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News and Commentary

Caspase-12: an overview

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In 1997, caspase-12 was cloned from a murine L929r2 fibrosarcoma cDNA library.[1] As depicted in Figure 1a, the caspase-12 gene is located on chromosome 9. The genes for caspase-1 and -11, the closest phylogenetic relatives of caspase-12, reside upstream (5′) of the caspase-12 gene, while the genes for the D polypeptide of platelet-derived growth factor (PDGF-D) and for two members of the large matrix metalloprotease (MMP) family, that is, MMP-13 and -12, are localized downstream (3′) of *caspase-12*. The caspase-12 gene itself consists of 11 exons (Figure 1a). The entire N-terminal CARD is encoded by exons 1 and 2. The CARD is separated from the large catalytic subunit by a small linker region, encoded by the relatively small exons 3–5. The large and small catalytic subunits of caspase-12 arise from exons 5–8 and 8–10, respectively. The end of exon 10 also contains a small part of the 3′-untranslated region (3′UTR), while the remainder of the 3′UTR is encoded by exon 11 (Figure 1a). The mRNA of this caspase (Genbank accession code Y13090) encodes a protein of 48 kDa.[1] Based on primary sequence homology, caspase-12 can be classified as an inflammatory caspase, since it shares the highest amino-acid sequence identity with murine caspase-1 and -11 and human caspase-1, -4 and -5 (Table 1 and Figure 1c).[1]

Is caspase-12 implicated in inflammation?

A hallmark in the functional analysis of a gene is the generation of knockout animals. Caspase-1- and -11-deficient mice were challenged with proinflammatory stimuli, such as lipopolysaccharide (LPS) and tumor necrosis factor (TNF).[2,3] These and other experiments led to the conclusion that caspase-1 and -11 are essential for the maturation and release of the proinflammatory cytokines IL-1β and IL-18.[2,3] Both pro-IL-1β and pro-IL-18 are produced as biologically inactive precursor cytokines of 31 kDa that require cleavage by caspase-1 before the 17 kDa mature form is released from the cytosol. Monocytes from caspase-1- and -11-deficient mice display lower levels of other cytokines such as IL-1α, TNF-α, IL-6 and IFN-γ in endotoxemia.[2–4] Furthermore, both caspase-1 and -11 knockout mice are resistant to septic shock induced by the intraperitonial injection of LPS.[2,3] This is likely due to the lack of mature IL-18 in these mice, since protection from LPS-induced lethality is not observed in IL-1β-deficient animals, while neutralization of IL-18 confers complete resistance to lethal endotoxemia.[5,6]

Is caspase-12 involved in responses to infection and inflammation? Contrary to caspase-1, but similar to caspase-11, coexpression of caspase-12 with the precursor of the proinflammatory cytokine interleukin-1β (pro-IL-1β) does not result in the maturation of pro-IL-1β[1] (and Lamkanfi *et al.*, unpublished results). Moreover, in contrast to caspase-1 and -11, no caspase-12 protein expression or induction was detected in macrophage cell lines, or in isolated primary macrophages.[7] The absence of caspase-12 in macrophages, the inflammatory cells *par excellence*, suggests that the protein has a function distinct from that of caspase-1 and -11. Though induction of caspase-12 expression in macrophages in response to certain proinflammatory stimuli remains possible, caspase-12 may have a function related to inflammation in cells other than macrophages. Indeed, constitutive expression of caspase-12 has been observed in primary fibroblasts and in the L929 and AKR-2B fibrosarcoma cell lines.[7,8] Moreover, it was reported that the mRNA of caspase-12 is induced by the proinflammatory cytokine IFN-γ in primary hepatocytes[9] and the caspase-12 protein in murine primary fibroblasts, L929 fibrosarcoma and B16/B16 melanoma cells.[7] The induction of caspase-12 is specific for IFN-γ and does not occur with IFN-α or -β.[7] However, the exact role of caspase-12 in these cells remains elusive. Generation of caspase-12-deficient mice by replacement of a segment of intron 3 and exon 4 with a neomycin gene has been reported 3 years ago.[10] These mice undergo normal embryonic development and the adults have no apparent gross abnormalities. However, to the best of our knowledge, nothing has been reported yet on the response of these mice to a challenge with inflammatory stimuli such as LPS or TNF, experiments which would assess a possible inflammatory role for caspase-12.

Is caspase-12 implicated in apoptosis?

