

METHODS IN MOLECULAR BIOLOGY

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Cancer Immunosurveillance

Methods and Protocols

Editors

Alejandro López-Soto

*Departamento de Biología Funcional, Inmunología, Universidad De Oviedo,
Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, Spain*

Alicia R. Folgueras

*Departamento de Bioquímica y Biología Molecular, Universidad de Oviedo, Instituto Universitario
de Oncología del Principado de Asturias (IUOPA), Oviedo, Asturias, Spain*

Editors

Alejandro López-Soto
Departamento de Biología
Funcional, Inmunología
Universidad de Oviedo, Instituto Universitario de
Oncología del Principado de Asturias (IUOPA)
Oviedo, Spain

Alicia R. Folgueras
Departamento de Bioquímica y
Biología Molecular
Universidad de Oviedo, Instituto Universitario de
Oncología del Principado de Asturias (IUOPA)
Oviedo, Asturias, Spain

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Preface

During the past few years, the field of tumor immunology has been the subject of an exciting and growing interest in cancer research. In this sense, the encouraging results obtained in patients with certain types of cancer treated with therapies that ignite specific antitumor immune responses strongly support that immune-based treatments are likely to open new therapeutic opportunities for cancer patients. Moreover, compelling experimental and clinical evidence suggests that a functional immune system is required in order to achieve a protective response in cancer patients exposed to the therapeutic approaches that are currently being used.

Given the vast number of new scientific approaches that are constantly being incorporated and improved in the field of oncoimmunology, it is imperative to summarize the cutting-edge techniques devoted to unveil the mechanisms that regulate the immune response to cancer cells. This book of the series *Methods in Molecular Biology* aims to provide a selection of a number of techniques that will be of interest for both preclinical and clinical researchers in the field of tumor immunology.

The initial chapters of the book detail different methodologies for functional analysis and expansion of T lymphocytes for cancer research, including the droplet digital PCR assay of T cell quantification in cancer, as well as protocols to generate tumor antigen-specific cytotoxic T lymphocytes from pluripotent stem cells and to expand human V δ 2⁺ T cells for adoptive transfer. A chapter describes how single-cell RNA sequencing can be exploited to dissect heterogeneity and identify precursors of highly complex immune cell subsets with prominent roles in cancer, such as the recently identified innate lymphoid cells (ILCs). In addition, detailed protocols are described to isolate and expand natural killer (NK) cells and to evaluate their antitumor killing activity in vitro (CD107a degranulation assay and flow cytometric and radioactive NK cell-mediated cytotoxicity assays). Likewise, a collection of mouse models to study cancer immunosurveillance in vivo orchestrated by specific immune subsets are described in successive chapters. These include procedures to evaluate NK cell-mediated immunosurveillance of latent metastases, the tumor promoting activity of tumor-associated macrophages as well as the conditional genetic ablation in mice of genes required for the development of certain immune populations. Local tumor microenvironments are known to shape immunity and, therefore, it is essential to study the anti-cancer immune cells in the context of the tumor bed. Thus, procedures are described to isolate immune cells from brain and colitis-associated colorectal cancers tumor microenvironments, which are accompanied by assays to dissect the immune-tumor cell interplay, such as the establishment of slice cultures to mimic the cancer-immune microenvironment and the use of heterotypic tumor-stroma spheroids which, for instance, allow the evaluation of the impact of immune checkpoint blockade on antitumor immune responses.

A second group of chapters outline techniques that are devoted to gain insight into the cancer-intrinsic properties that modulate their immunogenicity and, therefore, the immune recognition and elimination of developing tumors. These include protocols to delve deeper into cancer signaling pathways by single-cell mass cytometry, to purify leukemia-derived exosomes, quantitatively identify senescent cells in cancer, and to characterize circulating

tumor cells to study tumor immune subversion. Furthermore, the use of HLA peptidomics for cancer exome-based identification of tumor neo-antigens by mass spectrometry is described, which is complementary to the protocol for high-throughput T cell receptor sequencing from tumors that is detailed in one of the initial chapters of the book.

