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Review

The mitochondrial serine protease HtrA2/Omi: an overview

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The HtrA family refers to a group of related oligomeric serine proteases that combine a trypsin-like protease domain with at least one PDZ interaction domain. Mammals encode four HtrA proteases, named HtrA1–4. The protease activity of the HtrA member HtrA2/Omi is required for mitochondrial homeostasis in mice and humans and inactivating mutations associated with neurodegenerative disorders such as Parkinson's disease. Moreover, HtrA2/Omi is released in the cytosol, where it contributes to apoptosis through both caspase-dependent and -independent pathways. Here, we review the current knowledge of HtrA2/Omi biology and discuss the signaling pathways that underlie its mitochondrial and apoptotic functions from an evolutionary perspective.
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The evolutionarily conserved high-temperature requirement (HtrA) family of oligomeric serine proteases has been classified in family S1B of the PA protease clan in the MEROPS protease database (http://merops.sanger.ac.uk), and its members are characterized by the combined presence of a trypsin-like protease domain and one or two C-terminal PDZ domains (Figure 1a). The PDZ domain functions as a protein–protein interaction motif that preferentially binds C-terminal peptides of the target protein to stabilize interactions and modulate the proteolytic activity of the trypsin-like protease domain.[1] The bacterial HtrA family members have been implicated in stress tolerance and pathogenicity.[2] Although the functions of their eukaryotic homologs have been less well studied, it has become apparent in recent years that the human HtrA member HtrA2/Omi executes essential roles in the mitochondria and contributes to apoptosis through caspase-dependent and -independent mechanisms. Here, we review the current knowledge of HtrA2/Omi biology and discuss its mitochondrial and apoptotic functions from an evolutionary perspective.

Phylogenetic Analysis of HtrA2/Omi and its Homologs

Members of the HtrA family are present in nearly all bacterial and eukaryotic genomes, with no less than eight paralogs identified in the α-proteobacterial species *Mesorhizobium loti*.[3] In contrast to the phylogenetic domains of Eukaryota and bacteria, HtrA homologs are absent from nearly all archaean genomes.[3] In line with Margulis's endosymbiosis theory,[4] these findings support a monophyletic origin of eukaryotic HtrA proteases in a mitochondrial ancestor of the α-proteobacterial lineage.[3] The apparent absence of HtrA proteases from the bacterial class Mollicutes, to which the human parasites *Mycoplasma pneumonia* and *M. genitalium* belong, and the presence of many HtrA homologs in the related phylogenetic classes *Clostridia* and *Bacilli* strongly suggests that *Mycoplasma* species lost their HtrA-encoding genes after their diversification from the remaining classes of the Firmicutes. In the animal kingdom, HtrA-like genes are absent from all sequenced genomes of the phylum Nematoda, including that of the well-studied model organism *Caenorhabditis elegans*. These findings are in marked contrast with a previous report that suggested the presence of six nematode HtrA genes, but failed to provide further information.[3] Our studies indicate that nematodes lack genes encoding trypsin-like protease domains, although PDZ-containing proteins are present. A possible explanation for the apparent discrepancy is that PDZ-encoding genes without trypsin-like domains were selected in the study of Koonin and Aravind.[3] As mutations in the genes encoding HtrA proteins correlate with decreased bacterial fitness,[2] perinatal lethality in mice[5] and human Parkinson's disease,[6] the apparent lack of HtrA homologs in mycobacteria and nematodes suggests the functional convergence of structurally unrelated proteins in the latter organisms. In contrast to nematodes, the arthropod *Drosophila melanogaster* and the amphibian model organism *Xenopus tropicalis* encode an HtrA homolog in their respective genomes. Animals of the vertebrate lineage have expanded their repertoire of HtrA homologs, with four paralogs present in humans and mice. Whereas HtrA2/Omi resides in the mitochondrial intermembrane space (IMS), its paralogs HtrA1, 3 and 4 are most likely targeted to the

