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Review

The multifaceted activity of insect caspases

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ABSTRACT

Caspases are frequently considered synonymous with apoptotic cell death. Increasing evidence demonstrates that these proteases may exert their activities in non-apoptotic functions. The non-apoptotic roles of caspases may include developmentally regulated autophagy during insect metamorphosis, as well as neuroblast self-renewal and the immune response. Here, we summarize the established knowledge and the recent advances in the multiple roles of insect caspases to highlight their relevance for physiological processes and survival.

Contents

1. Introduction

Among biological processes, cell death is one of the most described phenomena due to its importance in development, physiology and pathology of organisms. Aside from the accidental cell death that may occur in any tissue due to a sudden injury, most living organisms possess mechanisms that promote cell death through a highly regulated multi-step process. Consequently, programmed cell death is a term commonly adopted in all situations in which controlled cell death occurs. Based on the cell morphology in each case, the most common terms referring to programmed cell death are apoptosis (Maghsoudi et al., 2012) (also known as programmed cell death type 1), autophagic cell death (programmed cell death type 2) and necrosis. The latter has long been considered a passive and poorly controlled process, but this view has recently been challenged by the introduction of the term "programmed necrosis" (Jain et al., 2013). Other morphological typologies of cell death have been described, including mitotic catastrophe, anoikis and entosis (Galluzzi et al., 2012). The first has recently been defined in functional terms as an oncosuppressive process that can be started by perturbations of the mitotic apparatus during the M phase of the cell cycle and ending with cell death or senescence (Vitale et al., 2011). There is not a general consensus on the functional and morphological features of the other cell death modalities (Galluzzi et al., 2012), and because they are not well represented in invertebrate models, they will not be considered further in the present review.

Among invertebrates, holometabolous insects have been studied for a long time because the tissue rearrangements during metamorphosis frequently involve massive cell death. The fruit fly *Drosophila melanogaster* and the silkworm *Bombyx mori* are

Abbreviations: IAP, inhibitor of apoptosis; Ark, Apaf-1-related killer; PK, protein kinase; atg genes, autophagy-related genes; CARD, caspase activation and recruitment domain.

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invertebrate models frequently adopted for studying programmed cell death, although several other species are also used for elucidating basic cell death mechanisms (Malagoli et al., 2010).

Under physiological conditions, apoptosis is a tightly regulated process based on the action of cysteinyl aspartate-specific proteinases, better known as caspases (Denton et al., 2012). Caspases have been described in all metazoans, and they are principally considered as the main players of apoptosis, but they are also involved in additional processes. Indeed, these enzymes can be labeled inflammatory caspases or apoptotic caspases (Martinon and Tschopp, 2004), and the latter group includes initiator and effector caspases, which are defined based on their roles in the apoptotic process (Courtiade et al., 2011). Regarding structural characteristics, the caspase family has a canonical structure containing a prodomain and a catalytic domain named "peptidase C14". The prodomain has different structural specificities among caspases. It has been determined that the binding sites (L/S–T/S–H–G) and active sites (Q–A/R–C–R/Q–G) are conserved in all the caspases except for *D. melanogaster* Dronc (Dorstyn et al., 1999a) and *Aedes* Dronc, identified in *Aedes aegypti* (Cooper et al., 2007). A caspase sequence search with BLAST performed on January, 12 2015 using *D. melanogaster* Dronc as a template (Accession No. NP_524017.1) and the subsequent application of the Cobalt Constraint-based Multiple Protein Alignment Tool (http://www.st-va.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi) with default settings revealed that several other Dipterans, *e.g.*, *Musca domestica* (Accession No. XP_005178429.1), *Ceratitis capitata* (Accession No. XP_004519893.1), *Anopheles gambiae* (Accession No. XP_318061.4) and *Culex quinquefasciatus* (Accession No. XP_001844541.1), present a different amino acid sequence in their binding (M/L–T/S/A–H–G) and active (P/S–F/I–C–R–G) sites. The specific binding and active sites may be conserved in the order Diptera because the most similar sequence belongs to *B. mori*, but it does not present the dipteran hallmark. There are approximately 30 amino acid residues in the prodomains of effector caspases and 80 or more in those of initiator caspases (Fuentes-Prior and Salvesen, 2004). The catalytic domain of caspases contains two subunits, which are known as p20 and p10 on the basis of their molecular weights. The proteolytic processing of the proenzyme re-arranges these two subunits as a heterodimer to produce active caspases.

