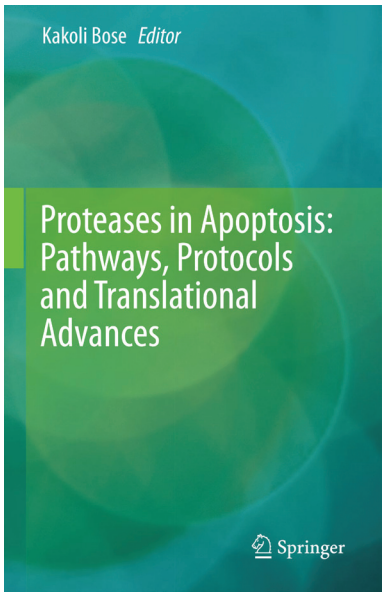
BOOK REVIEW

CASPASES, CALPAINS, GRANZYMES, CATHEPSINS, HTRAs: FAR MORE THAN JUST KILLER ENZYMES

Book Review of "Proteases in Apoptosis: Pathways, Protocols and Translational Advances", Kakoli Bose, Ed., Publisher: Springer International Publishing, ISBN 978-3-319-19496-7, 2015

The limited proteolysis of proteins and peptides is an integral part of various basic vital functions of Protozoa as well as higher organisms. As known, the hydrolysis of peptide bonds is catalyzed by specific proteases both inside the cell and in the extracellular space. In the past two decades, the study of the role of proteolytic enzymes in the initiation and execution of the cell death has been in the spotlight in large part because of the disclosure of the molecular mechanisms of apoptosis and the discovery of a dozen non-apoptotic forms of the regulated cell death. Nowadays, it is clear that numerous proteases of different clans and families are involved in the apoptotic cell death machinery. The recent developments in the field are nicely treated in the book under review.



The book starts with the explanation of the general concepts pertaining to the principal molecular mechanisms of the initiation and execution of apoptosis. In particular, three apoptotic pathways are considered, namely apoptosis mediated by death receptors, mitochondrial or endoplasmic reticulum pathways. More frequently, the activation of the cysteine proteases of the unique caspase family occurs whichever is the pathway initiated by apoptosis inducer. Apart from caspase-dependent apoptosis, the mechanisms of alternative, caspase-independent apoptotic cell death pathway are also presented. The chapter ends with a short section on the analysis of impairment of apoptosis induction in various human illnesses and injury states. Of particular value is the discussion of the advantages and drawbacks of the prospective therapeutics developed for the treatment of cancer and neurological disorders.

In the following chapters, the authors cover five different protease families, such as caspases (Chapter 2), calpains and granzymes (Chapter 3), cathepsins and high temperature requirement A proteases (HtrAs) (Chapter 4) responsible for the proteolytic cascades in apoptosis. Three chapters listed above are structured in similar way comprising such sections as "Classification", "Structural Assembly", "Activation Mechanism", "Catalytic Mechanism", "Functions", "Substrates and Inhibitors". The Chapter 2 deals with the cysteine-dependent aspartate-driven proteases of the caspase family (Clan CD, Family C14A*) as the most widely studied mediators of apoptosis. As for myself, the most interesting is the recently discovered phenomenon termed apoptosis-induced compensatory cell proliferation wherein apoptotic cells induce proliferation of surviving neighboring cells. Apoptotic caspases are known to play a critical role for such apoptosis-induced proliferation. This phenomenon may have important implications for mechanisms of stem cell activation, tissue regeneration as well as oncogenesis.

The Chapter 3 deals in detail with calpains (Clan CA, Family C2A) as intracellular Ca^{2+}-dependent cysteine proteases and granzymes (Clan PA, Family S1A), which are members of a family of serine proteases. The latter are synthesized in cytotoxic T lymphocytes (CTL) or natural killer (NK) cells and are important for elimination of virus-infected and malignant cells. Contrary to caspases,

* Hereinafter, only identifiers of clans and families of human proteases are given according to the MEROPS database (http://merops.sanger.ac.uk).

calpain activation requires Ca^{2+} and phospholipids or phosphoinositides. One more distinction between caspases and calpains is the lack of protease activity in several calpains (7, 10, and 15). It should be mentioned that caspases are the substrates of calpains while caspase-3 exerts a proteolytic degradation of calpastatin (endogenous inhibitor of calpains). Another pathway of activation of caspase-independent cell death by calpains involves calpain-mediated proteolysis and translocation to the nucleus of the truncated form of AIF (apoptosis-inducing factor) where the latter initiates chromatin condensation and DNA fragmentation. Of note, the ATG5 (autophagy-related gene 5) cleaved by calpains is unable to induce autophagy. Nevertheless, the binding of the truncated ATG5 with antiapoptotic protein BCL-X_L (B-cell lymphoma-extra large) allows for apoptogenic factors to release from mitochondria with the subsequent activation of caspases. Therefore, synergistic interactions between calpains and caspases in apoptotic pathways are evident.