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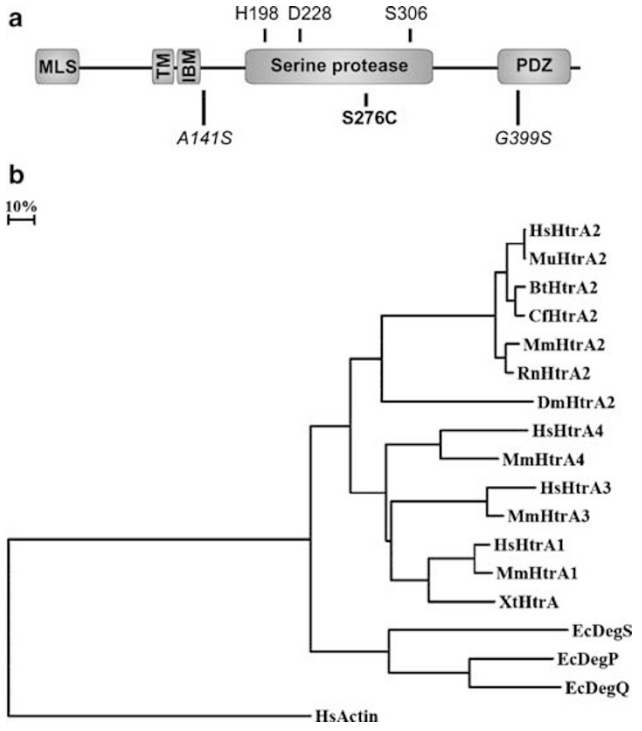

Figure 1 Domain organization and phylogenetic analysis of human HtrA2/Omi. (**a**) Full-length HtrA2/Omi consists of five functional domains and motifs: the N-terminal mitochondrial localization signal (MLS), the transmembrane (TM) segment, the IAP-binding motif (IBM), the serine protease domain and the C-terminal PDZ domain. Amino-acid substitutions associated with Parkinson's disease and the Parkinsonian phenotype of the Mnd2 mice are indicated below the functional domains in italic and bold, respectively. The catalytic triad residues are depicted above the functional domains. (**b**) Phylogenetic relationship of the HtrA family members. The sequences were aligned using the CLUSTAL X (gap weight = 10.00; gap length weight = 0.20) and trees were visualized in TreeCon. Bt, *Bos taurus*; Cf, *Canis familiaris*; Dm, *Drosophila melanogaster*; Ec, *Escherichia coli*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Mu, *Macaca mulatta*; Rn, *Rattus norvegicus*; Xt, *Xenopus tropicalis*

secretory pathway. Indeed, whereas the HtrA2/Omi precursor contains a mitochondrial localization signal (MLS) in its N terminus, HtrA1, 3 and 4 all harbor secretion signals in addition to insulin-like growth factor binding motifs and KAZAL domains in their N terminus. Interestingly, the HtrA2/Omi orthologs from human, rhesus monkey, dog, cow, mouse and rat also segregate phylogenetically from the cluster harboring HtrA1, 3 and 4 (Figure 1b). Moreover, the identified HtrA homolog in the fruitfly represents an ortholog of HtrA2/Omi (Figure 1b), in accordance with a recent report describing its cloning and functional characterization.[7,8] In contrast, the frog most likely expresses an HtrA1 ortholog. Although HtrA2/Omi segregates phylogenetically from the other metazoan HtrA proteins, the eukaryotic HtrA proteins, nevertheless, relate more to each other than to their bacterial homologs DegP, DegQ and DegS (Figure 1b). *Escherichia coli* HtrA/DegP functions as a chaperonin at normal temperatures, but relies on its proteolytic activity to prevent the accumulation of misfolded proteins in the periplasmic space at higher temperatures.[9] In line with this function, its protease activity displays only limited substrate selectivity.[10] In contrast, the anti-σ factor RseA is the only known target of bacterial DegS, which cleaves its substrate to initiate the transcription of stress-responsive genes when misfolded outer membrane proteins bind to its C-terminal PDZ domain.[11] The physiological function of bacterial DegQ is less well understood, although it is believed to fulfill roles redundant with those of DegP and DegS as many bacteria lack DegP and DegS, but encode *DegQ* genes.[12] Additionally, its protease activity displays a substrate specificity profile resembling that of DegP[10] and it may functionally substitute for DegP when overexpressed in *E. coli*.[13]

