

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>

Suicide Gene Therapy

Methods and Protocols

Edited by

Nejat Düzgüneş

*Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific,
San Francisco, CA, USA*

 **Humana Press**

Editor

Nejat Düzgüneş
Department of Biomedical Sciences
Arthur A. Dugoni School of Dentistry
University of the Pacific
San Francisco, CA, USA

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-8921-8 ISBN 978-1-4939-8922-5 (eBook)
<https://doi.org/10.1007/978-1-4939-8922-5>

Library of Congress Control Number: 2018961739

© Springer Science+Business Media, LLC, part of Springer Nature 2019

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. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Humana Press imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.

The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

The concept of suicide gene therapy most likely originated in 1986 with the work of Frederick Moolten at the Hubert H. Humphrey Cancer Research Center at Boston University. He exposed neoplastic BALB/c murine cell lines carrying the herpes simplex virus thymidine kinase gene to the herpes thymidine kinase-specific substrate 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy] methyl]guanine, which inhibited the clonogenic potential of the cells *in vitro*. Tumors produced by these cell lines in BALB/c mice underwent complete regression following exposure to this drug.

Subsequently, in 1988, Ronald Evans and collaborators at the Salk Institute in La Jolla used a procedure to kill transfected cells in culture or to selectively delete immune cells in transgenic mice upon administration of acyclovir or 1-(2-deoxy-2-fluoro-beta-D-arabino-furanosyl)-5-iodouracil (FIAU). In 1991, Knudsen and Karlström at the University of Copenhagen used the *Escherichia coli relF* gene driven by *lac*-derived promoters to devise a biological containment system for genetically engineered bacteria. In the same year, the Pardoll laboratory at Johns Hopkins University in Baltimore utilized the HSV-*tk* + ganciclovir system in tumor cell vaccines as a means to eliminate the cells in case the immune system was unable to clear them. Klatzman and colleagues at the Hôpital de la Pitié-Salpêtrière, Paris, in 1992, employed the HSV-*tk* + ganciclovir system to kill liver tumor cells *in vivo*. They also engineered CD4+ T-lymphocytes to express HSV-*tk* under the control of the HIV promoter (5'-LTR) and showed the elimination of these cells upon HIV infection. The Blaese laboratory at the National Cancer Institute showed in 1994 that tumor cells expressing the cytosine deaminase gene are eliminated *in vivo* by the administration of 5-fluorocytosine, which is converted to the toxic compound, 5-fluorouracil.

This volume includes the methods used for most of the recent approaches to suicide gene therapy of cancer. It also contains chapters describing methods to improve the safety of cell therapy and those utilizing bone marrow mesenchymal cells. The goal of cancer treatment is to eliminate, or at least to greatly reduce, the number of cancer cells without harming normal cells. In addition to utilizing cell surface markers overexpressed on cancer cells, targeted suicide gene therapy exploits promoters that are specific to cancer cells, thereby ensuring (or greatly increasing the likelihood) that the therapeutic gene is expressed only in cancer cells.

In the first chapter, we outline the origins of the different systems used for suicide gene therapy. In Chapter 2, Fehse, Miletic, and collaborators provide guidelines for the preparation of high-titer 3rd-generation lentiviral vectors that encode a genetically improved HSV-*tk* version (TK.007) and its application *in vitro* and *in vivo*. The success of suicide gene therapy can be greatly enhanced by the use of tissue-specific or tumor-specific gene expression and efficient gene delivery. Thus, Chung and Hsieh and co-authors provide the details of osteonectin promoter-mediated suicide gene therapy of prostate cancer in Chapter 3. Pedroso de Lima and collaborators describe in Chapter 4 the methods for the use of the HSV-*tk*/ganciclovir system to achieve antitumor activity both in cultured oral cancer cells and in orthotopic and subcutaneous murine models of oral squamous cell carcinoma, using ligand-associated lipoplexes for enhancing therapeutic delivery.

Although chimeric antigen receptor (CAR)-redirected T-cells can be used for the treatment of cancers, they can have adverse effects such as the cytokine release syndrome and off-target effects that can cause organ damage. In Chapter 5, Di Stasi and co-authors describe an inducible caspase 9 suicide gene system that can eliminate a large percentage of CAR T-cells when necessary. Altaner and Altanerova outline their methods to prepare exosomes derived from human mesenchymal stem cells engineered to express cytosine deaminase and uracil phosphoribosyl transferase mRNA, and their use in inhibiting the growth of cancer cells following the administration of 5-fluorocytosine as a prodrug (Chapter 6). In Chapter 7, Saydam and colleagues describe a similar system, which also expresses the green fluorescent protein in HEK293T cells, to produce extracellular vesicles that they have used to inhibit the growth of glioblastoma cell lines and spheroids, and glioblastoma tumors in vivo.

