METHODS IN MOLECULAR BIOLOGY

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Cancer Immunosurveillance

Methods and Protocols

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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 $V\delta2^+$ 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 anticancer 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

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