Mitochondrial HtrA2/Omi and Neurodegenerative Disorders

HtrA2/Omi is expressed as a 49-kDa proenzyme that is targeted to the mitochondrial IMS,[14,15] although a fraction of the endogenous HtrA2/Omi pool has been detected in the nucleus of resting cells.[15–17] The transmembrane anchor behind the N-terminal MLS most likely attaches the precursor protein into the mitochondrial inner membrane, where it undergoes proteolytic maturation. The fully processed protein is devoid of the first 133 amino acids encompassing the MLS and the transmembrane anchor, thus exposing an N-terminal inhibitor of apoptosis protein (IAP)-binding motif (IBM) related to those found in the *Drosophila* IAP inhibitors Reaper, Hid and Grim, and the mammalian IAP antagonist Smac/DIABLO.[14,15,18–20] Although it is evident that the HtrA2/Omi proenzyme undergoes proteolytic maturation within the IMS, the mechanism involved requires further analysis. Autocatalysis is suggested by the observation that purified recombinant HtrA2/Omi undergoes autoprocessing at Ala133 *in vitro*, whereas the enzymatically inactive S306A mutant remains uncleaved.[21,22] However, HtrA2/Omi appears to be correctly processed in cells derived from *Mnd2* mice (motor neuron degeneration 2), which are homozygous for a naturally occurring Ser276Cys mutation in the HtrA2/Omi protease domain that greatly reduces its catalytic activity.[5] The latter observation suggests that the HtrA2/Omi zymogen may be cleaved by another protease in the IMS, although it cannot be ruled out that residual HtrA2/Omi activity is responsible for the unaffected HtrA2/Omi maturation observed in *Mnd2* mice.[5] Studies on the maturation of HtrA2/Omi zymogens containing mutations in the residues of the catalytic triad that are performed in a HtrA2/Omi-deficient background may clarify this important issue.

Although studies in *Mnd2* mice were not conclusive enough to elucidate the mechanism involved in HtrA2/Omi maturation, the striking Parkinsonian phenotype displayed by these mice clearly demonstrated the essential role of HtrA2/Omi *in vivo*.[5] In addition to this neurodegenerative phenotype, *Mnd2* mice failed to gain weight, and organs such as the heart, thymus and spleen were dramatically smaller when compared to wild-type littermates.[5] The reduced body weight and progressive loss of neurons in the striatum of the basal ganglia were also evident in mice with a targeted deletion of the HtrA2/Omi gene,[23] hence confirming the results obtained in *Mnd2* mice. Before the neuronal cell loss became lethal approximately 30 days after birth, HtrA2/Omi$^{-/-}$ mice displayed a lack of coordination, decreased mobility and tremor,[23] resembling the clinical manifestations of Parkinson's disease. Indeed, two

single nucleotide polymorphisms in the *HtrA2/Omi* gene that cause missense mutations (A141S and G399S; Figure 1a) and affect the enzymatic activity of the protease have been associated with the development of Parkinson's disease in humans (Table 1).[6] A recent study demonstrated the phosphorylation of HtrA2/Omi at a residue adjacent to a position found mutated in patients with Parkinson's disease.[35] HtrA2/Omi phosphorylation depended on the cytosolic MAP kinase p38 and required the putative mitochondrial protein kinase PTEN-induced putative kinase 1 (PINK1), a known susceptibility factor for early-onset Parkinson's disease.[39] Interestingly, lower HtrA2/Omi phosphorylation levels were detected in brains of patients with Parkinson's disease carrying mutations in PINK1.[35] These findings suggest that PINK1-dependent phosphorylation of HtrA2/Omi might modulate HtrA2/Omi protease activity. Mutations in HtrA2/Omi or PINK1 that affect HtrA2/Omi phosphorylation might abolish the induction of HtrA2/Omi protease activity in patients with Parkinson's disease, possibly causing an increased susceptibility to mitochondrial stress and neuronal cell death.

