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Caspase-7: a protease involved in apoptosis and inflammation

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Abstract

Caspase-7 was considered to be redundant with caspase-3 because these related cystein proteases share an optimal peptide recognition sequence and have several endogenous protein substrates in common. In addition, both caspases are proteolytically activated by the initiator caspases-8 and -9 during death receptor- and DNA-damage-induced apoptosis, respectively. However, a growing body of biochemical and physiological data indicate that caspase-7 also differs in significant ways from caspase-3. For instance, several substrates are specifically cleaved by caspase-7, but not caspase-3. Moreover, caspase-7 activation requires caspase-1 inflammasomes under inflammatory conditions, while caspase-3 processing proceeds independently of caspase-1. Finally, caspase-7 deficient mice are resistant to endotoxemia, whereas caspase-3 knockout mice are susceptible. These findings suggest that specifically interfering with caspase-7 activation may hold therapeutic value for the treatment of cancer and inflammatory ailments.

Keywords

caspase-7; caspase-3; apoptosis; inflammation

Introduction

Proteases are involved in a plethora of physiological reactions ranging from food digestion to highly-regulated cascades such as the apoptotic signalling pathways. Apoptosis has emerged as the default programmed cell death mode during embryonic development and is essential for the homeostasis of adult organisms. Caspases, an evolutionary conserved family of aspartate-specific cystein proteases, are at the heart of the apoptotic machinery (Lamkanfi et al., 2002). To prevent undesired cell death as a consequence of unscheduled caspase activity, these proteases are produced as latent zymogens with an N-terminal prodomain of variable length preceding the catalytic domain. Caspases typically signal in a two-step cascade (Figure 1): 'initiator caspases' with large prodomains (such as caspase-1, -8 and -9) are first recruited into large protein complexes in which they undergo proximity-induced autoactivation. Activated initiator caspases subsequently free 'executioner caspases' of their short inhibitory prodomain, allowing them to cleave a large set of cellular substrates (Boatright et al., 2003).

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Together with caspase-3 and -6, caspase-7 belongs to the subgroup of executioner caspases (Lamkanfi et al., 2002). It was originally cloned as ICE-LAP3, Mch3 and CMH-1 by three different groups, and later renamed caspase-7. This protease was long assumed to be functionally redundant with caspase-3, but detailed biochemical studies and the recent availability of caspase-7 deficient mice revealed that it also performs distinct, non-redundant roles in apoptosis and inflammation (Walsh et al., 2008, Slee et al., 2001, Lamkanfi et al., 2009, Akhter et al., 2009). This review focuses on the structure and functions of caspase-7, while addressing its emerging potential as a therapeutic target in cancer and inflammation.

Structure

The three-dimensional structure and the structural requirements for caspase-7 activation have been characterized in detail thanks to the availability of crystal structures of both procaspase-7 (Riedl et al., 2001, Chai et al., 2001) and the active enzyme in its free (Chai et al., 2001) and inhibitor-bound (Wei et al., 2000) forms. Procaspase-7 is generated as a protein of 303 amino acids (Figure 2A) that resides in the cytosol as a pre-assembled homodimer (Boatright et al., 2003). Structurally, the homodimer consists of a centrally located 12-stranded β-sheet surrounded by 10 α-helices (Riedl et al., 2001, Chai et al., 2001). This so-called 'open α/β barrel fold' comprises two identical anti-parallel arranged enzymatic units (Figure 2B), each harboring a singular active site. Each enzymatic unit is composed of a large and a small catalytic subunit of respectively 20 kDa and 11 kDa, which are connected by a linker sequence in the caspase-7 zymogen (Figure 2A). The linker region hinders ordering of the active site. Hence, its removal by initiator caspases is a prerequisite for caspase-7 activation (Chai et al., 2001, Riedl et al., 2001). Removal of the linker region is achieved by cleavage of the peptide bond between Asp198 and Ser199 and between Asp206 and Ala207, respectively (Figure 2A). This allows spatial reorganization of loops L2, L3 and L4 to form the active site and substrate binding pocket (Figure 2C). Unlike the linker region, the N-terminal propeptide (Figure 2A) does not interfere with procaspase-7 activation *in vitro* (Denault and Salvesen, 2003). Nevertheless, the prodomain negatively affects caspase-7 enzymatic activity in cells, although the mechanism remains unclear.