Viral vectors with suicide genes are very difficult to produce, because trace amounts of toxins (such as diphtheria toxin or the enzyme barnase) can kill the cells used for viral vector production. In Chapter 8, Chen describes a method to overcome this problem. In this method, insect cells are used, since mammalian introns are not recognized by these cells, and the open reading frames of the toxic genes are not broken up. Thus, the insect cells do not produce the toxins but generate normal levels of baculovirus and adeno-associated viral vectors carrying the toxin genes. Bhatia and Shi (Chapter 9) describe a vector containing resveratrol-responsive CC(A+T rich)6GG (referred to as a “CARg box”) elements from the promoter of the early growth response protein 1 (Egr-1) and the GADD45 α open reading frame. After delivery into lung cancer cells and treatment with resveratrol, the vector expresses GADD45 α , leading to cell cycle arrest.

Suicide gene expression may be used as a safety mechanism in regenerative medicine. Unwanted multipotent stem cells could thus be removed before they cause side effects, such as teratoma formation. The location of such cells can be monitored by imaging approaches. Himmelreich and co-authors describe in Chapter 10 how therapeutic cells can be engineered to express a suicide gene as well as genes that can be used for visualization in vivo. Demidyuk and colleagues provide the methods to create and evaluate the cytotoxic action of a bicistronic plasmid expressing the yeast cytosine deaminase/uracil phosphoribosyltransferase fusion protein and the hepatitis A virus 3C protease (Chapter 11).

Suicide gene therapy can result in the elimination of cancer cells within a tumor beyond those cells that actually express the transgene, in a process termed the “bystander effect.” This is achieved by the spread (via gap junctions) of cytotoxic anti-metabolites produced by the transgene to neighboring cells that may not have been transfected or transduced. Neschadim and Medin have developed a suicide gene therapy system that mediates a highly effective bystander effect, based on a variant of human deoxycytidine kinase that can phosphorylate the unusual nucleoside analogs bromovinyl deoxyuridine and L-deoxythymidine, which they describe in Chapter 12. Hirschberg and colleagues describe a modified form of photodynamic therapy that enhances the delivery of the cytosine deaminase gene into tumor cells and the cytotoxic effect of the locally produced 5-FU (Chapter 13). Finally, in Chapter 14, we provide the methods for TransfeX-mediated transfection of the plasmid pNGVL1-*tk* encoding HSV-tk under the control of the CMV promoter into HeLa cervical carcinoma, HSC-3 and H357 oral squamous cell carcinoma, and FaDu pharyngeal carcinoma cell lines, and the resulting cytotoxicity upon administration of ganciclovir.

I would like to thank Professor John Walker, the series editor, for his patient and unwavering support and advice in moving the editing along. I am also grateful to my wife Diana and my children Avery and Maxine who always supported me in my editing and writing endeavors.

I would like to dedicate this volume to the memory of my Ph.D. advisor, Professor Shinpei Ohki at the State University of New York at Buffalo (now University at Buffalo).

San Francisco, CA, USA

Nejat Düzgüneş

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>xi</i>
1 Origins of Suicide Gene Therapy	1
<i>Nejat Düzgüneş</i>	
2 Cancer Suicide Gene Therapy with TK.007	11
<i>Jubayer A. Hossain, Kristoffer Riecken, Hrvoje Miletic, and Boris Fehse</i>	
3 Osteonectin Promoter-Mediated Suicide Gene Therapy of Prostate Cancer	27
<i>Wan-Chi Hsiao, Shian-Ying Sung, Leland W. K. Chung, and Chia-Ling Hsieh</i>	
4 Suicide Gene Therapy for Oral Squamous Cell Carcinoma	43
<i>Henrique Faneca, Nejat Düzgüneş, and Maria C. Pedroso de Lima</i>	
5 Generation of Suicide Gene-Modified Chimeric Antigen Receptor-Redirected T-Cells for Cancer Immunotherapy	57
<i>Kentaro Minagawa, Mustafa Al-Obaidi, and Antonio Di Stasi</i>	
6 Mesenchymal Stem Cell Exosome-Mediated Prodrug Gene Therapy for Cancer	75
<i>Cestmir Altaner and Ursula Altanerova</i>	
7 Extracellular Vesicles as Carriers of Suicide mRNA and/or Protein in Cancer Therapy	87
<i>Erdogan Pekcan Erkan, Nurten Saydam, Clark C. Chen, and Okay Saydam</i>	
8 Production of Viral Vectors with Suicide Genes by Utilizing the Intron-Splicing Mechanism of Insect Cells	97
<i>Haifeng Chen</i>	
9 Resveratrol-Responsive CARG Elements from the Egr-1 Promoter for the Induction of GADD45 α to Arrest the G2/M Transition	111
<i>Qiwen Shi and Deepak Bhatia</i>	
10 Noninvasive Monitoring of Suicide Gene Therapy by Using Multimodal Molecular Imaging	123
<i>Bryan Holvoet, Cindy Leten, Christophe M. Deroose, and Uwe Himmelreich</i>	
11 In Vitro Assay for the Evaluation of Cytotoxic Effects Provided by a Combination of Suicide and Killer Genes in a Bicistronic Vector	135
<i>Alexey A. Komissarov, Sergey V. Kostrov, and Ilya V. Demidyuk</i>	
12 Engineered Thymidine-Active Deoxycytidine Kinase for Bystander Killing of Malignant Cells	149
<i>Anton Neschadim and Jeffrey A. Medin</i>	