Albeit less well established, some studies have suggested a link between HtrA2/Omi and Alzheimer's disease. The precursor of the β-amyloid protein that forms the plaques associated with Alzheimer disease undergoes post-translational processing by the mutually exclusive α- and β/γ-secretase pathways.[40] Cathepsin B was identified as the α-secretase,[41] whereas γ-secretase-mediated cleavage of amyloid precursor protein (APP) requires presenilin-1.[42] Interestingly, one study identified HtrA2/Omi as a presenilin-1-interacting factor in a yeast two-hybrid screen.[16] The association of endogenous HtrA2/Omi with presenilin-1 was later confirmed in cell lysates of untreated 293T cells.[36] Notably, presenilin-1 localizes primarily to the plasma membrane, endoplasmic reticulum, Golgi and nucleus,[43,44] whereas HtrA2/Omi resides mostly inside mitochondria, questioning the physiological context in which the interaction between HtrA2/Omi and presenilin-1 might occur. However, a fraction of the endogenous HtrA2/Omi pool may be targeted to the nucleus[15–17] and presenilin-1 may also traffic to mitochondrial membranes,[36,45] thus providing possible cellular niches for interaction. Alternatively, presenilin-1 and HtrA2/Omi may interact in the cytosol of apoptotic cells. Indeed, presenilin-1-derived peptides that bind to the PDZ domain of HtrA2/Omi induce apoptosis by upregulating its enzymatic activity.[36] In addition to its interaction with γ-secretase factor presenilin-1,[16,36] HtrA2/Omi was reported to generate a 28-kDa APP fragment *in vitro* and upon ectopic expression in 293T cells.[37] In accordance with APP-processing activity, the occurrence of this APP fragment was greatly reduced in brain extracts of *mnd2* mice carrying the Ser276Cys missense mutation in HtrA2/Omi,[37] suggesting a role for HtrA2/Omi in the turnover of APP that is targeted to the mitochondria by virtue of an N-terminal signal sequence.[46] It would be interesting to determine the fate of this 28-kDa APP fragment in the brains of mice with deficiencies in α-, β- and γ-secretase activities. Clearly, studies addressing the *in vivo* context in which endogenous HtrA2/Omi interacts with presenilin-1 and cleaves APP would greatly improve our understanding of these links to Alzheimer's disease.

Is HtrA2/Omi a Mitochondrial Chaperone?

The neurodegenerative phenotype of mice entirely lacking HtrA2/Omi or expressing the enzymatically inactive *Mnd2* mutant indicates that the protease activity of HtrA2/Omi fulfills a protective role in the mitochondria of neuronal cells.[5,23] Although the mechanism by which HtrA2/Omi exerts its protective effect is not clear, a role for HtrA2/Omi in the regulation of mitochondrial energy metabolism is excluded because the activity of the mitochondrial electron transport chain complexes was not affected in HtrA2/Omi-deficient cells.[23] It is tempting to speculate that HtrA2/Omi monitors and controls protein folding in the mitochondria, similar to the role of its homolog DegP in the bacterial periplasmic space. In this respect, HtrA2/Omi protein levels were shown to be upregulated several fold when the unfolded protein response was triggered by tunicamycin or heat shock.[16] Additionally, elevated HtrA2/Omi expression occurred following activation of the p53 stress pathway with etoposide.[47] Similar to the bacterial chaperone DegP, a transient exposure to elevated temperatures augments the protease activity of human HtrA2/Omi.[48] Moreover, the serine protease domains of both HtrA2/Omi and DegP favor the aliphatic residues Val or Ile in the P1 position.[10,48,49] In spite of these similarities between HtrA2/Omi and bacterial DegP, some important functional and structural characteristics of HtrA2/Omi are shared with DegS,

Table 1 Diseases associated with human HtrA homologs

Name	Synonym	Chromosome	Amino-acid identity with HtrA2	Localization	Disease	References
HtrA1	HtrA, PRSS11	10q26	53	Secreted	Arthritis	24,25
					Duchenne muscular dystrophy	26
					Alzheimer's disease	27
					Ovarian cancer	28,29
					Melanoma	30
					Endometrial cancer	31
					Lung cancer	32
					Age-related macular degeneration	33,34
HtrA2	Omi, PRSS25	2p12	100	Mitochondria, nucleus	Parkinson's disease	6,35
					Alzheimer's disease	36–38
HtrA3	PRSP	4p16	44	Secreted	Endometrial cancer	31
HtrA4		8p11	49	Secreted		