Data in the literature provide a confusing picture of the role of caspase-12 in apoptosis. On the one hand, it has been proposed that caspase-12 is not processed and activated following serum deprivation in rat PC12 pheochromocytoma cells, in etoposide-treated ST14A cells, a rat striatum-derived neuronal progenitor cell line,[11,12] or during TNF- and FasL-induced apoptosis in rat W4 fibrosarcoma cells.[10] Serum withdrawal and etoposide treatment are well-known inducers of the intrinsic mitochondrial apoptotic pathway, while TNF- and FasL-induced apoptosis are examples of the well-studied extrinsic death receptor pathway. On the other hand, caspase-12 processing and activation has been detected in FasL-treated L929sAhFas fibrosarcoma cells and TNF-α-treated B16/Bl6 melanoma cells.[7] During the mitochondrial apoptotic pathway, caspase-12 is processed in serum-deprived AKR-2B fibrosarcoma cells, in staurosporine-treated

murine cortical neurons and MEF cells, and in etoposide-treated PC12 cells.[8,13,14]

Is caspase-12 implicated in ER-stress induced apoptosis?

Evidence is emerging that ER stress causes apoptosis.[15–17] Caspase-12 has been localized on the cytoplasmic side of the ER using immunofluorescence and subcellular fractionation techniques.[10,11,13] ER stress can be induced by chemicals such as brefeldin A, tunicamycin, thapsigargin and the calcium ionophore A23187. Brefeldin A induces ER stress by blocking the transport of proteins from the ER to the Golgi complex. Tunicamycin is an inhibitor of N-glycosylation in the ER, while thapsigargin and A23187 disrupt intracellular calcium homeostasis. All these drugs lead to the accumulation of miss-folded proteins in the lumen of the ER. The observation that caspase-12 processing occurs during ER-stress induced apoptosis[7,10,11,18] has favored the idea that caspase-12 could be the initiator caspase in ER-stress-mediated apoptosis. However, the phenotype of MEF cells from caspase-12-deficient mice concerning this aspect is moderate as the absence of caspase-12 causes only a reduction of about 12% in ER-stress-induced apoptosis,[10] suggesting that other mechanisms may be operating. In agreement with this, caspase-12-deficient P19 EC cells do not display altered levels of tunicamycin-induced DNA fragmentation.[18] Furthermore, although caspase-12 is processed during ER-stress-mediated cell death in B16/B16 melanoma cells, the cells die to the same extent in the absence of caspase-12.[7] These data suggest that although caspase-12 is processed in ER-stress-mediated apoptosis, it may be dispensable for the execution of cell death prompted by ER stress, and that other molecular mechanisms may be operating.

Indeed, other ER-associated proapoptotic molecules have been reported. Bap31, a polytopic integral protein of the ER membrane, can bind caspase-8 and the proapoptotic protein spike.[19,20] Bap31 is a caspase-8 substrate. Caspase-8-cleaved Bap31 plays a role during Fas-mediated cell death.[15,17] Scotin and NRADD are two additional ER-residing proapoptotic molecules that have recently been discovered.[12,17] Scotin has been implicated in p53-mediated apoptosis.[17] In ST14A cells, CrmA and the vFLIP proteins E8 and MC159 inhibit NRADD-induced apoptosis. Interestingly, a dominant-negative form of NRADD exerts significant protection against ER-stress-inducing agents (thapsigargin,

