Methods in ENZYMOLOGY

Volume 545

Regulated Cell Death Part B: Necroptotic, Autophagic and other Non-apoptotic Mechanisms

Edited by

Avi Ashkenazi
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VOLUME FIVE HUNDRED AND FORTY FIVE

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NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

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525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK
32 Jamestown Road, London NW1 7BY, UK

First edition 2014

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ISBN: 978-0-12-801430-1
ISSN: 0076-6879

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METHODS IN ENZYMOLOGY

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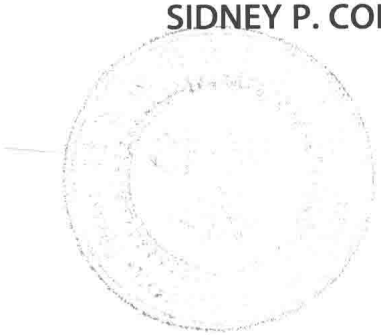

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PREFACE

Cell turnover is a fundamental feature of metazoan biology. Severe damage to cellular integrity usually causes passive, nonregulated cell death. In contrast, more confined disruption can lead to more deliberate cell elimination, through specific mechanisms of Regulated Cell Death. In these two volumes of *Methods in Enzymology*, we aim to highlight the current molecular understanding of the major processes of Regulated Cell Death and to illustrate basic and advanced methodologies to study them. Volume A focuses on the most extensively studied mode of cell death—apoptosis. Volume B covers several nonapoptotic mechanisms. These include necroptosis, which shares certain signal transduction aspects with apoptosis but is unique in its execution phase, and autophagic cell death, which is an offshoot of autophagy—a more basic prosurvival metabolic adaptation mechanism. Chapters 1–4 cover how to measure necroptosis and various molecular components and complexes that signal this process. Chapter 5 discusses approaches to interrogating interactions between tumor necrosis factor superfamily ligands and receptors. Chapters 6–8 highlight nonapoptotic cell death mechanisms in the model organisms, *C. elegans* and *D. melanogaster*. Chapters 9 and 10 discuss structural aspects of death receptor complexes and strategies to study posttranslational modification of downstream signaling components by RING E3 ubiquitin ligases. Finally, Chapter 11 describes a multidimensional profiling approach to studying small-molecule-induced cell death. We hope these chapters will be both conceptually informative and practically useful for readers interested in the current understanding and the key open questions in each area, as well as in experimental strategies and techniques to interrogate nonapoptotic regulated cell death mechanisms.

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Adam J. Wolpaw and Brent R. Stockwell, Figure 11.6 Comparing and clustering modulatory profiles. (A) Heat map of the similarity matrix showing the Spearman correlation between modulatory profiles of both characterized and uncharacterized lethal compounds. (B) Dendrogram derived from clustering the similarity matrix shown in (A). Five broad clusters are highlighted and lettered. In addition, microtubule destabilizers are shown in black, a cluster that includes three previously uncharacterized compounds. Other features that are not highlighted include clustering of characterized compounds according to their known mechanisms of action—alkylating agents, mitochondrial poisons, topoisomerase inhibitors, histone deacetylase inhibitors, and proteasome inhibitors. *Reproduced with permission from Wolpaw et al. (2011) and slightly altered.*

CONTENTS

CHAPTER ONE

Assays for Necroptosis and Activity of RIP Kinases

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Methods in Enzymology, Volume 545
ISSN 0076-6879
http://dx.doi.org/10.1016/B978-0-12-801430-1.00001-9

Abstract

Necrosis is a primary form of cell death in a variety of human pathologies. The deleterious nature of necrosis, including its propensity to promote inflammation, and the relative lack of the cells displaying necrotic morphology under physiologic settings, such as during development, have contributed to the notion that necrosis represents a form of pathologic stress-induced nonspecific cell lysis. However, this notion has been challenged in recent years by the discovery of a highly regulated form of necrosis, termed regulated necrosis or necroptosis. Necroptosis is now recognized by the work of multiple labs, as an important, drug-targetable contributor to necrotic injury in many pathologies, including ischemia–reperfusion injuries (heart, brain, kidney, liver), brain trauma, eye diseases, and acute inflammatory conditions. In this review, we describe the methods to analyze cellular necroptosis and activity of its key mediator, RIP1 kinase.

1. INTRODUCTION

1.1. Distinguishing features of necroptotic cell death

Discovery of regulated necrosis originates from the observations that "canonical" inducers of apoptosis, such as agonists TNFα family of death domain receptors (DRs), can trigger cell death morphologically resembling necrosis in cells either intrinsically deficient in caspase activation (e.g., mouse fibrosarcoma L929 cells) or under conditions when caspase activation is inhibited (e.g., caspase-8-deficient Jurkat cells or cells treated with pan-caspase inhibitor zVAD.fmk) (Holler et al., 2000; Matsumura et al., 2000; Vercammen, Vandenabeele, Beyaert, Declercq, & Fiers, 1997). The lack of caspase activation as well as the absence of other typical features of apoptosis, such as cytochrome *c* release, membrane blebbing, phosphatidylserine (PS) exposure, and intranucleosomal DNA cleavage, served as important initial differentiators between necroptosis and apoptosis (Tait & Green, 2008).

Electron microscopy has also proved very useful in distinguishing necroptosis from apoptosis in morphology. Necroptotic cells are characterized by the lack of typical nuclear fragmentation, swelling of cellular organelles especially mitochondria, and the loss of plasma membrane integrity, whereas apoptotic cells exhibit shrinkage, blebbing, nuclear fragmentation, and chromatin condensation (Degterev et al., 2005). Robust activation of

autophagy is another feature of necroptosis which provides useful means to distinguish this form of cell death *in vitro* and *in vivo* both morphologically (e.g., by EM) and at the molecular level (e.g., by measuring of LC3II formation) (Degterev et al., 2005; Yu et al., 2004). This leads to necroptosis in some cases being referred to as "autophagic cell death," such as zVAD-induced death of L929 cells (Yu et al., 2004). It should be noted, however, that functional role of autophagy varies greatly depending on the specifics of necroptosis activation, with instances where this process promotes, inhibits, or does not affect cell death (Degterev et al., 2005; Shen & Codogno, 2012; Yu et al., 2004). Furthermore, activation of necroptosis-inducing necrosome complex (discussed below) can also happen downstream from autophagosome formation (Basit, Cristofanon & Fulda, 2013).

A detailed comparison of TNF-induced necroptosis and H_2O_2-induced necrosis was performed by Vanden Berghe et al. (2010). Despite the different kinetics of cellular events including ROS production, mitochondrial polarization changes, and lysosomal membrane permeabilization, the major hallmarks of necroptosis and oxidant-induced necrosis were remarkably similar, leading to an important conclusion that necroptosis is a subtype of necrosis, morphologically indistinguishable from other types of necrosis but defined by a specific mode of activation (discussed below).

Generation of DAMPs as a result of cell lysis is an important consequence of necroptotic death both *in vitro* and *in vivo* (Duprez et al., 2011; Murakami et al., 2013). In addition, recent evidence suggests that synthesis of TNFα occurs independently of cell death as a result of specific signaling by key necroptosis initiator RIP1 kinases (RIPK1) (Christofferson et al., 2012; Kaiser et al., 2013; McNamara et al., 2013). Autocrine TNFα can promote cell death dependent on a cytosolic complex "ripoptosome" consisting of RIPK1, FADD, and caspase-8 (Biton & Ashkenazi, 2011; Hitomi et al., 2008; Kaiser et al., 2013; Tenev et al., 2011). Several instances have also been reported where RIPK1 and RIPK3 promote inflammatory signaling through the production of IL-1α and IL-1β/IL-18 in the absence of cell death (Kang, Yang, Toth, Kovalenko, & Wallach, 2013; Lukens et al., 2013). These data highlight complex interrelationship between necroptosis and inflammation.

1.2. Pathways and mediators of necroptosis

We refer the readers to a number of in-depth reviews on the subject (Christofferson et al., 2012; Christofferson, Li, & Yuan, 2014;