but not with DegP, thus arguing against a DegP-like chaperone function for HtrA2/Omi and suggesting a role closer to that of DegS. Particularly, HtrA2/Omi displays protease activity at room temperature,[21] whereas DegP requires elevated temperatures to become activated.[9] These functional differences are mirrored by extensive structural dissimilarities. For instance, DegP possesses two PDZ domains and oligomerizes into a hexameric cage in which the inner cavity is occupied by the protease domains with the side walls being constructed by the 12 PDZ domains.[50] In contrast, HtrA2/Omi and DegS form a trimeric pyramid-like structure with the N termini on top and the three PDZ domains at the bottom.[51–53] The overall stability of the HtrA2/Omi complex is ensured by extensive van der Waals interactions involving residues of the protease domains and a large hydrophobic interface formed by aromatic residues in the N-terminal segment of each monomer, which is referred to as the trimerization motif (Figure 2).[51] Second, both HtrA2/Omi and DegS lack the extended LA loop that ensures the dimerization of two DegP homotrimers and controls the height of the large central cavity of the hexamer to prevent properly folded proteins from entering the proteolytic sites.[1,54] In marked contrast to DegP, the short LA loop found in HtrA2/Omi and DegS does not interfere with proper formation of the active site.[1,12,53] Indeed, the relative orientation of the PDZ domain was suggested to modulate proteolytic activity in these proteases.[51–53] In the inactive state, the PDZ domain is directed back to the body of the protease domain through non-canonical interactions, trapping both the active site of the protease and its own peptide binding site in a distorted state.[51,52] Upon ligand binding, the PDZ domain would release the flexible L3 loop in the vicinity of the active site, thus triggering conformational reorganizations that result in the formation of a functional and accessible active site.[52] This mechanistic model is supported by the observation that the proteolytic activity of HtrA2/Omi is significantly augmented in the absence of its PDZ domain.[51] In addition, the protease activity of both HtrA2/Omi and DegS are upregulated in the presence of peptides that bind to their PDZ domains.[11,48] Notably, the PDZ domains of HtrA2/Omi and DegS have similar ligand specification, with both displaying a high affinity for peptides ending with the hydrophobic peptide sequence YYF(V) in the C terminus.[11,48] In addition to recognizing C-terminal peptide sequences, the HtrA2/Omi PDZ domain was recently reported to recognize internal stretches of extended, hydrophobic polypeptides as well.[55] In conclusion, arguments have been provided in favor of a role for HtrA2/Omi as a mitochondrial chaperone, as well as against it. Therefore, studies targeting the capacity of HtrA2/Omi to resolve the aggregation of unfolded proteins are required and may reveal more clues to its putative role in protein quality control.

Role of HtrA2/Omi in Apoptosis

As discussed above, proteolytic maturation of the HtrA2/Omi zymogen in mitochondria exposes an N-terminal IBM homologous to those of the *Drosophila* IAP inhibitors Reaper, Hid and Grim and the mammalian IAP antagonist Smac/DIABLO.[14,15,18–20] Nuclear DNA damage, death receptor activation and numerous other apoptotic insults trigger the

