

Caspases

Key References

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Overview

Caspases (Cysteine dependant Aspartate Specific Proteases) form a family of closely related enzymes (family C14; clan CD) involved in regulating a type of cell death known as apoptosis, inflammatory responses, cellular proliferation and differentiation. Distinct caspases and caspase combinations are employed in these pathways dependent upon stimulus and cell type.

Caspases are synthesized as inactive precursors (zymogens). These procaspases consist of a prodomain, followed by a large subunit, a linker, and a small subunit. Maximally active caspases are dimeric and stabilized by cleavage between the large and small subunit. In the case of apoptotic "initiator" caspases (caspases 2/8/9/10), activation is thought to be triggered by dimerization of zymogen monomers, which is mediated by scaffold proteins. Auto-cleavage follows dimerization and stabilizes the active confirmation or procures further regulation properties. In contrast, "effector" caspases (caspases 3/6/7) exist as inactive dimers and cleavage of the inter-subunit linker by initiator caspases results in their activation. Four multiprotein complexes involved in the activation of initiator caspases have been described so far: the DISC (death inducing signaling complex, caspases 8/10), the apoptosome (caspase-9), the inflammasomes complexes (inflammatory caspases-1 and -5) and the PIDDosome (caspase-2).

Caspases exert their activity by cleaving a limited set of proteins, mostly at a single site. Cleavage by caspases can result in protein or enzyme activation, loss of function and/or generation of new activities. In this context, identification, characterization and elucidation of the physiological significance of cellular caspase substrates remains an intensely studied area.

Like other members of clan CD, the caspase catalytic dyad involves coordination of a histidine-cysteine pair. All caspases have a strict requirement for an aspartate residue in P1 position of the scissile bond and weaker preferences for P2-P4. Roughly, inflammatory caspases and caspase-14 prefer WEHD peptidic sequences (aromatic in P4). Caspases 2/3/7 prefer DEVD containing substrates (aspartate in P4). Caspases 6/8/10 prefer (V/I/L)ETD (hydrophobic residues in P4), whereas caspase-9 cleaves after LEHD motif (histidine in P2 is strongly preferred). Notably, all caspases share preference for glutamate in P3 and small residues (Ser/Gly/Ala) in P1' of the recognition sequence. Cleavage is not limited to these preferred motifs, as, for example, both caspases 8/9 can activate pro-caspase-7 by cleavage at a non-optimal site, IQAD↓S. The context within the structure of the substrate, cellular localization and timing might influence the ability of a given caspase to cleave a given protein as much as the substrate sequence itself.

Human IAPs (Inhibitors of Apoptosis) were the first identified endogenous caspase inhibitors of which, the X-linked IAP (XIAP) is the best characterized so far. Sequences encompassing XIAP's Baculovirus Inhibitory Repeat domains (Bir2 and Bir3) inhibit caspases 3/7 and caspase-9 respectively. These XIAP derived domains are the most specific and potent caspase inhibitors known to date. Although other IAPs (cIAP1, cIAP2, NAIP, ML-IAP, ILP2, Bruce and Survivin) have been shown to inhibit caspases, their physiological roles may involve other functions. Regardless, these and other cellular regulators of caspases have been shown to be misregulated in cancer, thereby providing a survival advantage during the oncogenic process and facilitating resistance to thera-

pies. Pathogens have evolved inhibitors of caspases as well. The cowpox viral serpin CrmA, for example, is a powerful inhibitor of caspases 1/8, allowing the virus to evade the host inflammatory response and block initiation of host cell apoptosis induced by immune cells.

Currently available synthetic caspase inhibitors are based on the peptidic sequences preferred as substrates and include an active warhead such as aldehyde, chloro- or fluoro-methylketone or epoxide group. Specific non-peptidic inhibitors of caspases are nonexistent with the exception of isatin sulfonamides derivatives demonstrated to bind selectively to caspases 3/7. Finally, like many proteases of the CD clan, caspases are not inhibited by E-64, but are sensitive to cysteine alkylating agents. Caspase inhibitors have served as valuable tools in the elucidation of apoptosis pathways and have been proposed as therapeutics in a variety of diseases where inappropriate or excessive apoptosis occurs. Small molecule caspase inhibitors are currently in clinical trails for treatment of liver diseases and transplantation.

Many agents have been reported to activate cellular caspases including chemotherapeutic drugs, TNF receptor agonists and other enzymes such as granzyme B. Aside from direct activation by other enzymes, caspase activation by small molecules, TNF ligands and antibody agonists is indirect with the complete elucidation of these pathways under investigation. Development of directly targeted drugs that result in caspase activation and apoptosis specifically in cancer cells is an ongoing strategy in oncology.

Caspases

CURRENT NAME	Caspase-1 (C5482)	Caspase-2 (C2854)	Caspase-3 (C1224)	Caspase-4 (C6357)	Caspase-5 (C6482)	Caspase-6 (C6977)
MEROPS ID	C14.001	C14.006	C14.003	C14.007	C14.008	C14.005
PREVIOUS NAMES	ICE, IL1BC, p45	Ich-IL, Ich-1	CPP32, Yama, Apopain, Sca-1	TX, Ich-2, ICE-rel II	ICE-rel III, Ich-3, TY	Mch2
GENE AND CHROMOSOMAL LOCATION	CASP1 11q22.2-q22.3	CASP2 7q34-q35	CASP3 4q33-q35.1	CASP4 11q22.2-q22.3	CASP5 11q22.2-q22.3	CASP6 4q25
TISSUE EXPRESSION	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous
GROUP FUNCTION	Inflammatory	Initiator/effector?	Effector	Inflammatory	Inflammatory	Effector?
PHYSIOLOGICAL FUNCTION	Inflammation	Apoptosis	Apoptosis	Inflammation	Inflammation	Apoptosis
DISEASE RELEVANCE	Viral infection, inflammatory diseases, early onset of Parkinson's disease	Not known	Cancer, Alzheimer's, Parkinson's and Huntington's diseases, ischemias	Viral infection?, inflammatory diseases?	Not known	Alzheimer's disease
SUBSTRATES	Pro-caspase-4 Pro-IL-β D4-GD1, parkin	Itself?	Pro-caspase-7, itself, PARP (P0996), ICAD, Rb, PKC-δ, Huntington's	Pro-caspase-1	Not known	Lamins A/B1, parkin
SUBSTRATE GROUP	Group I	Group II	Group II	Group I	Group I	Group III
PREFERRED PEPTIDIC SEQUENCE(S)^a	WEHD (A0216, A6720) YVAD (A2452, A9965, C6234)	VDVAD (A5345) DEVD (A1086, A0466, A2559, C1609)	DEVD (A1086, A0466, A2559, C1609)	WEHD (A0216, A6720) YVAD (A2452, A9965, C6234)	WEHD (A0216, A6720) YVAD (A2452, A9965, C6234)	IETD (A4188, C5599, C6484) LETD (A6095) VEHD (A9973, A2097)
COMMONLY USED INHIBITOR^b	YVAD (A1466)	VDVAD (C1605, A2222)	DEVD (C0605, A0835)	YVAD (A1466)	YVAD (A1466)	IETD (A1216, C1230) LETD (C8734)
INHIBITORS	CrmA (low nM) p35	p35	XIAP (Bir2), cIAP1 and 2 (Bir2), p35, ML-IAP, MMPSI	CrmA (low nM) p35	CrmA?	CrmA (mid nM)
PRODOMAIN	CARD	CARD	N-peptide	CARD	CARD	N-peptide
ACTIVATORS	Inflammasome	PIDDosome	Caspases 8/9, GrB, caspase-10?	Inflammasome	Inflammasome?	Caspases 8/9, GrB caspase-10?

FOOTNOTES

Caspases

CURRENT NAME	Caspase-7 (C2979)	Caspase-8 (C1099)	Caspase-9 (C8726)	Caspase-10 (C6607)	Caspase-14
MEROPS ID ^a	C14.004	C14.009	C14.010	C14.011	C14.018
PREVIOUS NAMES	Mch3, ICE-Lap3, CMH-1	MACH, FLICE, Mch5	ICE-Lap6, Mch6	Mch 4, FLICE2	MICE, mini-ICE
GENE & CHROMOSOMAL LOCATION	CASP7 10q25.1-q25.2	CASP8 2q33-q34	CASP9 1p36.1-p36.3	CASP10 2q33-q34	CASP14 19p13.1
TISSUE EXPRESSION	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous	Epidermis, choroid plexus, retinal pigment epithelium, thymic Hassall's bodies
GROUP FUNCTION	Effector	Initiator	Initiator	Initiator	Inflammatory?
PHYSIOLOGICAL FUNCTION	Apoptosis	Apoptosis	Apoptosis	Apoptosis	Keratinocyte differentiation?
DISEASE RELEVANCE	Cancer?	Cancer, Auto-immune diseases, early onset of Parkinson's and Huntington's disease, immunodeficiency	Cancer	Huntington's disease, autoimmune lymphoproliferative syndrome	Psoriasis?
SUBSTRATES	PARP, same as caspase-3?	Pro-caspases 3/7, itself, Bid, FLIP, pro-caspase-6?	Pro-caspases 3/7, itself, pro-caspase-6?	Itself, pro-caspases 3/6/7?	Not known
SUBSTRATE GROUP	Group II	Group III	Group III	Group III	Group I
PREFERRED PEPTIDIC SEQUENCE(S) ^b	DEVD	(I/L)ETD > DEVD	LEHD (A5845)	(I/L)ETD > DEVD	WEHD
COMMONLY USED INHIBITOR ^b	DEVD	(I/L)ETD	LEHD (C1355)	(I/L)ETD	
INHIBITORS	XIAP (Bir2), p35, ML-IAP, MIMPSI	CrmA (low nM)	XIAP (Bir3), CrmA (high nM), ML-IAP, ILP2	CrmA?	p35
PRODOMAIN	N-peptide	Tandem DEDs	CARD	Tandem DEDs	None
ACTIVATORS	Caspases 8/9 caspase-10? GrB	DISC	Apoptosome	DISC	Not known

Abbreviations

Bir: Baculovirus inhibitory repeat
CARD: Caspase recruitment domain
CHO: Aldehyde
CrmA: Cytokine responsive modifier A
DED: Death effector domain
DISC: Death inducing signaling complex
GrB: Granzyme B
IAP: Inhibitor of apoptosis protein

ICAD: Inhibitor of caspase-activated DNase
ILP2: IAP-like protein-2
ML-IAP: Melanoma IAP
MIMPSI: (S)-(+)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin
PARP: Poly (ADP-ribose) polymerase
PIDD: P53 induced protein with a death domain
XIAP: X-linked inhibitor of apoptotic proteases

FOOTNOTES

An earlier report suggested the existence of a human caspase-13 (ERICE, a novel FLICE-activatable caspase. *J. Biol. Chem.*, **273**,15702-15707 (1998), but further analysis revealed that the original library was contaminated with bovine cDNAs. Mice also express caspase-12 that seems implicated in endoplasmic reticulum stress response. This protein is catalytically inactive because it lacks a critical residue (Arg³⁴¹ in caspase-1 numbering system). In human a pseudogene exist but is expressed only in a subpopulation of African descents as an inactive protein. Saleh, M, et al., Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms., *Nature*, **429**, 75-79 (2004).

a The MEROPS (<http://merops.sanger.ac.uk/index.htm>) database contains information about peptidases and the proteins that inhibit them. It is maintained by Neil D. Rawlings, Fraser R. Morton and Alan J. Barrett at the Wellcome Trust Sanger Institute, Cambs CB10 1SA, UK.

b Many substrates carrying various fluorogenic and chromogenic groups are normally used. Those include Afc (7-amino-4-trifluoro methylcoumarin), Amc (7-amino-4-methylcoumarin) and pNA (p-nitroanilide). In principle, inhibitors composed of the preferred substrate peptide moiety and an inhibitor warhead constitute the preferred inhibitor. Warhead may include but is not limited to aldehyde (CHO), fmk (fluoromethylketone) and epoxides. Chloromethylketone are not recommended since General inhibitors (pan-caspase inhibitors) are z-VAD-fmk (**V116**, **C2105**) and z-EVD-fmk and to a lesser extend BAF (z-D-fmk).