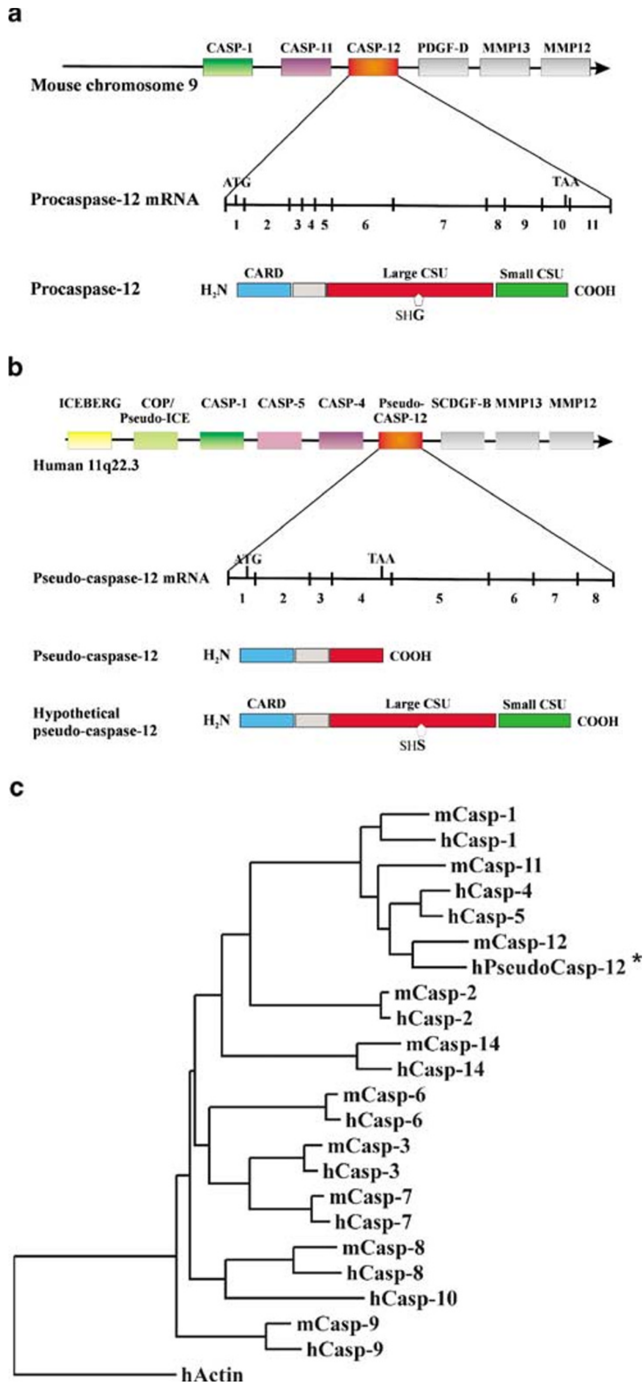

Figure 1 Structural organization of murine caspase-12 and human pseudo-caspase-12 and their phylogenetic relationship to other caspases. (**a**) The *caspase-12* gene is localized on murine chromosome 9 downstream (3′) of the genes encoding caspase-1 and -11. The lower panel shows the organization of caspase-12 mRNA and protein. The conserved catalytic SHG box is shown. (**b**) The *pseudo-caspase-12* gene is localized on human chromosome 11q22.3. The locus is depicted from telomere to centromere. The lower panel represents the organization of pseudo-caspase-12 mRNA and hypothetical protein. The hypothetical CARD-only and full-length pseudo-caspase-12 forms are depicted. The mutated SHS box is indicated. The translational start (ATG) and stop (TAA) codons are depicted. The size and intergenic distances are not to scale. Exons are numbered and are drawn to a scale collinear with the protein domains. Catalytic subunit (CSU) (**c**) phylogenetic analysis of the catalytic part of the human and murine caspases. The catalytic parts of the inflammatory caspases cluster separately from the apoptotic caspases. In the cluster of the inflammatory caspases, two separate subclusters can be identified. The first contains only caspase-1, the second combines murine caspase-11 with human caspases-4 and -5 on the one hand, and murine caspase-12 and human pseudo-caspase-12, on the other hand. The sequences were aligned using the CLUSTAL X (gap weight = 20.00; gap length weight = 0.10) and trees were visualized in TreeCon. *Mus musculus* (m), *Homo sapiens* (h), *the catalytic part of the hypothetical full-length human pseudo-caspase-12 was used

Table 1 Amino-acid sequence identity between the murine and human inflammatory caspases

	mC-1	mC-11	mC-12	hC-1	hC-4	hC-5	hpC-12
mC-1	100						
mC-11	43	100					
mC-12	42	41	100				
hC-1	62	41	40	100			
hC-4	45	59	48	48	100		
hC-5	45	54	45	47	74	100	
hpC-12	43	52	54	42	52	49	100

Murine caspase (mC), human caspase (hC), hypothetical human full-length pseudo-caspase-12 (hpC-12)

tunicamycin and brefeldin A) in ST14A and Schwann cells.[12] Taken together, the results reported for Bap31, NRADD and caspase-8 suggest that caspase-8 may be another ER-stress-associated protease.[12] In support of an important role for caspase-8 in ER stress is the observation that caspase-8 deficiency significantly delays the onset of tunicamycin-induced DNA fragmentation, while caspase-12 deficiency hardly has any effect on both DNA fragmentation and caspase activation.[18] Nevertheless, the exact roles of these proapoptotic factors during ER-stress-induced apoptosis need further investigation.