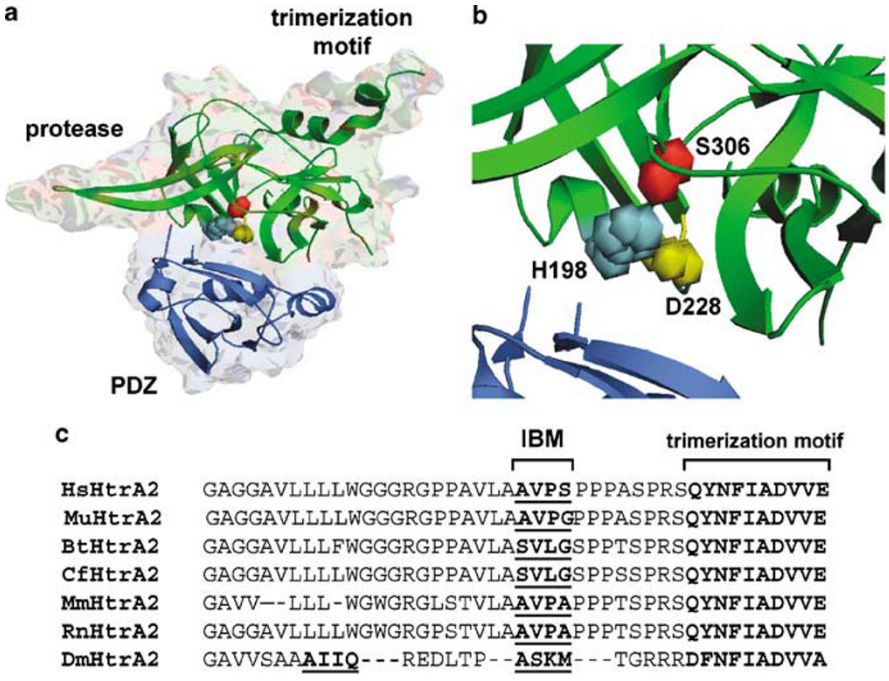

Figure 2 Three-dimensional structure of HtrA2/Omi and primary structure of the IBM and trimerization motifs. (**a**) Schematic representation of the HtrA2/Omi monomer. The serine protease and PDZ domains are indicated in green and blue, respectively. (**b**) A close-up view of the catalytic triad. The catalytic residues His198 (blue), Asp228 (yellow) and Ser306 (red) are shown in space fill. (**c**) The amino-acid sequences of the conserved trimerization motifs in human HtrA2/Omi and its orthologs are indicated. The amino-acid sequences of confirmed and putative IBM motifs are underlined. Bt, *Bos taurus*; Cf, *Canis familiaris*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Mu, *Macaca mulatta*; Rn, *Rattus norvegicus*

translocation of the mature protease into the cytosol, where it contributes to apoptosis through both caspase-dependent and -independent mechanisms. In this respect, antisense- and RNAi-mediated knockdown of HtrA2/Omi increases the resistance of multiple cell lines against apoptotic stimuli, such as anoikis, staurosporine, cisplatin, UV irradiation, anti-Fas and TRAIL.[14,15,56–60] Moreover, the synthetic HtrA2/Omi inhibitor Ucf-101[61] partially protects from cell death induced by cisplatin,[56,62] TNF-α[63] and staurosporine.[64]

HtrA2/Omi unleashes caspase activity in a biphasic process that frees the active forms of caspases-3, -7 and -9 by proteolytically removing their natural inhibitors.[58,59] Indeed, RNAi-mediated downregulation of HtrA2/Omi diminished the degradation of XIAP and cIAP1 in cells undergoing apoptosis in response to etoposide, staurosporine and TRAIL.[57–59] Mechanistically, the Reaper-like IBM sequesters IAP proteins in a first step.[14,15,18–20] The protease activity of HtrA2/Omi may then steer the reaction into the favorable thermodynamic direction by actively degrading bound IAP proteins.[58,59] In line with the two-step model for the degradation of IAP proteins by HtrA2/Omi, IBM-deficient mutants of HtrA2/Omi cleaved recombinant cIAP1 10 times less efficiently than the wild-type protease.[58] In addition, blocking the proteolytic activity of HtrA2/Omi attenuated post-ischemic myocardial apoptosis *in vivo* by preventing HtrA2/Omi-mediated XIAP degradation and subsequent caspase activity.[65] The HtrA2/Omi inhibitor Ucf-101 did not alter caspase-3-like activity in *Mnd2* MEF cells that underwent hypoxia/reoxygenation, in line with the proposal that Ucf-101 exerted its cardioprotective role by specifically inhibiting the protease activity of HtrA2/Omi.[65] A recent report confirmed the evolutionary conservation of this function by demonstrating that the *Drosophila* HtrA2/Omi ortholog harbors two IBM motifs to recruit DIAP1, easing its removal by the serine protease activity.[8] IBM motifs closely resembling that of human HtrA2/Omi have been retained in the rhesus monkey and rodent orthologs of this protease (Figure 2c). Serine substitutes for the N-terminal alanine in the putative IBM motif of bovine and canine HtrA2/Omi (Figure 2c). As noted for the IBM motifs of caspase-7 and glutamate dehydrogenase,[66] a serine residue may be tolerated in interactions with the BIR2 domain of IAP proteins, thus suggesting that the IAP-binding capacity of HtrA2/Omi is conserved in mammals. As discussed above, the genomes of nematodes, such as the model organism *C. elegans*, lack HtrA2/Omi homologs. Interestingly, the two IAP-like proteins in *C. elegans* do not seem to be implicated in the regulation of apoptosis,[67,68] suggesting that IAP proteins and the concomitant emergence of IAP-antagonistic proteins such as HtrA2/Omi and *Drosophila* Reaper, Hid and Grim represent more recent additions to the repertoire of apoptotic molecules.

Although multiple IAP family members were shown to be targeted and degraded by human HtrA2/Omi and its evolutionary paralogs, a recent study demonstrated that XIAP is the only bona fide inhibitor of caspases-3, -7 and -9.[69] Indeed, the BIR2 and BIR3 domains of cIAP1, cIAP2 and XIAP all bind the IBM motifs in the N terminus of the small catalytic subunits of active caspases-3, -7 and -9, but only XIAP engages a second interaction surface that allows potent inhibition of caspases.[70] The lack of this key feature in cIAP1 and 2 results in a catalytic inhibition that is 100- to 1000-fold less efficient.[69] Nevertheless, cIAP1 and 2, as well as XIAP, may prevent caspase activation by targeting bound caspases for ubiquitin-mediated proteasomal degradation,[71] providing an explanation why HtrA2/Omi targets all three IAP members. In marked contrast to its effect on caspases, XIAP binding enhanced the proteolytic activity of HtrA2/Omi.[48] Although IBM-mediated catalytic inhibition of caspases is a current focus for therapeutic exploitation in cancer,[72,73] XIAP-deficient mice lacked a clear apoptotic phenotype.[74,75] The only apoptotic phenotype is that sympathetic neurons from these mice are more sensitive to cytochrome *c* injection.[76] The absence of a major apoptotic phenotype may point to a limited physiological role for IAP proteins in the control of apoptosis or may be explained by the functional redundancy of XIAP with cIAP1 and 2. Double and triple knockout mice lacking these IAP members may reveal the extent to which these proteins protect against apoptosis. Similarly, the endogenous role of HtrA2/Omi in the sequestration of IAP proteins during apoptosis may be masked by its functional redundancy with IAP-binding proteins such as Smac/DIABLO[77,78] and the endoplasmic reticulum-associated protein GSPT1.[79] Moreover, a number of caspase substrates[80] and mitochondrial proteins that are released into the cytosol during apoptosis[66] have been proposed to contain XIAP-antagonizing IBM motifs. In addition, a detailed study of the physiological role of HtrA2/Omi in apoptosis has been hampered by the concomitant loss of its mitochondrial function in knockout mice.[23] A knock-in strategy that introduces a functionally defective IBM motif may circumvent this caveat by preserving its intramitochondrial function.

The retinoic acid/IFNβ-induced cell death activator Grim-19[81] was proposed to interact with the PDZ domain of HtrA2/Omi to enhance the proteolytic degradation of XIAP.[82] Grim-19 physically associates with the PDZ domain of HtrA2/Omi, and their interaction is enhanced by the combination of retinoic acid and IFNβ.[82] Grim-19 augmented HtrA2/Omi activity *in vitro*, thus providing an explanation for the reduced cell death and impaired HtrA2/Omi-mediated degradation of XIAP in antisense Grim-19-expressing MCF7 cells.[82] Similar to the results obtained with Grim-19, antisense-mediated downregulation of HtrA2/Omi conferred resistance to retinoic acid/IFNβ-induced cell death.[82] Grim-19 was previously shown to contribute to the activity of mitochondrial complex I[83] and homozygous deletion of Grim-19 caused embryonic death.[84] Therefore, both Grim-19 and HtrA2/Omi exert life-essential functions in mitochondria, but interact in the cytosol of apoptotic cells to promote HtrA2/Omi-mediated degradation of XIAP. In contrast to Grim-19, the death effector domain-containing protein Ped/Pea-15 was identified as a substrate of recombinant HtrA2/Omi *in vitro* and the HtrA2/Omi inhibitor Ucf-101 prevented Ped/Pea-15 degradation in UV-irradiated 293T and HeLa cells.[85] As Ped/Pea-15 interfered with XIAP binding on HtrA2/Omi and prevented UV-induced caspase-3 activity, cellular Ped/Pea-15 levels were proposed to modulate the ability of HtrA2/Omi to relieve XIAP-mediated inhibition of caspases.[85] Similar to the results obtained with Ped/Pea-15, siRNA-mediated knockdown of the tumor suppressor and mitotic regulator WARTS protected HeLa cells against HtrA2/Omi-induced cellular toxicity and