Biological function

a. Caspase-7 in apoptosis

Two independent apoptotic signaling cascades are frequently distinguished: the extrinsic and intrinsic pathway. The extrinsic pathway is often triggered by binding of extracellular death receptor ligands such as Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) to their respective transmembrane receptors. The death signal is transmitted to the cytosol by receptor clustering, which leads to recruitment and activation of caspase-8 and -10 (Figure 1). On the other hand, DNA damage induced by UV irradiation and chemotherapeutic drugs triggers the release of mitochondrial cytochrome *c* into the cytosol, where the latter associates with the adaptor protein Apaf-1 to form the 'apoptosome'. This large (<700 kDa) protein complex mediates activation of caspase-9 (Figure 1). Once activated, caspases-8,-9 and -10 process the executioner caspases-3 and -7. Mature caspases-3 and -7 cleave a large set of substrates, ultimately resulting in the characteristic morphological and biochemical hallmarks of apoptosis such as phosphatidylserine exposure, nuclear condensation and genomic DNA fragmentation.

The generation of mice lacking caspase-3 (Leonard et al., 2002), caspase-7 or both caspase-3 and -7 (Lakhani et al., 2006) has contributed significantly to our understanding of the physiological roles of these caspases. Interestingly, C57BL/6 mice deficient for both caspase-3 and -7 die shortly after birth, while mice lacking only caspase-3 or -7 have a normal life span and display a limited apoptotic phenotype in this genetic background (Lakhani et al., 2006,

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Leonard et al., 2002). This points to the functional redundancy between caspase-3 and -7 during embryogenesis. However, several observations suggest that this overlap is not complete and that caspase-3 and -7 also fulfill non-redundant roles in apoptosis. For instance, eye lenses of caspase-7 knockout mice are grossly normal, whereas those of caspase-3 deficient mice display marked cataracts at the anterior lens pole (Zandy et al., 2005). Further support for this notion stems from biochemical studies demonstrating that caspase-3 and -7 exhibit differential activities toward multiple protein substrates, with caspase-7 being more selective (Slee et al., 2001, Walsh et al., 2008). Nevertheless, certain substrates such as cochaperone p23 are more prone to proteolytic processing by caspase-7 than caspase-3 (Walsh et al., 2008). These differential cleavage activities may underlie the interesting observation that mouse embryonic fibroblasts (MEFs) lacking caspase-3 or caspase-7 behave distinctly during ultraviolet (UV)- and FasL-induced apoptosis. Caspase-$7^{-/-}$ MEFs are more resistant to FasL- and UV-induced apoptosis than caspase $3^{-/-}$ MEFs, although double knockout MEFs are even more resistant (Lakhani et al., 2006). Nevertheless it is caspase-3, and not caspase-7, that is essential for the appearance of certain characteristic apoptotic features such as DNA fragmentation and PARP-1 cleavage under these conditions (Lakhani et al., 2006). These observations demonstrate that caspase-3 and -7 have overlapping, but also distinct roles in apoptosis. However, it should be noted that the importance of caspase-3 and -7 in apoptosis appears to be cell type- and stimulus-dependent. For instance, caspase-3/-7 double knockout thymocytes are highly resistant to staurosporine- and etoposide-induced apoptosis, but they remain susceptible to FasL. In sharp contrast to thymocytes, caspase-3/-7 double knockout MEFs are highly resistant to Fas-induced apoptosis (Lakhani et al., 2006). This may suggest that initiator caspases are sufficient to complete the apoptotic program under particular conditions or that alternative cell death modes such as necrosis may operate in the absence of executioner caspases.

b. Caspase-7 in inflammation

Besides its activation during apoptosis, proteolytic maturation of caspase-7 has also been observed under inflammatory conditions. Interestingly, in macrophages stimulated with lipopolysaccharides (LPS) and ATP or infected with the Gram-negative pathogens *Salmonella typhymurium* and *Legionella pneumophila*, caspase-7 activation requires caspase-1 complexes named ‘inflammasomes’ (Figure 1) rather than the caspase-8 and -9 protein complexes involved in apoptosis (Lamkanfi et al., 2008,Akhter et al., 2009). Biochemical studies demonstrated that caspase-7 is a direct substrate of caspase-1 with maturation occurring after the canonical activation sites Asp23 and Asp198 (Lamkanfi et al., 2008). In contrast to caspase-7, caspase-3 activation is not hampered in caspase-1 deficient macrophages, suggesting that activation of caspase-3 and -7 is differentially regulated during inflammation (Lamkanfi et al., 2008).

In addition to the distinct activation mechanisms described above, non-redundant functions of caspase-3 and -7 in infection and inflammation models are emerging as well. For instance, caspase-7 deficient macrophages are less capable of restricting intracellular *Legionella pneumophila* replication, possibly due to defects in the fusion of bacteria-containing phagosomes with lysosomes and the delayed induction of macrophage cell death (Akhter et al., 2009). Caspase-7 knockout mice were also reported to be resistant to lethality triggered by intraperitoneal injections of LPS, a commonly used model for human shock syndrome (Lamkanfi et al., 2009). Interestingly, caspase-3 deficient mice are not protected from LPS-induced lethality. The lower mortality rate in caspase-7 knockout mice was linked to a significant protection from splenocyte death. Because caspase-1 is activated in splenocytes of LPS-treated mice (Lamkanfi et al., 2009) and caspase-1 knockout mice are protected from LPS-induced mortality and splenocyte death (Kuida et al., 1995), caspase-1 may function upstream of caspase-7 in the LPS-endotoxemia model. However, a direct link between these caspases remains to be formally established in this mouse model.

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Possible medical applications

The described roles of caspase-7 in apoptosis and inflammation suggest that interfering with caspase-7 activation may prove beneficial in conditions where excessive cell death and/or inflammation contribute to disease. Therapeutic inhibition can be achieved with synthetic inhibitors or through targeted delivery of natural caspase inhibitors such as XIAP and the baculoviral caspase inhibitor p35. Caspase-7 inhibition seems especially warranted in neurodegenerative disorders such as Alzheimer's disease and Huntington's disease, where increased caspase-7 expression correlates with excessive neuronal cell death (Ramasamy et al., 2006, Hermel et al., 2004). Another potentially promising application is the prevention of lymphocyte cell death in sepsis. Extensive leukocyte apoptosis is commonly observed in sepsis patients and was suggested to contribute to immune suppression and lethality (Hotchkiss and Nicholson, 2006). Synthetic caspase inhibitors and overexpression of the anti-apoptotic protein Bcl-2 have already shown promising results in experimental sepsis models (Hotchkiss and Nicholson, 2006). In addition to the potential roles of caspase-7 in sepsis and neurodegenerative disorders, single nucleotide polymorphisms (SNPs) in the *caspase-7* gene have been linked with rheumatoid arthritis (SNP rs2227309; K249R mutation) (Teixeira et al., 2008) and Insulin-Dependent Diabetes Mellitus (SNP rs144692; D251E mutation) (Babu et al., 2003). Additional studies are required to extend these promising findings and to unravel the mechanisms by which the resulting missense mutations in caspase-7 trigger disease.

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Abbreviations

DISC	death-inducing signalling complex
FasL	Fas ligand
LPS	lipopolysaccharide
MEF	mouse embryonic fibroblast
TRAIL	TNF-related apoptosis-inducing ligand
UV	ultraviolet

