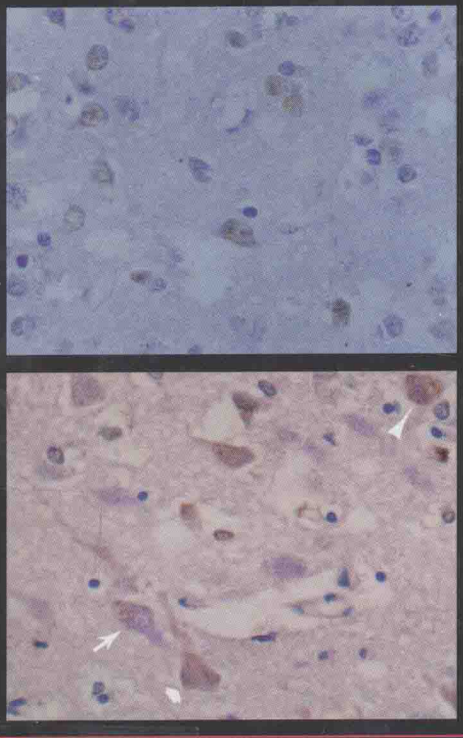
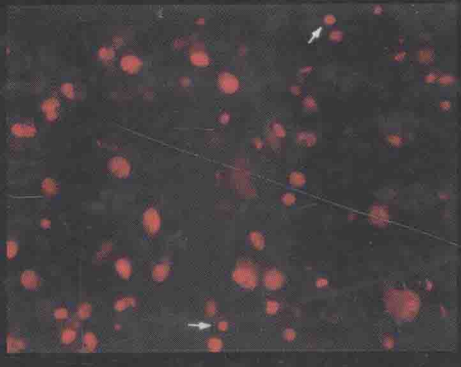
APOPTOSIS in NEUROBIOLOGY





Edited by
Yusuf A. Hannun
Rose-Mary Boustany

METHODS IN NEUROSCIENCE

CRC METHODS IN NEUROSCIENCE SERIES

APOPTOSIS in NEUROBIOLOGY

Edited by
Yusuf A. Hannun
Rose-Mary Boustany

CRC Press
Boca Raton London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Catalog record is available from the Library of Congress

Direct all inquiries to CRC Press LLC, 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-3352-0
Printed in the United States of America 1 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

Dedication

To Raymond D. Adams, MD, a mentor, friend, and guiding light for scores of neurologists, neuroscientists, and many others who are destined to carry the fields of neurobiology and applied neuroscience into the next millenium.

Rose-Mary Boustany

To my father, Awni Hannun, for his unwavering confidence and support.

Yusuf A. Hannun

Preface

In the last few years, the scientific community has synchronously and overwhelmingly come to the realization that the study of cell death is a highly rewarding and important endeavor. Relegated to the sidelines of modern cell biology research for most of the last century, cell death, nonetheless, has received some attention from investigators who noted several forms of morphologic cell death and speculated on the relevance of this process. Indeed, major breakthroughs in cell biology came from the investigation of neurotrophic factors that prevented the otherwise default cell death of neurons. Biologists had also noted the significance of programmed or predetermined cell death in developmental biology, and botanists had labeled the periodic death of leaves as senescence.

Understandably, general interest in cell death was lacking, due to the preconception that cell death is a default process that shows little if any regulation, and therefore, does not lend itself to investigation or interest. Major events and observations in cell biology occurred in the last three decades that slowly began to change this perception and ultimately created the current avalanche of interest in this field of study. First, different forms of cell death were clearly distinguished and defined: necrosis was applied to the usual forms of direct cell death due to (usually harsh) physical conditions, and apoptosis was applied to a more slowly developing process that could be distinguished morphologically from necrosis. This alerted keen observers to perceive that not all forms of cell death are identical, and therefore, by implication, there must be distinct mechanisms that operate during cell death. Second, it became appreciated that apoptosis is accompanied by activation of specific endonucleases that cleave DNA at internucleosomal junctions, whereas necrotic cells showed diffuse and generalized (nonspecific) breakdown of DNA. This singular observation heralded the biochemical approach to apoptosis since it demonstrated, and very clearly, that apoptotic stimuli generate signals that result in specific biochemical effects. This approach eventually led to the discovery of the role of proteases (the caspases) in apoptosis, and to the unraveling of mechanisms in receptro-mediated cell death. Third, evaluation of molecular mechanisms of oncongenesis disclosed that one prominent "anti-oncongene," p53, functioned primarily as a mediator of growth arrest and apoptosis whereas the oncongenic Bcl-2 functioned primarily as an inhibitor of apoptosis. Finally, genetic studies in *C. elegans* identified several genes specifically involved in apoptosis. Elucidation of the structure of those genes, as homologues of Bcl-2 and caspases, allowed for the convergence of these different approaches in the study of apoptosis. This convergence has catapulted the study of apoptosis

to its current heights, and it promises rapid unfolding of many of the remaining mysteries on the significance of apoptosis and its mechanisms.

The field of neurobiology is particularly rich in potential understanding and application of apoptosis study. It appears that disorders of neurodevelopment as well as neurodegenerative disorders are a direct result of activation of apoptotic programs (either due to primary defects in these programs or, more commonly, as a consequence of insults and injuries that activate these programs). Therefore, the study of apoptosis in neurobiology promises significant rewards in understanding such diverse disorders as Alzheimer's disease, Parkinson's disease, and the many neurodegenerative diseases of the central and peripheral nervous systems.

This volume was compiled with the singular purpose of allowing the uninitiated neuroscientist intellectual and practical access to the study of apoptosis, with special consideration to the nervous system. The book is divided into two major sections. The first concentrates on conceptual approaches to the study of apoptosis in neurobiology and its significance in the nervous system. The second part provides for a user-friendly approach to methods and techniques in the study of apoptosis and, where appropriate, as specifically applied to neurobiology.

We would like to take this opportunity to thank our contributors for outstanding and timely contributions. We would also like to thank our many colleagues and students who make these efforts worthwhile.

Yusuf A Hannun and Rose-Mary Boustany

The Editors

Yusuf Hannun, M.D., is currently the Ralph Hirschmann Professor of Biomedical Research and Chair, Department of Biochemistry and Molecular Biology and Professor of Medicine at the Medical University of South Carolina in Charleston. Dr. Hannun obtained his M.D. degree from the American University of Beirut in 1981 where he recieved training in internal medicine. He then trained in hematology and medical oncology at Duke University. He also trained in biochemistry with Dr. Robert Bell. He then joined the faculty of Duke University where he spent 15 years, becoming the R. Wayne Rundles Professor of Medical Oncology. His research interests are in the areas of lipid-mediated cell regulation, the chemistry and biochemistry of sphingolipids, mechanisms of cell death, and cancer biology. He has authored or co-authored more than 170 manuscripts, edited 4 books and holds three patents on the use of sphingolipid-derived molecules in the treatment of human diseases. He has been named a Pew Scholar in the biomedical sciences and he is the 13th Mallinckrodt Scholar in biomedical research.

Rose-Mary Boustany, M.D., is tenured Associate Professor in Pediatrics and Neurobiology at Duke University Medical Center. She obtained her M.D. in 1979 at the American University of Beirut where she completed her training in pediatrics. Dr. Boustany spent almost nine years (1980 to 1988) in Boston at Massachusetts General Hospital and the Shriver Center for Mental Retardation. There she trained in pediatric neurology and neurogenetics and later joined the neurology faculty at Massachusetts General Hospital and was associate director of the Lysosomal Storage Diseases Laboratory at the Shriver Center. She moved to Duke University at the end of 1988 where she joined the division of pediatric neurology. She also spent two years in the laboratory of Kuni Suzuki at the University of North Carolina at Chapel Hill. Her fields of interest include neurogentics, the cell and molecular biology of inherited neurodegenerative diseases, and basic mechanisms of neuronal apoptosis.

Contributors

Andrea Amalfitano, Duke University Medical Center, Durham, North Carolina

Tim R. Bilderback, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas

Rose–Mary Boustany, Department of Pediatrics, Duke Medical Center, Durham, North Carolina

Joseph M. Corless, Duke University, Durham, North Carolina

Rick T. Dobrowsky, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas

William C. Earnshaw, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, U.K.

Harris A. Gelbard, University of Rochester Medical Center, Rochester, New York

Yussef A. Hannun, Medical University of South Carolina, Charleston, South Carolina

Kam M. Hoffman, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas

Scott H. Kaufmann, Division of Oncology Research and Department of Pharmacology, Mayo Medical School, Rochester, Minnesota

Timothy J. Kottke, Division of Oncology Research, Mayo Medical School, Rochester, Minnesota

L. Miquel Martins, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, U.K.

Peter W. Mesner, Jr., Division of Oncology Research, Mayo Medical School, Rochester, Minnesota

Vinodh Narayanan, Department of Pediatrics, Neurology, and Neurobiology, The Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania

Kasturi L. Puranam, Department of Pediatrics, Duke University Medical Center, Durham, North Carolina

Donald E. Schmechel, Division of Neurology, Departments of Medicine and Neurobiology, Duke University Medical Center, Durham, North Carolina

Nina Felice Schor, Departments of Pediatrics, Neurology, and Pharmacology, University of Pittsburgh, and Division of Child Neurology, The Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania

Sidney A. Simon, Department of Neurobiology, Duke University Medical Center, Durham, North Carolina

Miriam J. Smyth, Department of Medicine, Duke University Medical Center, and Geriatric Educational and Clinical Center, Department of Veterans Affairs Medical Center, Durham, North Carolina

Contents

Section A Diseases and Concepts

Section B *Methods*

Section A

Diseases and Concepts

1

Introduction: Occurrence, Mechanisms, and Role of Apoptosis in Neurobiology and in Neurologic Disorders

Rose-Mary Boustany and Yusuf A. Hannun

CONTENTS

Apoptosis in the developing nervous system results in naturally occurring cell death (NOCD), a necessary and desirable process. NOCD effectively eliminates neurons that have made faulty synapses or have not reached appropriate targets.[1] In the rest of the organism, apoptosis is essential for organogenesis, sculpts digits and extremities, and plays a role in determining polarity of structures by contributing to directional growth of cell populations.[2]

Failure of carefully orchestrated and effective apoptosis in the developing fetus can have serious and long-lasting effects in the adult. Congenital brain malformations such as heterotopias, schizencephaly, myelomeningocoele, and many others probably represent poorly designed and/or incomplete apoptosis.

An accelerated rate of apoptosis is purposefully induced when cancers are treated with radiation and various chemotherapeutic agents. In fact, cancers are frequently thought of as failure of enactment of apoptosis. Mutations in *p53*, that normally is a suppressor of growth, occur in a large number of human tumors.[3] In addition, there are numerous endogenous factors that protect normal and tumor cells from apoptotic death. Nerve growth factor (NGF) bound to its low affinity P75 or high affinity Trk A receptors is an example.[4] NGF binding to the p75 receptor on neuroblastoma tumor cells

0-8493-3352-0/99/$0.00+$.50

explains their resistance to chemotherapy induced apoptosis. Chapter 4 on neurooncology delves into this issue in greater detail.

If cancer is a state of transformation, unbridled cell proliferation, or failure of enactment of apoptosis, neurodegenerative diseases on the other hand represent accelerated apoptosis in the face of fully differentiated nondividing neurons. In fact, the repertoire of most neurons in the adult nervous system is limited to healthy quiescence, senescence, or death. Neurodegenerative disease is the phenotypic expression of undesirable and inappropriate neuronal death occurring in the adult brain. These diseases can be due to autosomal recessive defects in genes involved in the apoptotic pathway. Examples include defects in the antiapoptotic *CLN3* gene in the juvenile form of Batten disease or defects in the survival motor neuron (SMN) or neuronal apoptosis inhibitory protein (NAIP) defective in spinal muscular atrophy.[5-7] Alternatively, neurodegenerative disorders can result from defects in dominant genes, as seen in the expanded triplet repeat diseases. These represent a deleterious gain of function model where the expanded CAG/polyglutamine tract in the mutant protein results in novel toxic protein–protein interaction in part responsible for the death of neurons. Some of these diseases are Huntington disease (*huntingtin*), spinocerebellar ataxia type-1 (*ataxin-1*), Machado-Joseph disease (*ataxin-3*) and dentatorubro-pallipallidoluysian atrophy or DRPLA (*atrophin-1*). There are other neurodegenerative diseases where apoptosis has been implicated as the mechanism of neuronal death. These include a subset of Alzheimer cases, amyotrophic lateral sclerosis, Parkinson's disease, and various forms of retinitis pigmentosa resulting from mutations in rhodopsin or other retinal proteins. A more complete discussion of these disorders is addressed in Chapter 3 on neurodegenerative diseases.[8]

Acquired diseases representing neuronal apoptosis triggered by an infectious agent include HIV-1 encephalitis and prionic encephalopathies. It is thought that the HIV-1 infection initiates an apoptosis-signaling cascade in the central nervous system. The reader is referred to Chapter 5 on HIV-1.[9]

1.1 Molecular Mechanisms of Apoptosis

We are just beginning to unravel the complexities and intricacies of the regulation of apoptosis. Insight has developed rapidly in the last decade from (1) studies on cytokine- and chemotherapeutic agent-induced cell death, (2) genetic regulation of cell death in the nematode *C. elegans*,[11] and (3) studies on proapoptotic tumor suppressor genes such as *p53* and antiapoptotic oncongenes, most notably *bcl*-2.[12]

Control of apoptosis is possible at many levels. This regulation can be expressed as a positive or negative modulating effect (Table 1.1): transcriptional regulation, induction of early intermediate genes; stage of the cell cycle

TABLE 1.1

Positive and Negative Modulators of Apoptosis

Negative	Positive
Bcl-2	Bax
Bcl-x_L	Bcl-x_s
Bag	Bcl-$_{x\beta}$
Baculovirus p35	Bag, Bak, Mcl-1, Bok
Cowpox virus serpin crm A	TNF superfamily (Fas, TNFR-1, Reaper)
NAIP?	Chemotherapeutic agents
SMN?	Radiation
CLN3	ceramide
NGF	p53
IL-6, IL-3, erythropoetin	*c-myc*

and relative levels of cyclins; presence or absence of nerve growth factor and its receptors; TNF-α and related receptors[13]; Fas–Fas-L interactions,[14] ceramide as proapoptotic lipid second messenger and the sphingomyelin cycle[15]; the neuroprotective *bcl-2* oncogene and its homologues,[16] *p53* and retinoblastoma genes as inducers of growth arrest and apoptosis[17]; the early initiator and later executionary caspase cascades and their triggers and inhibitors[18]; the role of the mitochondrion as central processor of incoming messages, and the role of translocation of inner mitochondrial membrane proteins such as cytochrome c, Apaf-1, and other factors to be found.[19] A hypothetical and simplified choreography depicting possible interactions, as best illustrated with apoptosis-inducing cytokines, is outlined in the scheme shown. According to this model, the action of proapoptotic cytokines, such as TNF, Fas-L, or NGF, on their membrane receptors (P75 receptor in the case of NGF) results in recruitment/activation of a number of adapter proteins such as FADD, TRAFs, and TRADs. These proteins, though poorly understood mechanisms, couple the occupied receptors to distinct pathways of signaling and cell regulation. Whereas Fas appears to be a more dedicated proapoptotic receptor, the TNF receptors couple to apoptotic, antiapoptotic, and inflammatory pathways. Thus, TNF can activate the following: (1) NF-kB, which predominantly functions as antiapoptotic transcription factor; (2) the jun kinase (JNK) or stress-activated kinase (SAPK) pathway, which primarily functions in the regulation of stress, at times promoting apoptosis and at other times inhibiting it; and (3) the MACH/Flice protease, a member of the caspase family of proteases, which launches the apoptotic functions of TNF.[20]

It is not yet clear how MACH/Flice turns on the apoptotic program. In the case of Fas, it has been proposed that a cascade of proteases is turned on, and that it is necessary and sufficient to cause apoptosis. This proposed mechanism now appears as an over-simplified explanation, especially in the case of TNF, where many endogenous pathways are activated and regulated in response to TNF and Fas and contribute to the terminal apoptotic outcome. These pathways include the formation of reactive oxygen intermediates and changes in mitochondrial permeability and function.[21] Also implicated are

ceramide- and sphingolipid-derived molecules as stress-induced mediators that promote and enhance the apoptotic program.

Noncytokine stresses, such as heat, oxidative damage, and DNA-damaging agents also activate apoptosis by generating poorly understood internal signals. It is not yet determined whether these processes overlap cytokine-induced apoptosis, but in the case of DNA-damaging agents the proapoptotic protein P53 plays an important role in driving the response of the cells either through induction of cell cycle arrest or the induction of apoptosis.[22]

Significant results now implicate cytochrome c as a key mediator of the apoptotic pathways (Figure 1.1). Many, but not all, inducers of apoptosis cause the release of cytochrome c from the mitochondria. Also, it is now assumed that the mitochondrial membrane is the site of action of members of the Bcl-2 family of pro- and antiapoptotic proteins.[22] It is suggested that *bcl-2*, the mammalian homologue of the *ced-9* gene from *C. elegans*, functions primarily by inhibiting the release of cytochrome c, whereas proapoptotic relatives of *bcl-2* may promote this event. The released cytochrome c interacts with Apaf-1, a positive regulator of apoptosis with homology to the *C. elegans ced-4* proapoptotic gene. This collaboration results in activation of downstream caspases such as caspase 3, which are homologues of the *C. elegans ced-3* gene. It is the action of these caspases on their substrates that results in the systematic degradation of key substrates such as nuclear lamins, PARP, fodrin, protein kinases, and other structural or regulatory proteins. This process culminates in the organized collapse of the nucleus, membranes, and cellular organelles. Many neuronal proteins are now recognized as substrates of caspases, including presenilins and huntingtin.[23,24] The orderly breakdown of dying cells through the apoptotic mechanisms results in the packaging of cellular debris into apoptotic bodies which are then cleared by reticuloendothelial cells as well as normal adjacent cells, thus preventing inflammatory reactions to cell fragments.

The study of existing apoptotic developmental and neurodegenerative diseases, be they caused by a genetic defect or a triggering environmental factor, provide us with naturally occurring human models that validate existing hypotheses in neuronal culture systems and provide new information pertinent to basic cell biology.

1.2 Mechanisms of Apoptosis in Neurological Disorders

One theory invoked to explain Alzheimer cases that are apoptosis positive is that the accumulation of amyloidogenic protein results in excess intracellular calcium, a known trigger for the endonuclease responsible for the DNA fragmentation seen during the final stages of apoptosis.[25] Oxidative stress due to defects in energy and/or mitochondrial metabolism contributes to apoptosis in anterior horn cells in amyotrophic lateral sclerosis, in the substantia