As a general mechanism, it has been observed that unactivated caspases are present in the cytoplasm until a cell death cue activates the cell death signaling pathway. This promotes the activation of initiator caspases, leading to the cleavage and/or the activation of effector (or executioner) caspases. Upon their activation, the effector caspases attack and cleave key intracellular components, promoting several of the morphological and biochemical features associated with apoptosis, including plasma membrane blebbing and DNA fragmentation (Nicholson, 1999). This series of events results in the regulated dismantling of the cell, the remnants of which will be phagocytosed by the neighboring cells in the absence of an inflammatory process (Inohara and Nuñez, 2003). As observed during *D. melanogaster* metamorphosis, the caspases are involved in the rearrangement of several tissues and organs (Denton et al., 2013). Aside from apoptotic cell death, in some cases, the disposal or rearrangement of these organs may also include widespread autophagic activity, a marker of type 2 programmed cell death. Recently, caspases have been seen to be involved also in this second type of programmed cell death (Mariño et al., 2014). In addition to their fundamental roles in programmed cell death, caspases are also fundamental regulators in non-apoptotic processes of insect cells, including neural differentiation and immune activation. This review will summarize the established knowledge and the recent advances regarding the various biological roles of caspases, particularly in *D. melanogaster* and Lepidoptera.

2. Caspase activation and apoptosis

In mammals, the pathways leading to apoptotic cell death have been separated into intrinsic (promoted by oxidative stress and having caspase 9 as the principal initiator caspase) and extrinsic (promoted by specific ligands such as TNF and having caspase 8 as the principal initiator caspase) pathways, respectively. In mammals, the activation of the intrinsic cell death pathway results in the assembly of Apaf-1 monomers into a ring-like platform that activates procaspases, such as procaspase 9 (Bratton and Salvesen, 2010; Rodriguez and Lazebnik, 1999; Zou et al., 1999, 2003). Active caspase 9 cleaves caspase 3, whose substrates are intracellular components, leading to cell death. Similarly to the intrinsic apoptotic pathway, the extrinsic pathway also converges on the effector caspases, but due to the formation of a specific ligand–receptor complex. These models of caspase activation have been described in great detail in mammals, but the fundamentals of metazoan cell death were initially discovered in *Caenorhabditis elegans* (Denton et al., 2013). In this nematode, four principal death-related molecules have been described: EGL-1, CED-9, CED-4 and CED-3 (Denning et al., 2013; Hengartner and Horvitz, 1994). The proposed scheme for the activation of the programmed cell death apparatus in *C. elegans* describes the antagonization of CED-9, a pro-survival molecule, by the pro-apoptotic factor EGL-1. Under normal conditions, CED-9 binds CED-4. The EGL-1-mediated sequestration of CED-9 allows the activating interaction between CED-4 and the effector caspase CED-3. Further, structural studies have demonstrated that EGL-1 promotes the release of dimers of CED-4 from a protein complex that includes CED-9. After the interaction between EGL-1 and CED-9, four free dimers of CED-4 assemble into an apoptosome that binds and activates CED-3 (Yuan and Akey, 2013).

Among insects, the regulation of caspase activation has been principally studied in *D. melanogaster*, which shows certain differences from mammals and *C. elegans* (Fig. 1). The apoptotic pathway in *Drosophila* involves effector caspases that can be considered homologous to *C. elegans* CED-3, but the proteolytic cascade activating the effector caspases in *D. melanogaster* involves players that are different from those involved in the activation of caspase 9 or EGL-1 (Chen and Abrams, 2000). The pro-apoptotic potential of *Drosophila* caspases is regulated by the inhibitor of apoptosis (IAP) proteins (Crook et al., 1993), whose action is based on their binding to key apoptotic players. The IAP levels determine the sensitivity to apoptotic stimuli, and their removal is necessary for caspase activation (Kang and Bashirullah, 2014). Although distinct intrinsic and extrinsic pathways were not described in *D. melanogaster*, molecules that may be connected to the extrinsic apoptotic pathways were identified, including orthologues of both the TNF ligand (Eiger) and the TNF-receptor (Wengen). The TNF orthologue Eiger induces apoptosis via a JNK-mediated signaling pathway, and this process can be blocked by the IAP Diap1. However, the role of *Drosophila* caspases in this cell death pathway has not yet been fully elucidated (Kauppila et al., 2003; Keller et al., 2011).

In the fruit fly, seven caspases have been reported and can be categorized based on the length of their prodomains, which seems to be involved in caspase activation. The caspases Dronc, Dredd and Strica present a long prodomain, whereas this is not the case for Drice, Dcp-1, Decay and Damm (Denton et al., 2013). The regulation of apoptotic machinery in *D. melanogaster* is different from that described in mammals and *C. elegans*, but the caspases and their enzymatic functions seem to be conserved. In *Drosophila*,