Human caspase-12 gene encodes an aberrant caspase

Until now, the functional orthologue of murine caspase-12 has only been cloned from rat cells (Genbank accession code AF317633). Nevertheless, many of the different reports on the ER localization of caspase-12 and on the molecular mechanisms of its activation during ER-stress-induced apoptosis have been proposed on the basis of studies in human cells and cell lines.[10,21–26] A biocomputational analysis of the human genome reveals that the human *caspase-12* gene on chromosome 11q22.3 has acquired several nonsense mutations, leading to a premature translational stop or to a loss-of-function mutation (Figure 1b). The premature stop may lead to the production of a CARD-only protein[27] (and Lamkanfi *et al.*, unpublished results). These mutations have been confirmed experimentally both on the genomic level[28] and the mRNA level.[27] Therefore, this type of human caspase-12 may be comparable with two other human CARD-only proteins, ICEBERG and COP/Pseudo-ICE, residing on the same chromosome (Figure 1b).[29–31] Similar to the human *pseudo-caspase-12* gene, ICEBERG and COP/Pseudo-ICE are encoded by caspase-like genes that have acquired premature nonsense mutations leading to the production of truncated molecules. Both ICEBERG and COP/Pseudo-ICE closely resemble the prodomain of caspase-1. Furthermore, both proteins interact with the CARD present in the prodomain of caspase-1, preventing the activation of this caspase and the subsequent generation of IL-1β.[29–31] Interestingly, COP/Pseudo-ICE is also capable of activating NF-κB and binding to the CARD of RIP2, a known inducer of NF-κB activation.[29,31] ICEBERG does not bind to RIP2 nor does it signal to NF-κB activation.[29,30] Since human pseudo-caspase-12 is structurally comparable to ICEBERG and COP/Pseudo-ICE, it would be interesting to study its involvement in similar pathways.

In certain people, an additional read-through mutation reverts the premature stop codon to an Arg codon, which may give rise to full-length human caspase-12 mRNA (Figure 1b). This particular variant of the human pseudo-caspase-12 gene can be found in the genomic SNP database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?search Type = adhoc_search&type = rs&rs = rs648264). However, this hypothetical full-length mRNA is unlikely to encode enzymatically active human caspase-12, since the conserved catalytic SHG box in the large catalytic domain remains mutated to SHS (Figure 1b). Introduction of the same SHS mutation in the catalytic SHG box of human caspase-1 completely abolishes the enzymatic activity.[28] Taken together, the existence of truncated human caspase-12 or enzymatically inactive caspase-12 makes it unlikely that it would play a major role in neurodegenerative diseases such as Alzheimer's disease, a role that has been suggested on the basis of the reduced cytotoxicity of the β amyloid peptide in caspase-12-deficient mice.[10]

Conclusion

Pseudo-caspase-12 is the human orthologue of murine caspase-12. Phylogenetic analysis based on the full-length proteins, the CARD prodomains or the enzymatic part of the caspases (Figure 1c), clearly classifies murine caspase-12 and the hypothetical human pseudocaspase-12 as the closest homologues residing in the cluster of the inflammatory caspase-1, -4, -5 and -11 (Figure 1c). Interestingly, the genes for murine caspase-1, -11 and -12 are also clustered on chromosome 9 in a region with high synteny to human chromosome 11q22, where the genes for human COP/Pseudo-ICE, ICEBERG, caspase-1, -4, -5 and pseudo-caspase-12 are located (Figure 1b). The region 11q22–q23 is often deleted in a wide range of cancers, including breast, lung, ovarian, cervical and colorectal carcinomas, in malignant melanomas and in hematologic malignancies.[32–35] In a number of neoplasias, the tumor-suppressor gene involved has been pinpointed to this region, but remains to be identified. After pseudo-caspase-12, the next closest human homologues of murine caspase-12 are caspase-4 and -5, sharing, respectively, 48 and 45% amino-acid sequence

identity with procaspase-12 (Table 1). There are several reasons favoring the idea that caspase-4 and -5 arose as gene duplications from an ancestral caspase-11 gene: (1) the three genes share the highest sequence homology; (2) they share comparable intron–exon structures and intron–exon boundaries (data not shown); (3) the proteins contain a high degree of sequence identity around the conserved QACRG box. However, as human caspase-12 is a pseudogene, it is conceivable that caspase-4 or -5 may be the functional counterpart of caspase-12 in man. Careful comparative analysis of the expression profiles and transcriptional regulation of human caspase-4 and -5, and murine caspase-11 and -12 may reveal the nature of the enigmatic functional orthologue of murine caspase-12 in humans. However, the conclusive identification of the human functional homologue of caspase-12 will require the identification of a specific substrate related to the conditions that lead to proper processing and activation of caspase-12. Moreover, the elucidation of the mechanism of caspase-12 activation may shed light on its physiological role. Until then, a possible role for caspase-12 in apoptosis and inflammation remains enigmatic.

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