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## Preface

The ongoing research on neuronal cell death is rapidly expanding after the recognition that not only do neurons die as a consequence of external insults, but also following activation of stereotyped genetic programs/intracellular pathways leading to death. However, a long time passed before the term “cell death” took over in the scientific literature: in the nineteenth century, when Rudolf Virchow published his famous book entitled *Lectures on Cellular Pathology in its Grounds on Physiological and Pathological Histology* first reporting on the occurrence of cell death in damaged tissues, the dominating idea was that only injured tissues and cells underwent some sort of degeneration when damaged. Thus, the concept that cell death took place also during the course of normal development or during growth and aging was still very far from general acceptance, and required a considerable amount of experimental work to convince the academic community. A significant step forward along this direction was done in the 1960s of the last century, when electron microscopists started describing the different modes of cell death. Since then the field has dramatically widened, particularly with the recognition of several forms of neuronal degeneration, such as necrosis, apoptosis, autophagy, pyroptosis, oncosis, etc. and of the close relationship of many of these with cell proliferation and aging. Today, the ultimate frontiers in neuronal cell death research lie in the development of novel approaches to monitor the phenomenon by the use of in vivo and/or ex vivo preparations, such as organotypic cultures, that are more closely related to the intact mammalian brain than primary cultures, and to better exploit the use of non-mammalian model organisms. In parallel, there is a need for understanding the type and role of cell death in neurodegenerative diseases, to develop pharmacologically active compounds that are capable to exert their biological role(s) in vivo, and to construct genetic vectors to be employed in gene therapy.

With such a wide array of exciting and rapidly expanding fields of research, this book, from its initial conception, had obviously to be limited in the choice of subjects, but we believe it represents a valuable and readily reproducible collection of established and emerging techniques for neuronal cell death research. Such a collection is preceded by a general introductory chapter (Chapter 1) that recalls the history of cell death and, to put things into perspective, discusses the main morphological features of the most diffuse types of cell death in neurons, in parallel with relevant cellular pathways and current assays for a proper recognition. The methods presented include immunocytochemical localization at light and electronic levels, biochemical characterization, and functional analysis in vivo or ex vivo by novel types of microscopy, as well as protocols for development and production of genetic probes. Although this book is primarily devoted to approaches for analysis of the mammalian brain, a few non-mammalian species are also taken into consideration to demonstrate specific methodologies that are of great value to boost cell death research by taking advantage from the use of less complex models.

As a general indication to the readers, the book is divided into four parts.

Part I (Chapters 2–12) is focused on a series of techniques for the molecular, structural, functional, and genomic characterization of dying neurons. They cover a broad range of protocols, such as epifluorescence and digital holography to monitor the cell volume

(Chapter 2); a series of techniques to study DNA synthesis/alterations and the morphological signs of nuclear sufferance (Chapters 3 and 4); a number of approaches to monitor parameters of primary importance in cell viability (Chapters 5–8), such as oxygen and calcium concentration, mitochondrial function, and activation of caspase-3 in single alive cells; and a series of molecular approaches for RNA silencing, genomic analysis, and high-throughput cell death assays (Chapters 9–12).

Part II (Chapters 13–18) groups together a number of protocols that are of primary interest in neuropathology (Chapters 13 and 14) and in experimental neuropathology (Chapters 15–18) by describing the current ameliorations to well-established diagnostic techniques such as the Golgi method for study of neuronal and glial death in autopsy material (Chapter 13), the use of optimized protocols and image analysis algorithms for reliable analysis of cell death in human and animal samples (Chapter 14), some specific experimental approaches such as oxygen-glucose deprivation (Chapter 15), single axon lesioning by laser microbeam targeting (Chapter 16), in vivo imaging of retinal apoptosis (Chapter 17), and use of neurotoxins to model neuronal death in Parkinson's disease (Chapter 18).

Part III (Chapters 19–22) is devoted to a series of gene engineering techniques to obtain and manipulate neuronal stem cells and progenitors (Chapter 19), to prepare HSV-1 vectors for the gene therapy (Chapters 20 and 21), and to CNS transplantation of bone marrow stem cells (Chapter 22).

Part IV (Chapters 23–26) describes some very interesting protocols for study of cell death in non-mammalian models, such as the analysis of caspase-3 activation in lamprey (Chapter 23), the generation of zebrafish models by genome editing (Chapter 24), and the assessment of cell death (Chapter 25) and phagocytosis (Chapter 26) in *Drosophila*.

All scientists who have excellently contributed to this book have a direct experience in one or more fields of neuronal cell death research. We are very much indebted to all of them for their time, the high standards of their contributions, and for successful effort in emphasizing the description of the more common pitfalls in the techniques that they have described, and of the hints to reduce the possibility of failure for beginners.

The collection of protocols that forms this book is surely not exhaustive of the wide range of approaches that today can be employed in top-level cell death research. Yet it is intended for a large audience of scientists, including histologists, biochemists, cellular and molecular biologists, and electrophysiologists that are currently active in the field or are willing to enter such an exciting and still expanding area of neurobiology.

As the two of us have been the first to benefit from such an excellent assemblage of information, we are confident that readers too will find this book very useful for their future work.

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Neuronal Cell Death

Methods and Protocols

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2015, XIV, 396 p. 82 illus., 48 illus. in color., Hardcover

ISBN: 978-1-4939-2151-5

A product of Humana Press