The remaining chapters describe methods for studying the therapeutic relevance of immune modulation in cancer. Hence, a gold standard to assess the so-called immunogenic cell death (a form of cancer cell demise triggered by certain forms of conventional anticancer agents, which ignites immunity) in mice and a novel vaccination protocol involving dendritic cells to enhance immune protective response to aggressive cancers are described. Finally, detailed protocols for the production of two forms of immunotherapies recently approved by the US Food and Drug Administration (FDA) for the treatment of patients with certain types of cancer—so-called bispecific antibody derivatives, and T cells engineered to express chimeric antigen receptors (CAR-T cells)—are also included.

We would like to take this opportunity to express our gratitude to all the authors for their excellent contribution. We sincerely hope that the protocols assembled in this book will serve the research community to better understand and to design their experiments on the exciting and relevant field of tumor immunology.

Oviedo, Spain

*Alejandro López-Soto
Alicia R. Folgueras*

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Contributors

- MAYKEL ARIAS • *Fundación Instituto de Investigación Sanitaria Aragón (IIS Aragón), Biomedical Research Centre of Aragón (CIBA), Zaragoza, Spain*
- ROBERT BERAHOVICH • *Promab Biotechnologies, Richmond, CA, USA*
- ANAT BIRAN • *Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel*
- PAULA D. BOS • *Department of Pathology, Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, VA, USA*
- EDWIN BREMER • *Department of Hematology, Section Immunohematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*
- GUIDO CARPINO • *Dipartimento di Anatomia, Istologia, Medicina Forense e Scienze Ortopediche, Sapienza Università di Roma, Rome, Italy*
- PAOLO CARREGA • *Laboratory of Immunology and Biotherapy, Department of Human Pathology, University of Messina, Messina, Italy; Cell Factory Center, University of Messina, Messina, Italy*
- VINCENZO CASOLARO • *Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Baronissi, Salerno, Italy*
- MARTA CASTRO • *Faculty of Veterinary, Department of Physiology, University of Zaragoza, Zaragoza, Spain*
- BENNY CHAIN • *Division of Infection and Immunity, UCL, London, UK*
- XIAONIAO CHEN • *Department of Ophthalmology, Chinese PLA General Hospital, Beijing, China; Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
- NICHOLAS M. CLARK • *Department of Pathology, Integrative Life Sciences Graduate Program, Virginia Commonwealth University School of Medicine, Richmond, VA, USA*
- JESSICA DAL COL • *Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Baronissi, Salerno, Italy*
- Y. DAVID SEO • *Department of Surgery, University of Washington School of Medicine, Seattle, WA, USA*
- MAGALI DUPONT • *Unité d'Immunité Innée, Département d'Immunologie, Institut Pasteur, Paris, France; INSERM U1223, Paris, France; Université Paris Diderot, Paris, France*
- ANNA FRÖMMING • *NOXXON Pharma, Berlin, Germany*
- ERNESTO GARGIULO • *Laboratory of Experimental Cancer Research, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- PAOLA GAZZANIGA • *Dipartimento di Medicina Molecolare, Sapienza Università di Roma, Rome, Italy*
- JAVIER GODINO • *Cell Separation and Flow Cytometry Core Facility, Aragon Institute of Health Sciences (IACS), Zaragoza, Spain*
- VITA GOLUBOVSKAYA • *Promab Biotechnologies, Richmond, CA, USA*