13	Photochemical Internalization Enhanced Nonviral Suicide Gene Therapy	165
	<i>Chung-Ho Sun, Kristian Berg, and Henry Hirschberg</i>	
14	Suicide Gene Therapy of Oral Squamous Cell Carcinoma and Cervical Carcinoma In Vitro	177
	<i>Nejat Düzgüneş, Jennifer Cheung, and Krystyna Konopka</i>	
	<i>Index</i>	185

Contributors

- MUSTAFA AL-OBAYDI • *Department of Hematology/Oncology, Bone Marrow Transplantation and Cell Therapy Unit, University of Alabama at Birmingham, Birmingham, AL, USA*
- CESTMIR ALTANER • *Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; Stem Cell Preparation Department, St. Elizabeth Cancer Institute, Bratislava, Slovakia*
- URSULA ALTANEROVA • *Stem Cell Preparation Department, St. Elizabeth Cancer Institute, Bratislava, Slovakia*
- KRISTIAN BERG • *Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*
- DEEPAK BHATIA • *Bernard J. Dunn School of Pharmacy, Shenandoah University, Fairfax, VA, USA*
- CLARK C. CHEN • *Department of Neurosurgery and School of Medicine, University of Minnesota, Minneapolis, MN, USA*
- HAIFENG CHEN • *R&D, Virovek, Inc., Hayward, CA, USA*
- JENNIFER CHEUNG • *Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA*
- LELAND W. K. CHUNG • *Uro-oncology Research Program, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
- ILYA V. DEMIDYUK • *Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia*
- CHRISTOPHE M. DEROOSE • *Nuclear Medicine and Molecular Imaging, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium; Molecular Small Animal Imaging Center (MoSAIC), University of Leuven, Leuven, Belgium*
- ANTONIO DI STASI • *Department of Hematology/Oncology, Bone Marrow Transplantation and Cell Therapy Unit, University of Alabama at Birmingham, Birmingham, AL, USA*
- NEJAT DÜZGÜNEŞ • *Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA*
- ERDOGAN PEKCAN ERKAN • *Department of Medical Genetics, Medicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland*
- HENRIQUE FANECA • *CNC—Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
- BORIS FEHSE • *Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
- UWE HIMMELREICH • *Molecular Small Animal Imaging Center (MoSAIC), University of Leuven, Leuven, Belgium; Biomedical MRI Unit, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium*
- HENRY HIRSCHBERG • *Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA, USA*
- BRYAN HOLVOET • *Nuclear Medicine and Molecular Imaging, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium; Molecular Small Animal Imaging Center (MoSAIC), University of Leuven, Leuven, Belgium*

- JUBAYER A. HOSSAIN • *Department of Biomedicine, University of Bergen, Bergen, Norway; KG Jebsen Brain Tumor Research Centre, University of Bergen, Bergen, Norway; Department of Pathology, Haukeland University Hospital, Bergen, Norway*
- WAN-CHI HSIAO • *The Ph.D. Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan*
- CHIA-LING HSIEH • *The Ph.D. Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan; TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan*
- ALEXEY A. KOMISSAROV • *Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia*
- KRYSZYNA KONOPKA • *Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA*
- SERGEY V. KOSTROV • *Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia*
- CINDY LETEN • *Molecular Small Animal Imaging Center (MoSAIC), University of Leuven, Leuven, Belgium; Biomedical MRI Unit, Department of Imaging and Pathology, University of Leuven, Leuven, Belgium*
- JEFFREY A. MEDIN • *Departments of Pediatrics and Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA*
- HRVOJE MILETIC • *Department of Biomedicine, University of Bergen, Bergen, Norway; KG Jebsen Brain Tumor Research Centre, University of Bergen, Bergen, Norway; Department of Pathology, Haukeland University Hospital, Bergen, Norway*
- KENTARO MINAGAWA • *Department of Hematology/Oncology, Bone Marrow Transplantation and Cell Therapy Unit, University of Alabama at Birmingham, Birmingham, AL, USA*
- ANTON NESCHADIM • *Centre for Innovation, Canadian Blood Services, Toronto, ON, Canada*
- MARIA C. PEDROSO DE LIMA • *CNC—Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
- KRISTOFFER RIECKEN • *Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
- NURTEN SAYDAM • *Department of Neurosurgery and School of Medicine, University of Minnesota, Minneapolis, MN, USA*
- OKAY SAYDAM • *Department of Neurosurgery and School of Medicine, University of Minnesota, Minneapolis, MN, USA*
- QIWEN SHI • *Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Zhejiang University of Technology, Hangzhou, Zhejiang, China*
- CHUNG-HO SUN • *Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA, USA*
- SHIAN-YING SUNG • *The Ph.D. Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan; Joint Clinical Research Center, Office of Human Research, Taipei Medical University, Taipei, Taiwan; TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan*