

# 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>



# Cancer Chemoprevention

## Methods and Protocols

Edited by

**Sabrina Strano**

*Molecular Cancer Chemoprevention Unit, Molecular Medicine Department, Regina Elena National  
Cancer Institute, Rome, Italy*

*Department of Oncology, Juravinski Cancer Center-McMaster University Hamilton, Hamilton,  
ON L8V 5C2, ON, Canada*

 **Humana Press**

*Editor*

Sabrina Strano  
Molecular Cancer Chemoprevention Unit  
Molecular Medicine Department  
Regina Elena National Cancer Institute  
Rome, Italy

Department of Oncology  
Juravinski Cancer Center-McMaster  
University Hamilton  
Hamilton, Ontario, Canada

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-3190-3              ISBN 978-1-4939-3191-0 (eBook)  
DOI 10.1007/978-1-4939-3191-0

Library of Congress Control Number: 2015957808

Springer New York Heidelberg Dordrecht London  
© 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

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

---

## **Preface**

Despite the advent of personalized therapies, cancer still remains a leading cause of death worldwide. The number of cancer cases is rising yearly and is expected to double in the next 20 years. The largest increase of new cases will occur mainly in lower income countries, where the access to cancer care is still inadequate. Thus, there is a strong and urgent need of additional and complementary therapies. The increasing healthcare costs of novel anti-cancer therapeutic agents and their toxic side effects in high-income countries pave the way for cancer chemoprevention strategies. Natural compounds and dietary supplement phytochemicals appear to be efficacious arms of intervention in cancer chemoprevention. The potential value of these agents has been demonstrated in preclinical and observational studies. Large-scale clinical trials of primary and secondary cancer chemoprevention are still in progress. As for any type of clinical trials, the monitoring of the endpoints through the evaluation of proper biomarkers is still the challenge to pursue. With the present book for Cancer Chemoprevention Protocols, we depict along the 18 chapters the state of the art of methods that can be useful for both basic and translational researchers to conduct chemoprevention preclinical studies. Each chapter includes an introduction to the specific technology and a detailed method section. Published and unpublished observations of the contributing authors are also included.

I take the occasion to thank all the authors for their great contributions and for the enjoyable scientific interaction.

*Rome, Italy*

*Sabrina Strano*



---

## Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>
1 Controlled Delivery of Chemopreventive Agents by Polymeric Implants . . . . . <i>Farrukh Aqil and Ramesh C. Gupta</i>	1
2 Use of Buffy Coat miRNA Profiling for Breast Cancer Prediction in Healthy Women . . . . . <i>Sara Donzelli, Giovanni Blandino, and Paola Muti</i>	13
3 microRNAs in Cancer Chemoprevention: Method to Isolate Them from Fresh Tissues . . . . . <i>Federica Ganci and Giovanni Blandino</i>	21
4 Application of RNA-Seq Technology in Cancer Chemoprevention . . . . . <i>Frauke Goeman and Maurizio Fanciulli</i>	31
5 Detection of Circulating Tumor DNA in the Blood of Cancer Patients: An Important Tool in Cancer Chemoprevention . . . . . <i>Peter Ulz, Martina Auer, and Ellen Heitzer</i>	45
6 The Methylated DNA Immunoprecipitation [MeDIP] to Investigate the Epigenetic Remodeling in Cell Fate Determination and Cancer Development . . . . . <i>Silvia Masciarelli, Teresa Bellissimo, Ilaria Iosue, and Francesco Fazi</i>	69
7 LC-MS-Based Metabolomic Investigation of Chemopreventive Phytochemical-Elicited Metabolic Events . . . . . <i>Lei Wang, Dan Yao, and Chi Chen</i>	77
8 <sup>1</sup> H NMR Metabolomic Footprinting Analysis for the In Vitro Screening of Potential Chemopreventive Agents . . . . . <i>Luca Casadei and Mariacristina Valerio</i>	89
9 Comet Assay in Cancer Chemoprevention. . . . . <i>Raffaella Santoro, Maria Ferraiuolo, Gian Paolo Morgano, Paola Muti, and Sabrina Strano</i>	99
10 Angiogenesis Assays . . . . . <i>Dhanya K. Nambiar, Praveen K. Kujur, and Rana P. Singh</i>	107
11 AlgiMatrix™-Based 3D Cell Culture System as an In Vitro Tumor Model: An Important Tool in Cancer Research . . . . . <i>Chandrayiah Godugu and Mandip Singh</i>	117
12 Cancer Gastric Chemoprevention: Isolation of Gastric Tumor-Initiating Cells . . . . . <i>Federica Mori, Valeria Cannu, Laura Lorenzon, Alfredo Garofalo, Giovanni Blandino, and Sabrina Strano</i>	129
13 Isolation of Chemoresistant Cell Subpopulations. . . . . <i>Claudia Canino and Mario Cioce</i>	139