- SEGUNDO GONZÁLEZ • *Departamento de Biología Funcional, Inmunología, Universidad of Oviedo, Oviedo, Spain; Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Instituto de Investigación Sanitaria del Principado de Asturias (IISPA), Oviedo, Spain*
- ANGELA GRADILONE • *Dipartimento di Medicina Molecolare, Sapienza Università di Roma, Rome, Italy*
- CORALIE GUERIN • *National Cytometry Platform, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- JENNY E. GUMPERZ • *Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*
- HIZKIA HARTO • *Promab Biotechnologies, Richmond, CA, USA*
- YUAN HE • *Department of Hematology, Section Immunohematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*
- JAMES M. HEATHER • *Division of Infection and Immunity, UCL, London, UK*
- WIJNAND HELFRICH • *Department of Surgery, Translational Surgical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*
- JULIETTE HUMEAU • *Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; INSERM, U1138, Paris, France; Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France; Université Pierre et Marie Curie/Paris VI, Paris, France; Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France*
- XIUYUN JIANG • *Department of Surgery, University of Washington School of Medicine, Seattle, WA, USA*
- KROOPA JOSHI • *Cancer Immunology Unit, UCL Cancer Institute, UCL, London, UK*
- SHELLY KALAORA • *Molecular Cell Biology Department, Weizmann Institute of Science, Rehovot, Israel*
- VALERY KRIZHANOVSKY • *Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel*
- GUIDO KROEMER • *Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; INSERM, U1138, Paris, France; Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France; Université Pierre et Marie Curie/Paris VI, Paris, France; Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France; Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden*
- KEN S. LAU • *Epithelial Biology Center and the Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA*
- SARAH LÉVESQUE • *Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; INSERM, U1138, Paris, France; Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France; Université Pierre et Marie Curie/Paris VI, Paris, France*
- FENGYANG LEI • *Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*
- PENGTAO LIU • *LKS Faculty of Medicine, School of Biomedical Sciences, The University of Hong Kong, Hong Kong, China*

- ALEJANDRO LÓPEZ-SOTO • *Departamento de Biología Funcional, Inmunología, Universidad de Oviedo, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, Spain*
- SEILA LORENZO-HERRERO • *Departamento de Biología Funcional, Inmunología, Universidad of Oviedo, Oviedo, Spain; Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Instituto de Investigación Sanitaria del Principado de Asturias (IISPA), Oviedo, Spain*
- SRINIVAS MALLADI • *Department of Pathology, Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX, USA*
- RAFFAELLA MEAZZA • *UOC Immunologia, IRCCS Ospedale Policlinico San Martino, Genova, Italy*
- BARBARA MONTICO • *Immunopathology and Cancer Biomarkers Unit, Department of Translational Research, CRO National Cancer Institute—IRCCS, Aviano, Pordenone, Italy*
- ETIENNE MOUSSAY • *Laboratory of Experimental Cancer Research, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- VIDHYA R. NAIR • *Department of Pathology, UT Southwestern Medical Center, Dallas, TX, USA*
- ROGIER J. NELL • *Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands*
- CHIARA NICOLAZZO • *Dipartimento di Medicina Molecolare, Sapienza Università di Roma, Rome, Italy*
- ANNUNZIATA NIGRO • *Department of Medicine, Surgery and Dentistry ‘Scuola Medica Salernitana’, University of Salerno, Baronissi, Salerno, Italy*
- THERES OAKES • *Division of Infection and Immunity, UCL, London, UK*
- JEROME PAGGETTI • *Laboratory of Experimental Cancer Research, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- JULIÁN PARDO • *Fundación Instituto de Investigación Sanitaria Aragón (IIS Aragón), Biomedical Research Centre of Aragon (CIBA), Zaragoza, Spain; Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Zaragoza, Spain; Department of Microbiology, Preventive Medicine and Public Health, University of Zaragoza, Zaragoza, Spain; Aragon I+D Foundation (ARAID), Zaragoza, Spain; Nanoscience Institute of Aragon (INA), University of Zaragoza, Zaragoza, Spain*
- MONICA PARODI • *UOC Immunologia, IRCCS Ospedale Policlinico San Martino, Genova, Italy*
- SANDRINE PIERSON • *Laboratory of Experimental Cancer Research, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- GABRIELLA PIETRA • *UOC Immunologia, IRCCS Ospedale Policlinico San Martino, Genova, Italy; Department of Experimental Medicine (DIMES), University of Genova, Genoa, Italy*
- venu G. PILLARISETTY • *Department of Surgery, University of Washington School of Medicine, Seattle, WA, USA*
- JONATHAN G. POL • *Gustave Roussy Comprehensive Cancer Institute, Villejuif, France; INSERM, U1138, Paris, France; Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France; Université Pierre et Marie Curie/Paris VI, Paris, France*