Table 2 Endogenous substrates of HtrA2/Omi

Name	Localization	Function	References
HtrA2/Omi?	Mitochondria, nucleus	Autoprocessing	17,21,22
APP	Mitochondria	Increase turnover of APP?	37
XIAP, cIAP1, cIAP2	Cytosol	Block IAP-mediated caspase inhibition	
		Enhance HtrA2/Omi protease activity	58,59
Ped/Pea-15	Cytosol	Antagonize Ped/Pea-15 anti-apoptotic function	85
WARTS	Nucleus?	Inhibit G1/S progression	17
HAX-1	Mitochondria	Antagonize HAX-1 anti-apoptotic function	62

XIAP degradation.[57] Interestingly, the kinase activity of WARTS was required for its association with the PDZ domain of HtrA2/Omi and the consequent increases in HtrA2/Omi protease activity and apoptosis.[57] It remains to be seen whether WARTS can phosphorylate HtrA2/Omi to modulate its protease activity as has been demonstrated for the serine/threonine kinases Akt1 and Akt2.[86] Indeed, phosphorylation of HtrA2/Omi on Ser212 attenuated its protease activity *in vitro* and impaired its pro-apoptotic function *in vivo*.[86] The authors demonstrated that phosphorylated HtrA2/Omi failed to cleave XIAP, although its binding with HtrA2/Omi was not affected.[86] These findings extend the anti-apoptotic role of Akt1 beyond the transcriptional upregulation of the anti-apoptotic proteins Bcl-2 and Mcl-1[87–90] and the inactivating phosphorylation of caspase-9[91] and the pro-apoptotic Bcl-2 members Bad and Bax.[92–95]

In addition to antagonizing IAP proteins to augment caspase activity, HtrA2/Omi contributes to apoptosis independently of its IBM. Indeed, siRNA-mediated knockdown of HtrA2/Omi combined with the pan-caspase inhibitor zVAD-fmk almost completely protected HeLa cells from undergoing staurosporine-induced cell death, whereas caspase inhibition alone was significantly less effective.[57] Additional evidence came from the observation that unlike HtrA2/Omi, caspase-9 and the IAP antagonist Smac/DIABLO were dispensable for detachment-induced anoikis in the epithelial cell line IEC-18.[60] Overexpression of IBM-defective HtrA2/Omi, but not the enzymatically inactive S306A mutant, induced morphological features of anoikis such as cell rounding and shrinkage.[14,15,18,20] This HtrA2/Omi-induced morphology persisted in Apaf-1$^{-/-}$ and caspase-9$^{-/-}$ cells as well as in the presence of the caspase inhibitors XIAP and zVAD-fmk, pointing to a caspase-independent mechanism.[14,18] A comprehensive proteome-wide analysis of Jurkat cell lysates led to the identification of 15 potential HtrA2/Omi substrates, 10 of which were validated *in vitro*.[49] Interestingly, this group included the cytoskeleton-associated proteins actin, α- and β-tubulin and vimentin, providing a likely explanation for the anoikis-like phenotype that occurs upon overexpression of HtrA2/Omi. In addition to these structural proteins, eIF-4G1 and EF-1α were identified as putative HtrA2/Omi targets.[49] The cleavage of these components of the translation machinery may contribute to the abrogation of *de novo* protein synthesis during apoptosis, next to the caspase-mediated cleavage of eIF-4G1.[96,97] The list of identified HtrA2/Omi substrates also included KIAA1967 and KIAA0251, two proteins that have recently been associated with apoptosis.[98,99] Another study demonstrated the mitochondrial anti-apoptotic protein HS1-associated protein X-1 (HAX-1) to be a HtrA2/Omi substrate.[62] Interestingly, experiments in *Mnd2* MEF cells reconstituted with wild-type HtrA2/Omi demonstrated that HtrA2/Omi-mediated degradation of HAX-1 correlated with extensive cell death in response to etoposide, cisplatin and H_2O_2.[62] In contrast to HtrA2/Omi, HAX-1 protein levels were significantly reduced but remained associated with mitochondria of cisplatin- and H_2O_2-treated 293T cells, suggesting that HtrA2/Omi degrades HAX-1 early in apoptosis.[62] Although more studies are required to decipher the physiological contribution of the identified HtrA2/Omi substrates, the results discussed above (summarized in Table 2) substantiate a role for HtrA2/Omi in apoptosis apart from antagonizing IAP proteins to induce caspase activity.

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