METHODS IN MOLECULAR BIOLOGY

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Programmed Cell Death

Methods and Protocols

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Cover illustration: A high resolution image of a *Drosophila* eye in which the pro-apoptotic gene, *hid*, was expressed using an eye-specific promoter, resulting in eye ablation.

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Preface

The field of programmed cell death is now one of the most dynamic and fast-moving areas of research in biology. Focus of this research field encompasses the basic science of delineating the molecular mechanisms underpinning the cell death process in both mammals and other model organisms (such as *C. elegans, Drosophila,* and *S. cerevisiae*) as well as understanding its role in various human pathologies such as cancer, degenerative diseases, autoimmunity, cardiomyopathy, and sepsis. In this edition of Methods in Molecular Biology, apart from protocols specifically designed for studying programmed cell death, we also have incorporated many of the recent advances in techniques that span broader areas of biology that have been recently used or that have the potential to be incorporated into cell death research. Though the protocols are mostly described in the context of mammalian systems, we also have incorporated other systems such as plants, *Drosophila*, and yeast as tool for studying metazoan apoptosis process.

The first five chapters describe apoptosis detection techniques. These include methods to discriminate between apoptotic and autophagic cell death processes and in vivo imaging of apoptosis either by Gallium-68 labeled annexin V or by using fluorescent, activity-based probes. The latter is also a useful technique for measuring caspase activity ex vivo in resected tissues. This section also covers a method for detecting caspase activity initiation at single-cell level and peptide-based techniques for detecting or inhibiting specific caspases. The next five chapters describe methods for studying apoptosis associated with various pathologies in different organs including the lymphoid compartment (for studying sepsis), intestinal epithelium (for studying gastric abnormalities), granulocytes (for studying allergies and hypersensitivity), hepatocytes (for studying HBV infection), and cardiomyocytes (for studying cardiomyopathy and heart failure).

Lower forms of eukaryotes such as *Drosophila* have been invaluable in studying apoptosis process during development and tumorigenesis owing to their extensive use as model organism at the cutting edge of genetic research, with exciting new technologies continuing to emerge. Similarly, programmed cell death is a critical component of plant development and immunity against pathogens. Metazoan apoptotic machinery can be reconstituted in bakers' yeast *Saccharomyces cerevisiae* to study the function of candidate or established apoptotic regulators. Chapters 11–13 cover protocols and techniques for studying apoptosis in these three non-mammalian systems. Chapters 14–16 cover biochemical and biophysical methods for studying Bcl-2 family protein dynamics and protein-protein interactions during apoptosis. The last section, i.e., Chapters 17–20 includes protocols that are useful not only in apoptosis research but also in the wider areas of biological research. This section covers methods for genome editing, inducible transgenes, and proteomics.

We hope the scope of this book is sufficiently comprehensive and useful for researchers who are beginners as well as advanced in the field. We are extremely grateful to all of the colleagues who provided such high-quality contributions to this book and to the Springer Publishers for their support, especially Professor Emeritus John Walker. Finally, we are indebted to our friend and colleague Dr. Anjali Sahasrabudhe for her exceptional and tireless editorial help.

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