- ZIV PORAT • *Flow Cytometry Unit, Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel*
- CRISTINA RAIMONDI • *Dipartimento di Medicina Molecolare, Sapienza Università di Roma, Rome, Italy; Dipartimento di Scienze Radiologiche, Oncologiche ed Anatomopatologiche, Sapienza Università di Roma, Rome, Italy*
- YARDENA SAMUELS • *Molecular Cell Biology Department, Weizmann Institute of Science, Rehovot, Israel*
- LLIPSY SANTIAGO • *Fundación Instituto de Investigación Sanitaria Aragón (IIS Aragón), Biomedical Research Centre of Aragon (CIBA), Zaragoza, Spain*
- ALBERTO J. SCHUHMACHER • *Molecular Oncology Group, Aragon Health Research Institute (IIS Aragón), Zaragoza, Spain*
- CHERIE' R. SCURRAH • *Epithelial Biology Center and the Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA*
- AKSHAT SHARMA • *Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*
- ALAN J. SIMMONS • *Epithelial Biology Center and the Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA*
- JIANXUN SONG • *Department of Microbial Pathogenesis and Immunology, Texas A&M University University Health Science Center, College Station, TX, USA*
- CHRISTIAN SORDO-BAHAMONDE • *Departamento de Biología Funcional, Inmunología, Universidad of Oviedo, Oviedo, Spain; Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Instituto de Investigación Sanitaria del Principado de Asturias (IISPA), Oviedo, Spain*
- KEVIN M. SULLIVAN • *Department of Surgery, University of Washington School of Medicine, Seattle, WA, USA*
- CHARLES SWANTON • *Cancer Research UK Lung Cancer Centre of Excellence, UCL Cancer Institute, UCL, London, UK; Translational Cancer Therapeutics Laboratory, The Francis Crick Institute, London, UK*
- IMRAN UDDIN • *Division of Infection and Immunity, UCL, London, UK*
- PIETER A. VAN DER VELDEN • *Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands*
- CHIARA VITALE • *UOC Immunologia, IRCCS Ospedale Policlinico San Martino, Genova, Italy; Department of Experimental Medicine (DIMES), University of Genova, Genoa, Italy*
- MASSIMO VITALE • *UOC Immunologia, IRCCS Ospedale Policlinico San Martino, Genova, Italy*
- CHRISTIAN A. J. VOSSHENRICH • *Unité d'Immunité Innée, Département d'Immunologie, Institut Pasteur, Paris, France; INSERM U1223, Paris, France*
- LIQIANG WANG • *Department of Ophthalmology, Chinese PLA General Hospital, Beijing, China*
- Marina Wierz • *Laboratory of Experimental Cancer Research, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg*
- Lijun Wu • *Promab Biotechnologies, Richmond, CA, USA*
- Xiaofang Xiong • *Department of Microbial Pathogenesis and Immunology, Texas A&M University University Health Science Center, College Station, TX, USA*
- QUMIAO XU • *Promab Biotechnologies, Richmond, CA, USA*
- Shirley Xu • *Promab Biotechnologies, Richmond, CA, USA*

YONG YU • *Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, School of Life Sciences and Technology, Tongji University, Shanghai, China*

DIRK ZBORALSKI • *3B Pharmaceuticals, Berlin, Germany*

HUA ZHOU • *Promab Biotechnologies, Richmond, CA, USA*

WILLEM H. ZOUTMAN • *Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands*

NICHOLAS A. ZUMWALDE • *Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*