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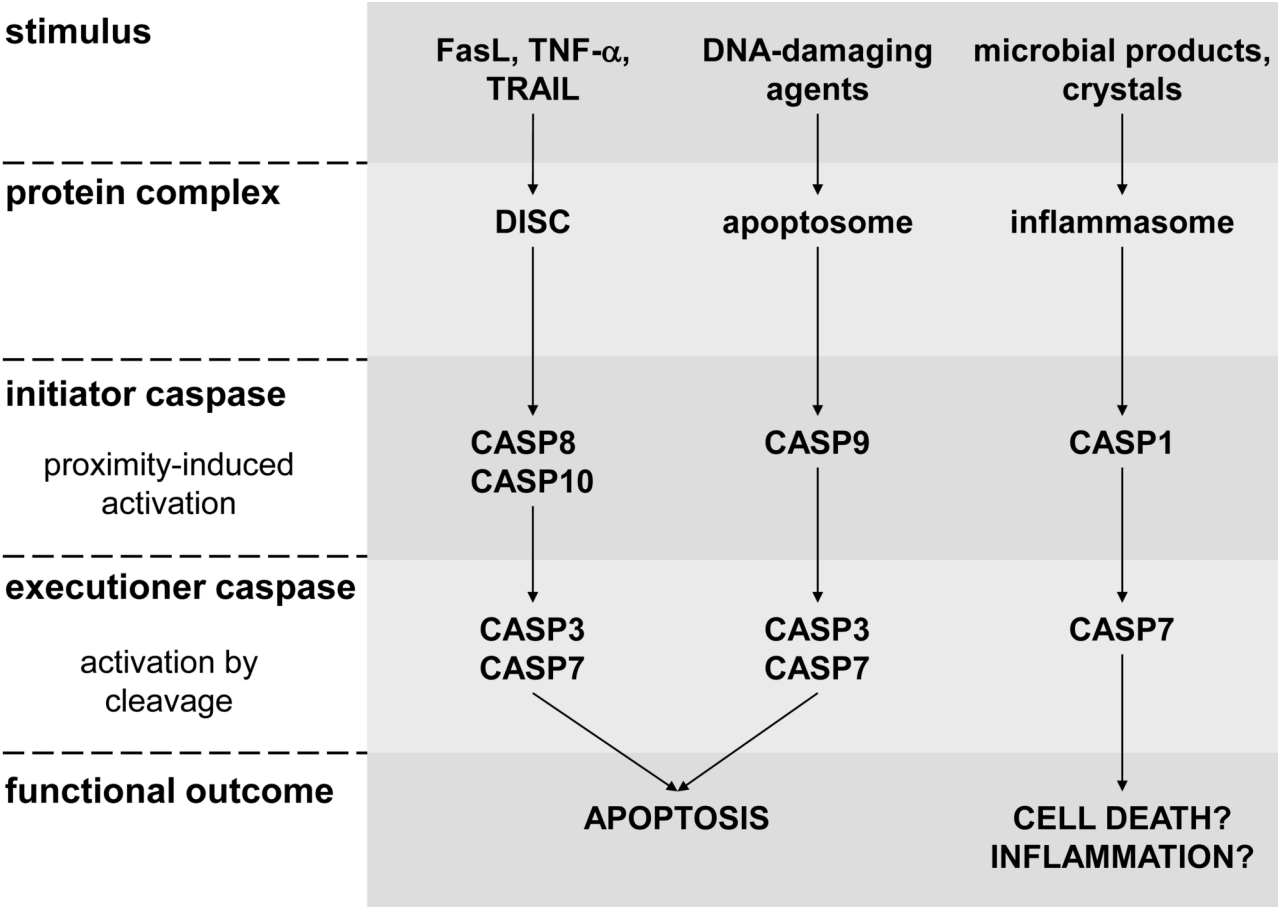

Figure 1.

Overview of caspase activation mechanisms. Caspases are activated in a two-step cascade, with initiator caspases (caspase-1, -8, -9 and -10) being first activated in large cytosolic protein complexes by proximity-induced autoactivation. Caspase-8 and -10 are activated in the death-inducing signaling complex (DISC) following death receptor stimulation, caspase-9 in the apoptosome in response to genotoxic stress, and caspase-1 in the inflammasome complex under inflammatory conditions. Activated initiator caspases directly activate the executioner caspases-3 and/or -7, which lead to apoptosis or inflammation by cleaving specific sets of substrates.

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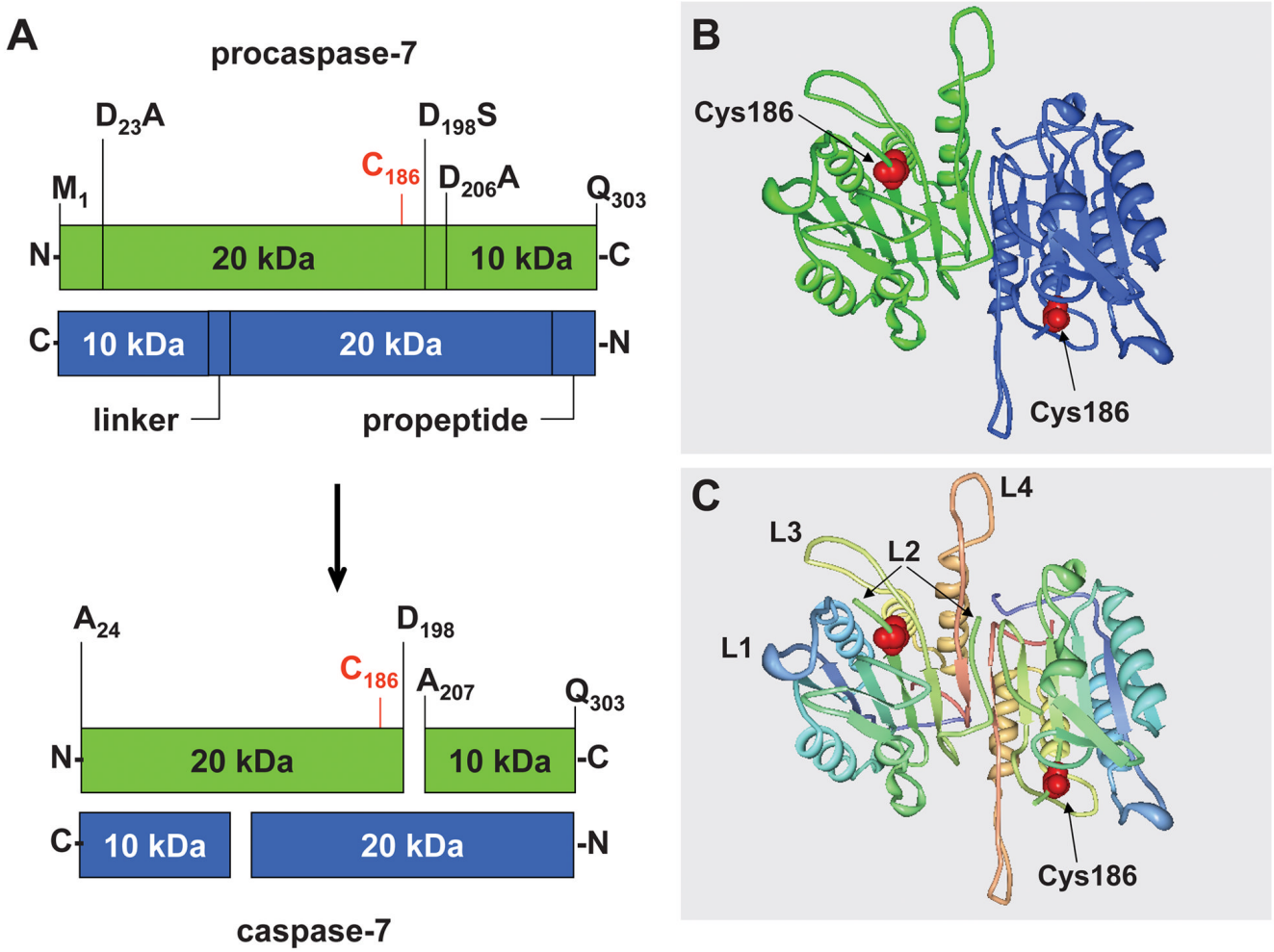

Figure 2.

Structure of procaspase-7. (A) Schematic representation of the procaspase-7 and active caspase-7 homodimer (shown in green and blue, respectively). The identity and position of the first (M_1) and last (Q_{303}) residues of the procaspase-7 amino acid sequence are indicated. The active site cystein residue Cys186 is shown in red. Initiator caspases cleave procaspase-7 after Asp_{23}, which is located between the propeptide and the p20 subunit. The linker region between the p20 and p10 subunits is removed by cleavage after Asp_{198} and Asp_{206}. Proteolytic removal of the propeptide and linker sequences leads to the active caspase-7 homodimer. (B) Three-dimensional structure of procaspase-7 (PDB entry 1K88) with the monomers of the homodimer shown in green and blue, respectively. The active site Cys186 is shown in red and space fill. (C) Three-dimensional structure of procaspase-7 (PDB entry 1K88) with backbone coloring by secondary structure. The positions of the flexible L1-L4 loops, which form the catalytic groove and substrate binding pockets, are indicated. The active site Cys186 is shown in red and space fill.