Preface

In Vitro and In Vivo Cell Senescence

Cell senescence, i.e., the process whereby cells permanently lose the possibility to proliferate without undergoing cell death, can be observed in vitro as well as in vivo, and occurs in a plethora of distinct model organisms. In both cases, cell senescence can be physiological, constituting a safeguard mechanism against cells that have accumulated potentially dangerous genetic alterations, or can be triggered by exogenous perturbations, such as the administration of DNA-damaging agents at low doses. This book provides a detailed description of the most common techniques for the investigation of cell senescence, in model organisms encompassing bacteria (Escherichia coli), fungi (Saccharomyces cerevisiae and Podospora anserina), worms (Caenorhabditis elegans), flies (Drosophila melanogaster), zebrafish (Danio rerio), and mammalian cells. The techniques presented in this book not only cover the study of all the biochemical and functional manifestations of senescence at the cellular level but also include protocols for population analysis and high-throughput approaches in suitable model organisms, as described by worldwide renowned experts of the field.

Chapter Organization

The book is composed of three types of chapters. Four review chapters open the book to provide a solid theoretical background on cell senescence, its morphological and biochemical manifestations and its pathophysiological relevance. Twenty-three protocol chapters follow, detailing the methods to investigate the morphological and biochemical features of senescence at the cellular level, in cultured mammalian cells. Finally, seven protocol chapters provide techniques for the study of cell senescence in lower model organisms, including methods for population studies. Each of these 30 protocols starts with an Abstract and includes four major sections: Introduction, Materials, Methods, and Notes. The “Abstract” presents an overview of the technique(s) detailed in the chapter. The “Introduction” provides a short theoretical view of the procedure and of its applications. “Materials” recapitulate the buffers, reagents, solutions, disposables, and equipments necessary to carry out the protocol(s). “Methods” describe step-by-step how the technique(s) must be carried out. Finally, the “Notes” section, which is the hallmark of Methods in Molecular Biology series, indicates not only the sources of problems and how to identify and overcome them, but also safety information, alternative procedures, and hints for the correct interpretation of experimental results.
Brief Content of the Chapters

Chapter 1 provides an overview on cell senescence and its dynamic links with autophagy, an important cytoprotective mechanism. Chapters 2 and 3 discuss the regulation of cell senescence by critical signaling molecules such as the mammalian target of rapamycin (mTOR) and p53. Chapter 4 summarizes the morphological and biochemical markers that have been associated with cell senescence. In Chapters 5–23, protocols for the investigation of senescence-associated alterations in cultured cells are provided, including the following: morphological features (Chapter 5), cell cycle blockage (Chapter 6), cell cycle-arresting proteins (Chapter 7), senescence-associated β-galactosidase (Chapters 8 and 9), senescence-associated secretory phenotype and chemokine signaling (Chapters 10 and 11), senescence-associated heterochromatin foci (Chapter 12), DNA damage (Chapter 13), telomerase activity and telomere length (Chapters 14 and 15), alterations of the nuclear envelope (Chapter 16), multiple markers of oxidative stress (Chapters 17–20), BRAF, sirtuin, and p66SHC signaling during senescence (Chapters 21–23). In Chapters 24–27, protocols for the study of cell senescence in global terms are detailed, including a method for the study of metabolomic alterations (Chapter 24), a technique to apply genome-wide RNAi approaches to cell senescence research (Chapter 25), and multiparametric strategies (Chapters 26 and 27). Finally, in Chapters 28–34, protocols applicable to lower model organisms are described, encompassing techniques to assess senescence in *Escherichia coli* (Chapter 28), *Podospora anserina* (Chapter 29), *Saccharomyces cerevisiae* (Chapter 30), *Caenorhabditis elegans* (Chapters 31 and 32), *Drosophila melanogaster* (Chapter 33), and *Danio rerio* (Chapter 34).

Potential Audience of This Book

In the first instance, this book will be of interest not only for undergraduate and graduate students but also for more experienced scientists who are approaching the study of cell senescence. In addition, the audience of this book encompasses:

- Libraries of universities and public biological/biomedical research institutions.
- Scientists interested in molecular and cell biology, biochemistry, pharmacology, genetics, systems biology, medicine, public health, and in life sciences in general.
- Specialists and experts in model organisms including bacteria, fungi, worms, flies, and mammals.
- Medical oncologists and scientists working in oncology.
- Pharmaceutical companies and developers of new drugs.

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