14 Autophagy in Cancer Chemoprevention: Identification of Novel Autophagy Modulators with Anticancer Potential . . . . . 151  
*Yuanzhi Lao and Naiban Xu*

15 Protocol for a Steady-State FRET Assay in Cancer Chemoprevention . . . . . 165  
*Marjolein C.A. Schaap, Andreia M.R. Guimarães, Andrew F. Wilderspin, and Geoffrey Wells*

16 3D Tumor Models and Time-Lapse Analysis by Multidimensional Microscopy . . . . . 181  
*Dimitri Scholz and Nobue Itasaki*

17 Antibody Array as a Tool for Screening of Natural Agents in Cancer Chemoprevention . . . . . 189  
*Claudio Pulito, Andrea Sacconi, Etleva Korita, Anna Maidecchi, and Sabrina Strano*

18 South African Herbal Extracts as Potential Chemopreventive Agents: Screening for Anticancer Splicing Activity . . . . . 201  
*Zodwa Dlamini, Zukile Mbita, and David Bates*

*Erratum* . . . . . E1

*Index* . . . . . 213



---

## Contributors

- FARRUKH AQIL • *James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA; Department of Medicine, University of Louisville, Louisville, KY, USA*
- MARTINA AUER • *Institute of Human Genetics, Medical University of Graz, Graz, Austria*
- DAVID BATES • *Faculty of Medicine and Health Sciences, Division of Pre-clinical Oncology, University of Nottingham, Nottingham, UK*
- TERESA BELLISSIMO • *Section of Histology and Medical Embryology, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, Rome, Italy*
- GIOVANNI BLANDINO • *Laboratory of Translational Oncogenomics, Molecular Medicine Department, Regina Elena National Cancer Institute, Rome, Italy*
- CLAUDIA CANINO • *Division of Thoracic Surgery, Department of Cardiothoracic Surgery, Langone Medical Center, New York University, New York, NY, USA*
- VALERIA CANU • *Translational Oncogenomic Unit, Molecular Medicine Department, Regina Elena National Cancer Institute, Rome, Italy*
- LUCA CASADEI • *Department of Chemistry, “Sapienza” University of Rome, Rome, Italy*
- CHI CHEN • *Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA*
- MARIO CIOCE • *Division of Thoracic Surgery, Department of Cardiothoracic Surgery, Langone Medical Center, New York University, New York, NY, USA*
- ZODWA DIAMINI • *Research, Innovation & Engagements, Mangosuthu University of Technology, Durban, South Africa*
- SARA DONZELLI • *Translational Oncogenomics Unit, Regina Elena Italian National Cancer Institute, Rome, Italy*
- MAURIZIO FANCIULLI • *Laboratory of Epigenetic, Molecular Medicine Area, Italian National Cancer Institute “Regina Elena”, Rome, Italy*
- FRANCESCO FAZI • *Section of Histology and Medical Embryology, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, “Sapienza” University of Rome, Rome, Italy*
- MARIA FERRAIUOLO • *Molecular Chemoprevention Unit, Molecular Medicine Area, Regina Elena National Cancer Institute, Rome, Italy*
- FEDERICA GANCI • *Translational Oncogenomics Unit, Italian National Cancer Institute “Regina Elena”, Rome, Italy*
- ALFREDO GAROFALO • *Department of GI Surgery, Regina Elena National Cancer Institute, Rome, Italy*
- CHANDRAIAH GODUGU • *Department of Regulatory Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, Telangana, India*
- FRAUKE GOEMAN • *Translational Oncogenomics Unit, Italian National Cancer Institute “Regina Elena”, Rome, Italy*
- ANDREIA M.R. GUIMARÃES • *UCL School of Pharmacy, University College London, London, UK*
- RAMESH C. GUPTA • *James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA; Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA*