As indicated above, granzymes released by CTL or NK cells are transferred into the cytosol of the target cells contacting with cytotoxic effectors. The mechanisms of the cell death induced by granzymes A, B, H, K, and M released by human CTL or NK cells are treated exhaustively in this chapter. The fact that granzymes may induce the apoptotic cell death both in caspase-dependent and caspase-independent modes makes some problems for searching the adequate molecular targets for therapeutic intervention. The novel information on pan-granzyme substrate coded by *HNRNPK* (heterogeneous nuclear ribonucleoprotein K) that may be well one of such targets is presented here.

Cathepsin family is the centerpiece of Chapter 4. In contrast to other families of apoptotic proteases, cathepsin family comprises proteases of different catalytic types (aspartic, serine or cysteine proteases) exhibiting both endo- and exopeptidase activities. Such diversity is coupled with the broad spectrum of underlying physiological processes where cathepsins are involved. Table 4.1 provided the exhaustive information not only on the functions of these lysosomal proteases but also on their substrates as well as endogenous and synthetic inhibitors. As to proapoptotic activity of cathepsins, all these enzymes except for those of O, V, W, and X types are involved in both intrinsic and receptor-mediated apoptotic pathways. Interestingly, procaspase-8 is a substrate for cathepsin D while granzyme B – for cathepsin C suggesting the close interplay between apoptotic proteases of different families in the complex regulatory network of apoptotic cell death.

Although only two of four known oligomeric serine proteases of high-temperature requirement A (HtrA) family, namely HtrA1 and HtrA2 (Clan PA, Family S1C) are known to be involved in apoptotic pathways, the mechanism of such involvement has been elucidated only for OMI/HtrA2. This protease is localized predominantly within the mitochondrial intermembrane space, and its endogenous inhibitors are largely unknown. Following the release of HtrA2 to cytosol, this protease is capable of cleaving and inactivating several antiapoptotic proteins including XIAP, cIAP1, cIAP2, cFLIP, and PEA15. Besides, numerous cytoskeletal proteins such as actin, tubulin-α, -β, and vimentin are substrates of HtrA2. The proteolytic degradation of such proteins is incompatible with maintaining cell viability. At present, the search for synthetic inhibitor of human mitochondrial protease HtrA2 is under way in the hope of developing the novel treatments for several neurological diseases.

Chapter 5, the longest one, covers in detail both general assays that characterize biochemistry of proteolytic enzymes and more specific assays used to discern apoptotic proteases including the techniques for detecting the apoptotic cells. The chapter contains a lot of practical examples demonstrating how one or another method is designed for specific tasks. Of particular interest are the methods suitable for studying cells with knockout/knockdown of apoptotic protease genes. In concluding section, the authors discuss the most advantageous strategies for the clinical application of our knowledge of apoptotic proteases such as the design of protease activated prodrugs, selective activators/inhibitors of apoptotic proteases, and use of nanomaterials for their targeted delivery.

The closing chapter describes the principles of the modern *in vivo* model systems (mainly mouse models of neurodegenerative diseases and cancer) used for the assessment of the efficacy of candidate drugs targeting apoptotic proteases. The examples of their use are also given. The chapter provides a very brief but useful outlook on a current methodology used for visualization and quantitative evaluation of apoptotic cells in the whole body. Several real-time imaging technologies such as PET, SPECT, CT, and MRI among others adapted for small laboratory animals are described as well.

In sum, it is worth noting that the book covers the comprehensive information about all the families of proteolytic enzymes involved in apoptosis. Such information proves convincingly that regulatory functions of apoptotic proteases are much broader than the removal of abundant, infected or potentially harmful cells. In particular, apoptotic proteases are implicated in an array of diverse physiological functions outside apoptosis such as cell proliferation and differentiation, cell cycle regulation, DNA damage response, maintenance of genomic stability, homeostasis of the adult T-cell population, tissue regeneration, embryonic development, modulation of inflammatory and immune responses, and aging. This adds a cautionary note to the development of the novel therapeutics for correction of proteolytic activity. The clear benefit of the book under review consists in highlighting many practical aspects of assaying the activity of the apoptotic proteases and visualizing the apoptotic cells in various model systems. The procedures are described in details and may be easily followed in the lab. Nevertheless, the book makes no mention of the alternative caspase-dependent mechanism of apoptosis initiation mediated by the so-called "dependence receptors". The authors did not address the role of apoptosis-associated proteases in realization of such non-apoptotic modes of cell death as necroptosis, autophagy, and immunogenic cell death. Again, no information about quantitative ultrasound modality as feasible technique for the detection of cell death *in vivo* is provided. However, such slight gaps do not take from the merits of the book and may be taken into account in the following editions.

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