raymond and Beverly Sackler Laboratory for the Convergence of Biomedical, Physical and Engineering Sciences, Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA, USA*
- RODERICK Y.H. LIM • *Biozentrum and The Swiss Nanoscience Institute, University of Basel, Basel, Switzerland*
- SULING LIU • *School of Life Sciences, University of Science and Technology of China, Hefei, China*
- MING LUO • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*
- NURIA MACIAS-CAMARA • *Genome Analysis Platform, CIC bioGUNE, Derio, Spain*
- TOMMASO DE MARCHI • *Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands; Postgraduate School of Molecular Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands*
- ANNA MARCINIAK-CZOCHRA • *Interdisciplinary Center for Scientific Computing and BIOQUANT, Institute of Applied Mathematics, Heidelberg University, Heidelberg, Germany*
- REBECCA MARLOW • *Breakthrough Breast Cancer Research Unit, Guy's Hospital, King's College London School of Medicine, London, UK*
- SUNITHA NAGRATH • *Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, USA*
- MILICA PAVLOVIC • *Oncology Program, Institute for Research in Biomedicine (IRB-Barcelona), Barcelona, Spain*
- MARIJA PLODINEC • *Biozentrum and The Swiss Nanoscience Institute, University of Basel, Basel, Switzerland*
- MIRIAM RÁBANO • *CIC bioGUNE, Technological Park of Bizkaia, Derio, Spain*
- ELODIE RICHARD • *Bergonié Cancer Institute, University of Bordeaux, Bordeaux, France*
- JOSÉ SCHNEIDER • *Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Madrid, Spain*
- ANIETA M. SIEUWERTS • *Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands; Postgraduate School of Molecular Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; Center for Translational Molecular Medicine, Eindhoven, The Netherlands*
- ARZU UMAR • *Laboratory of Breast Cancer Genomics and Proteomics, Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands; Postgraduate School of Molecular Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; Center for Translational Molecular Medicine, Eindhoven, The Netherlands*
- MARÍA d.M. VIVANCO • *CIC bioGUNE, Technological Park of Bizkaia, Derio, Spain*
- MAX S. WICHA • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*
- A. WRONSKI • *Developmental, Molecular and Chemical Biology Department, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA; Raymond and Beverly Sackler Laboratory for the Convergence of Biomedical, Physical and Engineering Sciences, Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA, USA*