- ELLEN HEITZER • *Institute of Human Genetics, Medical University of Graz, Graz, Austria*
- ILARIA IOSUE • *Section of Histology and Medical Embryology, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, “Sapienza” University of Rome, Rome, Italy*
- NOBUE ITASAKI • *School of Medicine and Medical Science, University College Dublin, Dublin, Ireland; Faculty of Health Sciences, University of Bristol, Bristol, UK*
- ETLEVA KORITA • *Molecular Chemoprevention Unit, Molecular Medicine Area, Italian National Cancer Institute “Regina Elena”, Rome, Italy*
- PRAVEEN K. KUJUR • *Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India*
- YUANZHI LAO • *School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China*
- LAURA LORENZON • *Surgical and Medical Department of Translational Medicine, Faculty of Medicine and Psychology, Sant’Andrea Hospital, University of Rome “La Sapienza”, Rome, Italy*
- ANNA MAIDECCHI • *Aboca SpA Società Agricola, Sansepolcro, Italy*
- SILVIA MASCIARELLI • *Section of Histology and Medical Embryology, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, Rome, Italy*
- ZUKILE MBITA • *Mangosuthu University of Technology Research, Innovation and Engagements Portfolio, Durban, South Africa*
- GIAN PAOLO MORGANO • *Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada*
- FEDERICA MORI • *Molecular Chemoprevention Unit, Molecular Medicine Department, Regina Elena National Cancer Institute, Rome, Italy*
- PAOLA MUTI • *Department of Oncology, Juravinski Cancer Center, McMaster University, Hamilton, ON, Canada*
- DHANYA K. NAMBIAR • *Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India*
- CLAUDIO PULITO • *Molecular Chemoprevention Unit, Molecular Medicine Area, Italian National Cancer Institute “Regina Elena”, Rome, Italy*
- ANDREA SACCONI • *Molecular Medicine Area, Regina Elena National Cancer Institute, Rome, Italy*
- RAFFAELA SANTORO • *Molecular Chemoprevention Unit, Molecular Medicine Area, Regina Elena National Cancer Institute, Rome, Italy*
- MARJOLEIN C.A. SCHAAP • *UCL School of Pharmacy, University College London, London, UK*
- DIMITRI SCHOLZ • *Conway Institute, University College Dublin, Dublin, Ireland*
- RANA P. SINGH • *Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India; School of Life Sciences, Central University of Gujarat, Gandhinagar, Gujarat, India*
- MANDIP SINGH • *College of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee, FL, USA*
- SABRINA STRANO • *Molecular Cancer Chemoprevention Unit, Molecular Medicine Department, Regina Elena National Cancer Institute, Rome, Italy; Department of Oncology, Juravinski Cancer Center-McMaster University Hamilton, Hamilton, ON, Canada*
- PETER ULZ • *Institute of Human Genetics, Medical University of Graz, Graz, Austria*
- MARIACRISTINA VALERIO • *Department of Chemistry, “Sapienza” University of Rome, Rome, Italy*

LEI WANG • *Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA*

GEOFFREY WELLS • *UCL School of Pharmacy, University College London, London, UK*

ANDREW F. WILDERSPIN • *UCL School of Pharmacy, University College London, London, UK*

NAIHAN XU • *Key Lab in Healthy Science and Technology, Division of Life Science, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China*

DAN YAO • *Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA*