Published in final edited form as:
Atherosclerosis. 2010 June ; 210(2): 422–429. doi:10.1016/j.atherosclerosis.2009.12.017.

CASPASE-1 RECOGNIZES EXTENDED CLEAVAGE SITES IN ITS NATURAL SUBSTRATES

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Abstract

Objective—The preferred amino acids in the proteolytic sites have been considered to be similar between caspase-1 and caspase-9, which do not support their differential functions in inflammatory pyroptosis and apoptosis. We attempted to solve this problem.

Methods—We analyzed the flanking 20 amino acid residues in the cleavage sites in 34 caspase-1 and 11 capase-9 experimentally identified substrates.

Results—This study has made the following findings: first, we verified that caspase-1 and caspase-9 shared 100% aspartic acid in the P1 position. However, the structures in the cleavage sites of most caspase-1 substrates are different from that of caspase-9 substrates in the following three aspects, *a)* the amino acid residues with the statistically high frequencies; *b)* the hydrophobic amino acid occurrence frequencies; and *c)* the charged amino acid occurrence frequencies; second, the amino acid pairs P1-P1′ are different; third, our identified cleavage site patterns are useful in the prediction for the 91.4% cleavage sites of 35 new caspase-1 substrates.

Conclusion—Since most caspase-1 substrates are involved in vascular function, inflammation and atherogenesis, our novel structural patterns for the caspases' substrates are significant in developing new diagnostics and therapeutics.

Keywords

inflammatory cell death (pyroptosis); apoptosis; inflammation; caspase-1; caspase-9

INTRODUCTION

A new form of cell death, pyroptosis, or caspase 1-dependent cell death, is inflammatory and is triggered by various pathological stimuli, both endogenous stimuli such as stroke, heart attack or cancer, and exogenous ones including bacterial and viral infections[1, 2, 3]. The recognition of pathogen-associated molecular patterns' (PAMPs) by PAMP-receptor families[4] leads to activation of caspase-1 (EC 3.4.22.36, a proinflammatory caspase), and subsequent proteolytic conversion of proinflammatory cytokines, interleukin-1β (IL-1β) and IL-18 from their precursors pro-IL-1β and pro-IL-18, respectively. Activation of pro-

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caspase-1 to form active caspase-1 is mediated by a cytosolic protein complex, termed inflammasome[4]. Therefore, PAMP-Rs-caspase-1-IL-1β pathway becomes an essential mechanism for sensing stimuli and initiating inflammation and pyroptosis. In contrast, in the activation of apoptosis, another form of cell death, the initiator caspases' activation platforms, such as death initiation signal complex (DISC) and apoptosome, sense pro-apoptotic stimuli and activate either death receptor/DISC-activated caspase-8 or mitochondrion/cytochrome c/apoptosome-activated caspase-9 pathway, respectively[5, 6]. Activation of caspase-9 results in apoptosis without inflammation. Since different cell death pathways require cellular substrates, therefore, the substrates cleaved by these two types of caspases have to be different.

The name caspase, derived from *c*ysteine-dependent *asp*artate specific prote*ase*, suggests its stringent specificity for cleaving substrates containing aspartic acid (Asp or D in abbreviation). The seminar work has identified the preferred amino acid residues with the Schechter and Berger's enzymatic cleavage nomenclature (Fig. 1)[7], P4-P3-P2-P1, in the cleavage sites of caspases' substrates as bulky X-Glu-X-Asp (X-E-X-D)[8, 9]. These short sequences are very similar among the proteolytic cleavage sites cleaved by different caspases[10]. However, the pathophysiological relevance of the amino acid preferences of caspases cleavage sites is limited, which could not explain the functional differences of caspases. The preferences of amino acid residues in the cleavage sites used by experimentally verified intracellular caspase-1 substrates remain to be analyzed. In addition, another important question also remains to be identified is that whether the flanking regions extended outside of P4-P1 motif in the natural caspase-1 substrates have any preferred amino acids. Therefore, we hypothesized that, caspase-1 cleaves different set of protein substrates from that of caspase-9, which is accomplished by preferring certain amino acid residues in the P10…P1-P1′…P10′ regions. Our results indicate that most extended cleavage sites of caspase-1 substrates are different from that of the extended cleavage sites of caspase-9 substrates.

MATERIALS AND METHODS

1. The experimentally verified cleavage sites of natural caspase-1 substrates and natural caspase-9 substrates

As we reported[11], a data mining strategy (suppl. Fig. 1) was adopted to analyze the amino acid occurrence frequencies in the flanking regions around the cleavage sites of experimentally identified human caspase-1 and caspase-9 substrates, according to the Schechter and Berger's enzymatic cleavage nomenclature (Fig. 1)[7]. The protein sequences of caspases' substrates were obtained from the protein database of the NIH/NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez) as we reported[12].

2. Amino acid occurrence frequencies in 30 randomly selected human proteins

Using a web based protein sequence software (http://www.expasy.ch/tools/protscale.html), the confidential intervals of the amino acid occurrence frequencies in the 30 randomly selected human house keeping gene proteins[13] were generated by calculating the mean ± 1.96 × the standard deviation. If the amino acid occurrence frequency of a given amino acid in the caspases' cleavage sites was larger than the upper limit of the confidential intervals (the mean ± 1.96 × the standard deviations) of the 30 proteins, the amino acid frequency in the position was statistically significant.

3. Prediction of the potential caspases' cleavage sites in experimentally verified caspases' substrates and potential caspases' substrates

The caspases' cleavage sites in the experimentally identified caspase-1 substrates and other proteins were predicted by analyzing caspases' cleavage sites in protein substrates using the caspase-1 cleavage site consensus sequence generated in this study and a web based protein alignment software (http://imed.med.ucm.es/PVS/).

4. Statistical analysis

The statistical analyses were performed using the functions of *t* test, confidential intervals and the chi-square in Microsoft Office Excel[14].

EXPERIMENTAL RESULTS

1. The amino acid occurrence frequencies in the flanking regions P10…P1-P1′-P10′ of natural caspase-1 substrates are different from that of natural caspase-9 substrates

To analyze the amino acid occurrence frequencies of the cleavage sites of caspase-1 substrates, we collected 34 experimentally characterized caspase-1 cleavage sites in the 23 human proteins (Table 1A). In addition, for comparison, we also collected 11 experimentally identified caspase-9 cleavage sites in the six human proteins (Table 1B).

In order to identify any amino acid residues having the higher frequencies with statistical significance than the normal amino acid occurrence frequencies, we generated the confidential intervals of amino acid occurrence frequency for each amino acid by calculating 12,467 amino acid positions in the 30 randomly selected human house keeping proteins[13] (Suppl. Table 1). The results showed that the mean amino acid occurrence frequencies derived by analyzing 1490 human proteins[15] were all within the confidential intervals that we generated ($p > 0.05$), suggesting that the confidential intervals of amino acid occurrence frequencies for each amino acid in Suppl. Table 1 are statistically unbiased representation of human proteins, and thus can be used in this study.

The significant group contained the amino acid residues with a frequency higher than the confidential interval of corresponding amino acid ($p < 0.05$). The non-significant group contained the amino acid residues with a frequency within the confidential interval in Suppl. Table 1 ($p > 0.05$). As expected, the P1 position of natural caspase-1 substrates had 100% stringently conserved amino acid Asp (Tables 2A and B). The positions of caspase-1 substrates P10, P7, P5, P2′, P4′, P6′, P7′, P8′, P9′ and P10′ had lower percentages of amino acids (≤40%) in the significant group. The positions of caspase-1 substrates P9, P8, P6, P4, P3, P2, P1, P1′, P3′ and P5′ had higher percentages of amino acids (>40%) in the significant group. Similarly, the P1 position of caspase-9 substrates had 100% stringently conserved Asp. In contrast to caspase-1 substrates, none of the positions in caspase-9 substrates had lower percentages of amino acids in the significant group (≤40%), suggesting that caspase-9 can cleave fewer substrates with more specialized cellular function than caspase-1. We then compared the percentages of amino acids in the P10-P10′ positions in the significant group of caspase-1 substrates to that of caspase-9 substrates. As shown in Table 2C, the percentages of amino acids in the positions P7, P5, P4, P3, P2′, P6′, P7′, P8′, P9′, and P10′ in the significant group of caspase-1 substrates were statistically different from that of caspase-9 substrates.

In addition, we examined the hydrophobic amino acid occurrence frequencies in these positions (Table 2C). The positions of caspase-1 substrates P7, P4, P2′, P3′, P5′ and P7′ had higher percentages of hydrophobic amino acids (≥50%). In comparison, the positions of caspase-9 substrates P8, P7, P4, P2, P2′, P3, P4′, and P9′ had higher percentages of

hydrophobic amino acids (≥50%). The percentages of amino acids in the position P3 in the hydrophobic amino acid group of caspase-1 substrates were statistically different from that of caspase-9 substrates.

Furthermore, we examined the charged amino acid occurrence frequencies in these positions (Table 2C). The position of caspase-1 substrates P1 had higher percentages of charged amino acids (≥50%). In comparison, the positions of caspase-9 substrates P9, P1, P5′ and P10′ had higher percentages of charged amino acids (≥50%). The percentages of amino acids in the positions P8 and P5′ in the charged amino acid group of caspase-1 substrates were statistically different from that of caspase-9 substrates.

2. The amino acid pairs in the cleavage sites P1-P1′ of caspase-1 substrates are, in as high as 55.9%, different from that of caspase-9 substrates

We compared occurrence frequencies of the amino acid pairs in the positions P1-P1′ of caspases' substrates[11], since they are primary structural features for the enzyme recognition and cleavage[7]. As shown in Suppl. Table 2, the three amino acid pairs in the cleavage sites P1-P1′ of caspase-1 substrates were shared 44.1% with that of caspase-9 substrates, including the amino acid pairs D–S, D-A and D–F. However, the amino acid pairs in the cleavage sites of caspase-1 were, in the rates as high as 55.9%, different from that of caspase-9 substrates. Therefore, these results suggest that the amino acid pairs used in the cleavage sites of caspase-1 substrates are different from that of caspase-9 substrates.

3. The extended cleavage site patterns P10-P10′ of caspase-1 substrates can be used in the prediction of cleavage sites for caspase-1 substrates

A recent report identified 41 new substrates of caspase-1, which characterized only one cleavage site in details (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) and left the cleavage sites in the rest of 40 caspase-1 substrates unidentified[16]. Of note, out of the other 40 substrates, four substrates including pro-caspase-1, β-actin, HnRNP A2, and HSP90 were previously characterized and listed in Table 1A. The calnexin's cleavage site was predicted using a recently reported prediction method (not shown) (http://us.expasy.org/tools/peptidecutter/)[17]. The uncharacterized cleavage sites of 35 substrates out of the 40 substrates could not be predicted by the existing algorithms, which were included in our studies. We hypothesized that the cleavage site patterns recognized in this study may be used in the prediction for the cleavage sites of caspase-1 substrates. To demonstrate the proof of principle, we used the amino acid sequences with the highest occurrence frequencies in all the P10-P10′ positions (Table 2A) for the initial prediction of caspase-1 cleavage sites including Asp in the P1 position. In the following fine tuning (Table 2A), the matches with other amino acids in the significant amino acid group and with amino acids in the non-significant amino acid group were also considered. In Table 3, our analysis of 35 caspase-1 substrates, with previously uncharacterized cleavage sites, showed that the P1 position of 32 cleavage sites (91.4%) out of 35 caspase-1 substrates were precisely predicted whereas the cleavage sites in the remaining 3 caspase-1 substrates (8.6%) were not predicted (Table 3A). The 60 predicted cleavage sites had more than 2 amino acid matches with ones from the significant amino acid group in the P10-P10′ positions of casapse-1 substrates (Table 2A). One substrate, adenyl cyclase, had nine amino acid matches with ones from the significant amino acid group in the positions. In addition, there were amino acid matches with the non-significant amino acids (Table 2A), which made the total amino acid matches up to 20 amino acids in the P10-P10′ region in four substrates (ARP3, SET translocation, calreticulin precursor and PHAP1/April). Of note, future experiments are required to verify the prediction of these cleavage sites of caspase-1. Taken together, the new cleavage site patterns can be used for prediction of the cleavage sites of caspase-1 substrates.

DISCUSSION

Current understanding of the specificity of caspases in the P4-P1 positions[9, 18, 8] has been employed in the various aspects: (1) as substrate reporter reagents; (2) as inhibitors; and (3) as experimental therapeutic agents for stroke and neurodegenerative diseases[19, 20]. However, these tetrapeptide-based approaches have a problem of a significant overlap between caspases' consensus sequences since caspases are promiscuous on these sequences[10]. This problem has hampered the characterization of functional differences between caspase-1-dependent inflammatory pyroptosis and caspase-9-dependent non-inflammatory apoptosis. To solve the problem, we analyzed the amino acid residues in the extended cleavage sites in the experimentally identified caspase-1 substrates and caspase-9 substrates. This study has made the following findings: first, caspase-1 and caspase-9 shared 100% stringently conserved Asp in the P1 position. However, the structures in most extended cleavage sites of caspase-1 substrates are different from that of caspase-9 substrates in the following three aspects: *a)* the cleavage site positions having amino acid residues with the statistically high frequencies; *b)* the positions with hydrophobic amino acid occurrence frequencies; and *c)* the positions with charged amino acid occurrence frequencies; second, the amino acid pairs P1-P1′ used in the cleavage sites of caspase-1 substrates are different from that of caspase-9 substrates; third, new cleavage site patterns may be used in the prediction of the uncharacterized cleavage sites of caspase-1 substrates. We have predicted the precise P1 position of 32 cleavage sites (91.4%) out of 35 experimentally identified caspase-1 substrates with previously uncharacterized cleavage sites[16], suggesting that our new cleavage site patterns have improved current caspases' cleavage site prediction methods. Our new cleavage patterns of caspase-1 could be used in the various aspects: (1) as substrate reporter reagents; (2) as inhibitors; and (3) as experimental therapeutic agents but with the accuracy and efficacy potentially much higher than the tetrapeptide-based compounds[19, 20]. In addition, our results in defining the cleavage site differences between caspase-1 substrates and caspase-9 substrates may also reflect the potential differences between caspase-1 enzymatic active site and the counterpart of caspase-9[10].

Increasing evidence suggests that caspases have important functions to regulate cell proliferation, differentiation, and migration in addition to cell death[5]. In contrast to 11 substrates identified for caspase-9, up to 70 caspase-1 substrates have been reported. Functions of caspase-1, in addition to regulate inflammatory pyroptosis (maturation of proinflammatory cytokines IL-1β and IL-18, activation of caspase-3[21] and caspase-7[22]), have also been identified including protein translation, ubiquitination-proteasome degradation, DNA repair, stabilization of cytoskeleton and glycolysis[16], etc (Table 1A). One unique function of caspase-1 substrates constitutes orchestrated caspase-1 dependent inflammatory pyroptosis phenotype. Indeed, we also found that most caspase-1 substrates play a role in vascular inflammation, function and atherogenesis (Suppl. Table 3). Although caspase-1 also activates effector caspase-3[21] and caspase-7[22] that are traditionally regarded as caspase-9 substrates (Table 2A), relative celerity of intracellular activation of caspase-3 and caspase-7 by caspase-1 are predicted to be slower than caspase-9 because of competition between pro-casapses-3 and -7 and many other proinflammatory caspase-1 substrates for caspase-1 cleavage. In addition, initiation of casapse-1 activation and inflammation is dependent on activation of NF-κB pathway[3]. However, activation of NF-κB also leads to inhibition of cell death by upregulating anti-apoptotic proteins such as Bcl-xL[23]. Therefore, comparing to apoptosis, caspase-1 dependent cell death has two unique features: *a)* it is slow in activating effector caspases-3 and 7; *b)* the cell survival mechanism associated with activation of NF-κB pathway serves as an additional "brake" for cell death aspect of pyroptosis. Our new working model in Fig. 2 emphasizes structural differences in the extended cleavage sites between caspase-1 and caspase-9. Our results on the extended

cleavage sites of caspase-1 and caspase-9 are significant in developing new detection tools, diagnostics and therapeutics for the pathology that caspases are involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was partially supported by the National Institutes of Health Grants HL094451 (X.-F. Yang) and HL67033, HL82774, and HL77288 (HW).

References

1. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–1695. [PubMed: 15843671]
2. Yang XF. Factors regulating apoptosis and homeostasis of CD4+CD25highFOXP3+ regulatory T cells are new therapeutic targets. Front Biosci 2008;13:1472–1499. [PubMed: 17981643]
3. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009;7:99–109. [PubMed: 19148178]
4. Tschopp J, Martinon F, Burns K. NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol 2003;4:95–104. [PubMed: 12563287]
5. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene 2008;27:6194–6206. [PubMed: 18931687]
6. Degterev A, Yuan J. Expansion and evolution of cell death programmes. Nat Rev Mol Cell Biol 2008;9:378–390. [PubMed: 18414491]
7. Schechter I, Berger A. On the size of the active site in proteases. I Papain. Biochemical and biophysical research communications 1967;27:157–162. [PubMed: 6035483]
8. Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 2002;9:459–470. [PubMed: 11931755]
9. Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J Biol Chem 1997;272:17907–17911. [PubMed: 9218414]
10. Timmer JC, Salvesen GS. Caspase substrates. Cell Death Differ 2007;14:66–72. [PubMed: 17082814]
11. Yang XF, Mirkovic D, Zhang S, Zhang QE, Yan Y, Xiong Z, Yang F, Chen IH, Li L, Wang H. Processing sites are different in the generation of HLA-A2.1-restricted, T cell reactive tumor antigen epitopes and viral epitopes. Int J Immunopathol Pharmacol 2006;19:853–870. [PubMed: 17166407]
12. Yin Y, Yan Y, Jiang X, Mai J, Chen NC, Wang H, Yang XF. Inflammasomes are differentially expressed in cardiovascular and other tissues. Int J Immunopathol Pharmacol 2009;22:311–322. [PubMed: 19505385]
13. Eisenberg E, Levanon EY. Human housekeeping genes are compact. Trends Genet 2003;19:362–365. [PubMed: 12850439]
14. Rosner, B. Hypothesis testing: one-sample inference. In: Rosner, B., editor. Fundamentals of Biostatistics. Australia, Canada, Mexico, Singapore, Spain, United Kingdom, United States: Duxbury; 2000. p. 211-271.
15. Wada K, Wada Y, Ishibashi F, Gojobori T, Ikemura T. Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res 1992;20 (Suppl):2111–2118. [PubMed: 1598238]
16. Shao W, Yeretssian G, Doiron K, Hussain SN, Saleh M. The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. J Biol Chem 2007;282:36321–36329. [PubMed: 17959595]

17. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, MR.; Appel, RD.; Bairoch, A. Protein Identification and Analysis Tools on the ExPASy Server. In: Walker, JM., editor. The Proteomics Protocols Handbook. Humana Press; 2005. p. 571-607.

18. Garcia-Calvo M, Peterson EP, Leiting B, Ruel R, Nicholson DW, Thornberry NA. Inhibition of human caspases by peptide-based and macromolecular inhibitors. J Biol Chem 1998;273:32608–32613. [PubMed: 9829999]

19. Schulz JB, Weller M, Moskowitz MA. Caspases as treatment targets in stroke and neurodegenerative diseases. Ann Neurol 1999;45:421–429. [PubMed: 10211465]

20. Li M, Ona VO, Guegan C, Chen M, Jackson-Lewis V, Andrews LJ, Olszewski AJ, Stieg PE, Lee JP, Przedborski S, Friedlander RM. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. Science 2000;288:335–339. [PubMed: 10764647]

21. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. N Engl J Med 2003;348:1365–1375. [PubMed: 12672865]

22. Lamkanfi M, Kanneganti TD, Van Damme P, Vanden Berghe T, Vanoverberghe I, Vandekerckhove J, Vandenabeele P, Gevaert K, Nunez G. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. Mol Cell Proteomics 2008;7:2350–2363. [PubMed: 18667412]

23. Basak S, Hoffmann A. Crosstalk via the NF-kappaB signaling system. Cytokine & growth factor reviews 2008;19:187–197. [PubMed: 18515173]

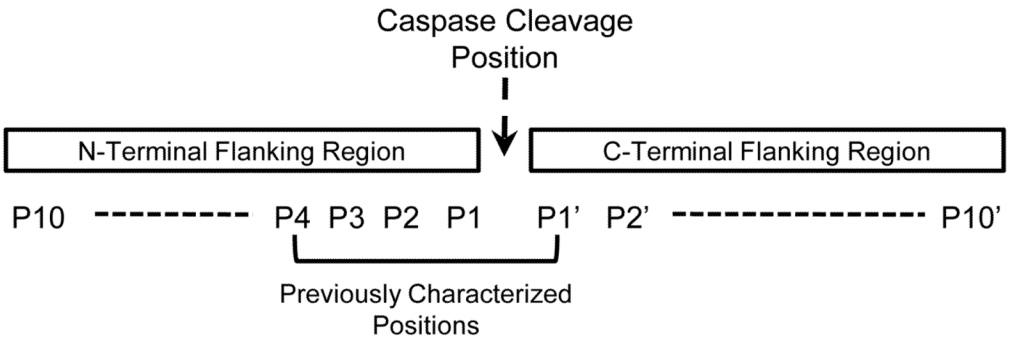

Figure 1. The position nomenclature for the caspases' cleavage sites and the flow chart of this study

The position nomenclature for the caspases' cleavage sites. The Schechter and Berger's enzymatic cleavage nomenclature is used in reference to the amino acid positions in the N-terminal and C-terminal flanking regions, relative to the caspases' cleavage sites. The amino acid preferences in the positions P4-P1′ have been previously characterized with synthetic peptide library screening, as marked.

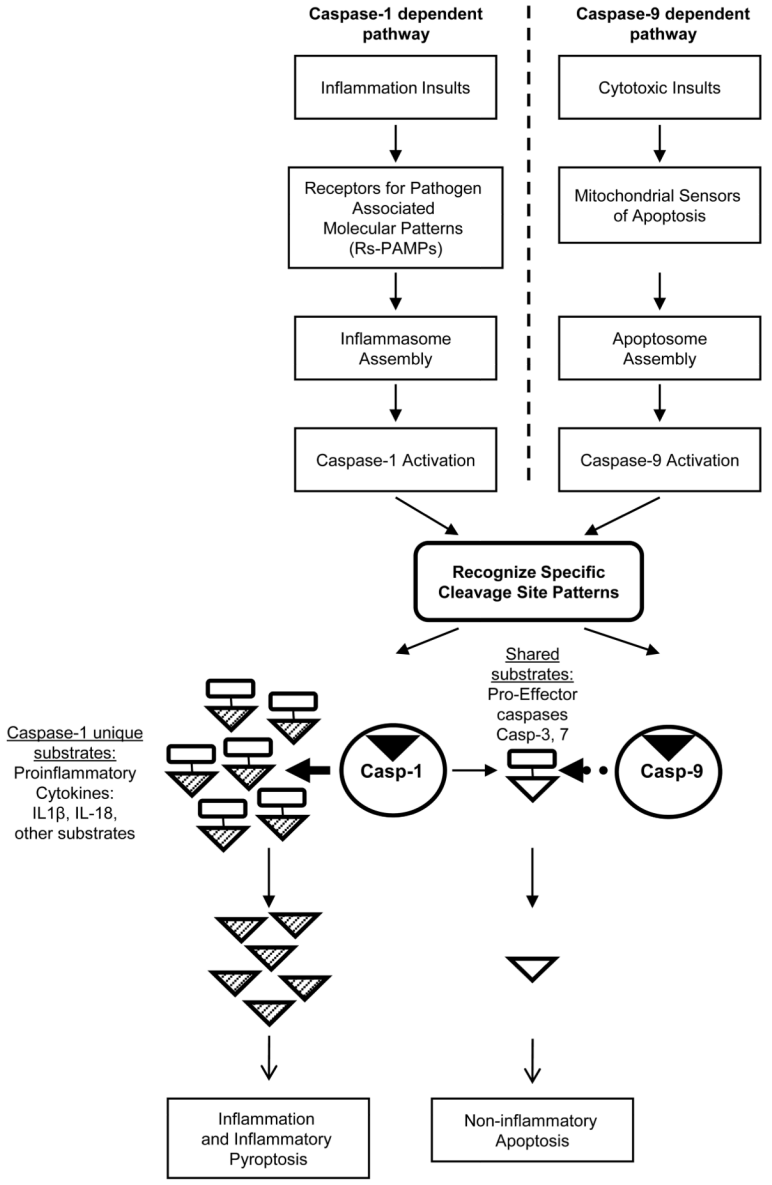

Figure 2.
The working model supported by our results presented in this paper.

Table 1
The experimentally identified caspase-1 substrates and caspase-9 substrates

The experimentally identified caspase-1 substrates and caspase-9 substrates are presented with the cleavage site position P1 of caspase, the NCBI protein database accession number, cellular function of the protein and the reference of the substrates.

Table 1A. Experimentally Identified Substrates of Caspase-1 with Characterized Cleavage Site

Substrate	Cleavage Position (P1 amino acid)	NCBI/Protein Database (GI) Accession No.	Cellular Function	Reference
caspase-1				
IL-1β - cut 1	27	28317372	Mediator of Inflammation	PMID: 10872455
IL-1β - cut 2	116	28317372	Mediator of Inflammation	PMID: 10872455
IL-18	36	4504653	Stimulation of INF-γ	PMID: 10872455
β-actin - cut 1	11	46397333	Cytoskeleton	PMID: 8700913
β-actin - cut 2	244	46397333	Cytoskeleton	PMID: 8700913
GADPH	189	31645	Glycolysis	PMID: 17959595
HnRNP A2-cut 1	49	500638	Translation	PMID: 17273173
HnRNP A2-cut 2	55	500638	Translation	PMID: 17273173
HnRNP A2-cut 3	76	500638	Translation	PMID: 17273173
Hsp90	259	306891	Chaperone	PMID: 17273173
Calpastatin - cut 1	137	303599	Calpain Inhibitor	PMID: 9705209
Calpastatin - cut 2	216	303599	Calpain Inhibitor	PMID: 9705209
Calpastatin - cut 3	417	303599	Calpain Inhibitor	PMID: 9705209
PPAR-γ	64	116284373	Transcription Factor	PMID: 18497737
Nedd4	237	32172435	Ubiquitin-protein Ligase	PMID: 9593687
Parkin	126	3063388	E3 ubiquitin ligase component	PMID: 12692130
Ataxin-3 - cut 1	241	14149093	Polyglutamine disease protein	PMID: 15140190
Ataxin-3 - cut 2	244	14149093	Polyglutamine disease protein	PMID: 15140190
Ataxin-3 - cut 3	248	14149093	Polyglutamine disease protein	PMID: 15140190
BCL-XL	61	510901	Anti-apoptotic protein	PMID: 9435230
PARP	214	116283598	DNA Repair	PMID: 7642516
TF AP-2α	19	4507441	Transcription Factor	PMID: 11438643
MAP-Tau Isoform 2	421	6754638	Stablization of Microtubules	PMID: 12888622
PSEN1	341	15079861	Regulation of APP	PMID: 10069390
PSEN2	329	13623517	Regulation of APP	PMID: 10069390
Pyrin	330	2407316	Mediterranean fever protein	PMID: 18577712
LMNA Isoform 2	230	5031875	Nuclear Membrane	PMID: 8978814
PLA2G4A	459	56202754	Phospholipid Hydrolysis	PMID: 9875225
SPTAN1	1185	55663122	Cytoskeleton	PMID: 9894612
IL1F7	20	20127524	Cytokine	PMID: 12096920
Caspase-1 - cut 1	103	266321	Pyroptosis	PMID: 7721861
Caspase-1 - cut 2	119	266321	Pyroptosis	PMID: 7721861
Caspase-1 - cut 3	297	266321	Pyroptosis	PMID: 7721861
Caspase-1 - cut 4	316	266321	Pyroptosis	PMID: 7721861

Table 1B. Experimentally Identified Substrates of Caspase-9 with Characterized Cleavage Site

Substrate	Cleavage Position (P1 amino acid)	NCBI/Protein Database (GI) Accession No.	Cellular Function	Reference
Caspase-9				
proCaspase 3 - cut 1	28	77416852	Apoptosis	PMID: 15274128
proCaspase 3 - cut 2	175	77416852	Apoptosis	PMID: 15274128
proCaspase 7 - cut 1	23	1730092	Apoptosis	PMID: 15274128
proCaspase 7 - cut 2	198	1730092	Apoptosis	PMID: 15274128
proCaspase 7 - cut 3	206	1730092	Apoptosis	PMID: 15274128
Vimentin - cut 1	85	62414289	Cytoskeleton	PMID: 11514563
Vimentin - cut 2	259	62414289	Cytoskeleton	PMID: 11514563
Vimentin - cut 3	429	62414289	Cytoskeleton	PMID: 11514563
DCC	1290	1169233	Transmembrane receptor	PMID: 11248093
RB1	270	132164	Tumor suppressor	PMID: 15735701
Raf-1	279	125651	Signal Transduction	PMID: 15674327

Table 2

The amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-1 and caspase-9 substrates

A. The amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-1 substrates. By comparing to the confidential intervals of human amino acid occurrence frequencies generated in supplemental Table 1, the amino acid residues occurred in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-1 are classified into the significant section with the frequencies higher than the upper limit of the confidential intervals ($p<0.05$) and the non-significant section with the frequencies in the range of the confidential intervals ($p>0.05$). **B.** The amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-9 substrates. By comparing to the confidential intervals of human amino acid occurrence frequencies generated in supplemental Table 1, the amino acid residues occurred in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-9 are classified into the significant section with the frequencies higher than the upper limit of the confidential intervals ($p<0.05$) and the non-significant section with the frequencies in the range of the confidential intervals ($p>0.05$). **C.** The amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites of human caspase-1 and caspase-9 substrates. The amino acid occurrence frequencies in the significant sections with the frequencies higher than the confidential intervals of each amino acid are presented in the left two columns. The hydrophobic amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites are presented in the central two columns. The charged amino acid occurrence frequencies in the flanking 20 amino acid positions P10-P10′ around the cleavage sites are presented in the right two columns. The features of amino acids are marked with different fonts, acidic = *italicized*; basic = underlined; hydrophobic = **bolded**; and neutral = normal font.

Table 2A. Significant Amino Acids in Experimentally Identified Caspase-1 Cleavage Sites

Position	Significant [a]	Non-significant[b]
P10	$D_{14.7}$ $Y_{5.9}$	$S_{8.8}$ $\underline{K}_{8.8}$ $E_{8.8}$ $\mathbf{A}_{8.8}$ $\mathbf{V}_{5.9}$ $T_{5.9}$ $\mathbf{P}_{5.9}$ $N_{5.9}$ $\mathbf{L}_{5.9}$ $\mathbf{I}_{5.9}$ $\mathbf{F}_{5.9}$ $\underline{R}_{2.9}$
P9	$S_{17.6}$ $\mathbf{G}_{14.7}$ $D_{11.8}$ $\mathbf{W}_{2.9}$	$\mathbf{P}_{8.8}$ $\mathbf{L}_{8.8}$ $E_{8.8}$ $T_{5.9}$ $\mathbf{I}_{5.9}$ $Y_{2.9}$ $\mathbf{M}_{2.9}$ $\underline{K}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{F}_{2.9}$
P8	$D_{20.6}$ $E_{14.7}$ $T_{11.8}$ $\mathbf{W}_{2.9}$	$\mathbf{G}_{8.8}$ $\mathbf{A}_{8.8}$ $\underline{R}_{5.9}$ $\mathbf{P}_{5.9}$ $Y_{2.9}$ $\mathbf{V}_{2.9}$ $N_{2.9}$ $\mathbf{M}_{2.9}$ $\mathbf{L}_{2.9}$ $\underline{K}_{2.9}$ $\mathbf{F}_{2.9}$
P7	$\mathbf{M}_{11.8}$ $D_{11.8}$ $T_{8.8}$	$\mathbf{A}_{11.8}$ $\mathbf{G}_{8.8}$ $\mathbf{V}_{5.9}$ $S_{5.9}$ $Q_{5.9}$ $N_{5.9}$ $\mathbf{I}_{5.9}$ $E_{5.9}$ $\underline{R}_{2.9}$ $\mathbf{P}_{2.9}$ $\mathbf{L}_{2.9}$ $\underline{K}_{2.9}$
P6	$E_{20.6}$ $S_{17.6}$ $\mathbf{V}_{11.8}$ $D_{8.8}$ $\mathbf{W}_{5.9}$ $\mathbf{C}_{5.9}$	$\mathbf{P}_{5.9}$ $\mathbf{G}_{5.9}$ $Y_{2.9}$ $T_{2.9}$ $\underline{R}_{2.9}$ $\mathbf{L}_{2.9}$ $\underline{K}_{2.9}$ $\mathbf{A}_{2.9}$
P5	$E_{14.7}$ $\mathbf{I}_{8.8}$ $D_{8.8}$ $\mathbf{W}_{2.9}$	$\mathbf{A}_{11.8}$ $\mathbf{I}_{8.8}$ $\mathbf{V}_{5.9}$ $\underline{R}_{5.9}$ $Q_{5.9}$ $\mathbf{G}_{5.9}$ $Y_{2.9}$ $T_{2.9}$ $S_{2.9}$ $\mathbf{P}_{2.9}$ $N_{2.9}$ $\mathbf{M}_{2.9}$ $\mathbf{F}_{2.9}$
P4	$D_{23.5}$ $\mathbf{A}_{14.7}$ $\mathbf{W}_{5.9}$ $Y_{5.9}$	$\mathbf{L}_{11.8}$ $\mathbf{V}_{8.8}$ $\underline{K}_{5.9}$ $\mathbf{F}_{5.9}$ $E_{5.9}$ $Q_{2.9}$ $N_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{C}_{2.9}$
P3	$E_{32.4}$ $\mathbf{V}_{14.7}$ $\mathbf{M}_{11.8}$ $Q_{8.8}$	$\mathbf{L}_{14.7}$ $S_{5.9}$ $T_{2.9}$ $\underline{R}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{F}_{2.9}$
P2	$\mathbf{A}_{14.7}$ $\mathbf{V}_{14.7}$ $T_{8.8}$ $H_{5.9}$	$S_{8.8}$ $\underline{R}_{8.8}$ $Q_{5.9}$ $\mathbf{P}_{5.9}$ $\underline{K}_{5.9}$ $\mathbf{I}_{5.9}$ $E_{5.9}$ $D_{5.9}$ $Y_{2.9}$
P1	D_{100}	
P1′	$S_{29.4}$ $\mathbf{G}_{14.7}$ $N_{11.8}$ $Y_{5.9}$	$\mathbf{A}_{11.8}$ $\mathbf{L}_{5.9}$ $E_{5.9}$ $\mathbf{P}_{2.9}$ $\mathbf{M}_{2.9}$ $\mathbf{F}_{2.9}$ $D_{2.9}$ $\mathbf{C}_{2.9}$
P2′	$\mathbf{P}_{20.6}$ $Q_{8.8}$	$\mathbf{V}_{8.8}$ $\mathbf{A}_{8.8}$ $T_{5.9}$ $\underline{R}_{5.9}$ $\underline{K}_{5.9}$ $\mathbf{I}_{5.9}$ $\mathbf{G}_{5.9}$ $\mathbf{F}_{5.9}$ $E_{5.9}$ $S_{2.9}$ $\mathbf{L}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{C}_{2.9}$
P3′	$\mathbf{G}_{17.6}$ $S_{14.7}$ $D_{8.8}$ $\mathbf{C}_{5.9}$	$\underline{K}_{11.8}$ $\mathbf{A}_{11.8}$ $\mathbf{V}_{8.8}$ $Q_{5.9}$ $Y_{2.9}$ $T_{2.9}$ $\underline{R}_{2.9}$ $\mathbf{M}_{2.9}$ $\mathbf{L}_{2.9}$

Table 2A. Significant Amino Acids in Experimentally Identified Caspase-1 Cleavage Sites

Position	Significant [a]	Non-significant[b]
P4′	$\underline{K}_{14.7}$ $D_{8.8}$ $\mathbf{M}_{5.9}$	$\mathbf{G}_{11.8}$ $\mathbf{V}_{8.8}$ $E_{8.8}$ $\mathbf{A}_{8.8}$ $Q_{5.9}$ $T_{2.9}$ $S_{2.9}$ $\underline{R}_{2.9}$ $N_{2.9}$ $\mathbf{L}_{2.9}$ $\mathbf{I}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{F}_{2.9}$ $\mathbf{C}_{2.9}$
P5′	$\mathbf{L}_{17.6}$ $S_{14.7}$ $\mathbf{M}_{5.9}$ $\mathbf{W}_{2.9}$	$\mathbf{P}_{11.8}$ $\underline{R}_{8.8}$ $\mathbf{A}_{8.8}$ $\mathbf{V}_{2.9}$ $T_{2.9}$ $N_{2.9}$ $\mathbf{I}_{2.9}$ $\mathbf{G}_{2.9}$ $E_{2.9}$ $D_{2.9}$ $\mathbf{C}_{2.9}$
P6′	$T_{8.8}$ $\underline{H}_{5.9}$	$E_{11.8}$ $S_{8.8}$ $\mathbf{P}_{8.8}$ $\mathbf{L}_{8.8}$ $\underline{K}_{8.8}$ $\mathbf{G}_{8.8}$ $\underline{R}_{5.9}$ $Q_{5.9}$ $\mathbf{I}_{5.9}$ $\mathbf{A}_{5.9}$ $D_{2.9}$ $\mathbf{C}_{2.9}$
P7′	$\mathbf{W}_{5.9}$	$\mathbf{A}_{11.8}$ $S_{8.8}$ $\underline{R}_{8.8}$ $\mathbf{P}_{8.8}$ $\mathbf{L}_{8.8}$ $\underline{K}_{8.8}$ $N_{5.9}$ $\mathbf{G}_{5.9}$ $Y_{2.9}$ $T_{2.9}$ $Q_{2.9}$ $\mathbf{I}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{F}_{2.9}$ $E_{2.9}$ $D_{2.9}$ $\mathbf{C}_{2.9}$
P8′	$\mathbf{A}_{17.6}$	$S_{11.8}$ $\underline{K}_{11.8}$ $R_{8.8}$ $Q_{5.9}$ $\mathbf{P}_{5.9}$ $\mathbf{L}_{5.9}$ $E_{5.9}$ $D_{5.9}$ $\mathbf{V}_{2.9}$ $T_{2.9}$ $N_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{G}_{2.9}$ $\mathbf{F}_{2.9}$ $\mathbf{C}_{2.9}$
P9′	$S_{17.6}$ $\mathbf{M}_{5.9}$	$E_{11.8}$ $\mathbf{P}_{8.8}$ $\underline{K}_{8.8}$ $\mathbf{G}_{8.8}$ $T_{5.9}$ $\mathbf{L}_{5.9}$ $\mathbf{F}_{5.9}$ $D_{5.9}$ $\mathbf{A}_{5.9}$ $\underline{R}_{2.9}$ $Q_{2.9}$ $\mathbf{I}_{2.9}$
P10′	$Q_{8.8}$	$S_{11.8}$ $\mathbf{G}_{11.8}$ $E_{11.8}$ $\underline{R}_{8.8}$ $\mathbf{A}_{8.8}$ $\mathbf{P}_{5.9}$ $\mathbf{L}_{5.9}$ $\mathbf{I}_{5.9}$ $Y_{2.9}$ $\mathbf{V}_{2.9}$ $T_{2.9}$ $\underline{K}_{2.9}$ $\underline{H}_{2.9}$ $\mathbf{F}_{2.9}$ $D_{2.9}$

Table 2B. Significant Amino Acids in Experimentally Identified Caspase-9 Cleavage Sites

Position	Significant[a]	Not Significant[b]
P10	$T_{18.2}$ $\mathbf{A}_{18.2}$ $Q_{9.1}$	$S_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $\underline{K}_{9.1}$ $\mathbf{G}_{9.1}$ $E_{9.1}$
P9	$E_{27.3}$ $D_{18.2}$ $\mathbf{V}_{18.2}$ $\mathbf{I}_{9.1}$	$\underline{R}_{9.1}$ $\mathbf{P}_{9.1}$ $\mathbf{L}_{9.1}$
P8	$\mathbf{I}_{18.2}$ $S_{18.2}$ $\mathbf{L}_{18.2}$ $N_{9.1}$ $Q_{9.1}$	$\mathbf{V}_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{P}_{9.1}$
P7	$D_{27.3}$ $\underline{H}_{18.2}$ $\mathbf{A}_{18.2}$ $\mathbf{L}_{18.2}$ $\mathbf{F}_{9.1}$	$\mathbf{G}_{9.1}$
P6	$\mathbf{P}_{18.2}$ $\mathbf{C}_{9.1}$ $N_{9.1}$ $D_{9.1}$	$\mathbf{V}_{9.1}$ $S_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $\underline{K}_{9.1}$
P5	$Q_{27.3}$ $E_{18.2}$ $\mathbf{G}_{18.2}$ $T_{9.1}$ $\mathbf{I}_{9.1}$	$S_{9.1}$ $\underline{R}_{9.1}$
P4	$\mathbf{I}_{27.3}$ $D_{18.2}$ $\mathbf{L}_{18.2}$ $\mathbf{M}_{9.1}$ $N_{9.1}$ $T_{9.1}$	$E_{9.1}$
P3	$S_{36.4}$ $D_{18.2}$ $E_{18.2}$ $N_{9.1}$ $Q_{9.1}$ $\mathbf{I}_{9.1}$	
P2	$\mathbf{V}_{36.4}$ $T_{18.2}$ $\mathbf{M}_{9.1}$ $N_{9.1}$	$\mathbf{L}_{9.1}$ $E_{9.1}$ $\mathbf{A}_{9.1}$
P1	D_{100}	
P1′	$S_{36.4}$ $\mathbf{A}_{27.3}$ $T_{9.1}$ $\mathbf{F}_{9.1}$	$\mathbf{V}_{9.1}$ $\underline{R}_{9.1}$
P2′	$\mathbf{G}_{36.4}$ $S_{18.2}$ $N_{9.1}$ $\mathbf{I}_{9.1}$	$\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $\underline{K}_{9.1}$
P3′	$\mathbf{P}_{36.4}$ $\mathbf{I}_{18.2}$ $\mathbf{F}_{9.1}$	$\mathbf{V}_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $\underline{K}_{9.1}$
P4′	$\mathbf{I}_{18.2}$ $D_{18.2}$ $S_{18.2}$	$\underline{R}_{9.1}$ $\mathbf{P}_{9.1}$ $\mathbf{L}_{9.1}$ $\mathbf{G}_{9.1}$ $\mathbf{A}_{9.1}$
P5′	$D_{27.3}$ $\underline{H}_{9.1}$ $Y_{9.1}$ $N_{9.1}$	$\mathbf{V}_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $E_{9.1}$ $\mathbf{A}_{9.1}$
P6′	$D_{36.4}$ $S_{18.2}$	$\mathbf{V}_{9.1}$ $\mathbf{L}_{9.1}$ $\underline{K}_{9.1}$ $\mathbf{G}_{9.1}$ $\mathbf{A}_{9.1}$
P7′	$T_{27.3}$ $\mathbf{I}_{18.2}$ $\mathbf{M}_{9.1}$ $N_{9.1}$	$S_{9.1}$ $\underline{R}_{9.1}$ $\mathbf{L}_{9.1}$ $E_{9.1}$
P8′	$S_{27.3}$ $\mathbf{A}_{18.2}$ $\mathbf{C}_{9.1}$ $\underline{H}_{9.1}$ $N_{9.1}$ $\mathbf{F}_{9.1}$ $D_{9.1}$	$\mathbf{P}_{9.1}$

Table 2B. Significant Amino Acids in Experimentally Identified Caspase-9 Cleavage Sites

Position	Significant[a]	Not Significant[b]
P9′	**A**$_{27.3}$ **V**$_{18.2}$ **C**$_{9.1}$ Y$_{9.1}$ T$_{9.1}$ Q$_{9.1}$	S$_{9.1}$ K$_{9.1}$
P10′	*E*$_{27.3}$ K$_{18.2}$ S$_{18.2}$ H$_{9.1}$ N$_{9.1}$	**P**$_{9.1}$ **L**$_{9.1}$

Table 2C. Comparison of Amino Acids in the Cleavage Sites of Caspase-1 substrates and Caspase-9 Substrates

	[a] Significant AA's Percentage		[b] Hydrophobic AA's Percentage		[c] Charged AA's Percentage	
Position	**Casp-1**	**Casp-9**	**Casp-1**	**Casp-9**	**Casp-1**	**Casp-9**
P10	20.6	45.5	38.3	36.4	35.2	27.3
P9	47.0	72.8	46.9	45.5	26.4	54.6
P8	50.0	72.8	38.0	54.6	44.1*	9.1*
P7	32.4*	91*	50.0	54.6	23.5	45.5
P6	70.6	45.5	41.2	45.5	35.2	27.3
P5	35.2*	81.9*	44.0	27.3	29.4	27.3
P4	50*	91*	50.0	54.6	38.2	27.3
P3	67.7*	100*	44.1*	9.1*	38.2	36.4
P2	44.1	72.8	41.2	63.7	32.4	9.1
P1	100.0	100.0	0.0	0.0	100	100
P1′	61.8	81.9	44.0	45.5	8.8	9.1
P2′	29.4*	72.8*	61.7	54.6	20.6	18.2
P3′	47.0	63.7	49.9	81.9	23.5	18.2
P4′	29.4	54.6	46.9	54.6	38.1	27.3
P5′	41.1	54.6	58.6	27.3	14.6*	54.6*
P6′	14.7*	54.6*	41.1	36.4	35.3	45.5
P7′	5.9*	63.7*	49.9	36.4	26.3	18.2
P8′	17.6*	91*	41.0	45.5	35.3	18.2
P9′	23.5*	81.9*	44.1	54.6	29.4	9.1
P10′	8.8*	81.9*	44.1	18.2	29.3	54.6

[a] Frequencies of AAs statistically higher than the background frequencies ($P<0.05$).

[b] Frequencies of AAs within 1.96×SD confidence intervals.

The amino acid features are indicated by different fonts: Acidic – italicized; Basic – underlined; Hydrophobic – bolded; Neutral - normal text

[a] Significant AA's total percentage

[b] Hydrophobic percentage

[c] Charged percentage

* and underlined fonts indicate the position has statistical difference between caspase-1 and capase-9 substrates ($P<0.05$)

Table 3

Prediction of caspase-1 cleavage sites in the experimentally identified 35 caspase-1 substrates

A. Prediction of the cleavage site of caspase-1 substrates. The predictions of caspase-1 cleavage sites in the experimentally identified caspase-1 substrates, in which the cleavage sites have not been mapped, are presented in Table 3A, which includes six columns: *(1)* substrate name; *(2)* the NCBI protein database accession number; *(3)* the cleavage site position P1 of caspase; *(4)* the numbers of the amino acid matches between the target sequences and the best cleavage site patterns; *(5)* the numbers of the amino acid matches between the target sequences and the cleavage site patterns including other amino acids in the significant section of the cleavage site patterns; and *(6)* the total numbers of the amino acid matches between the target sequences and the cleavage site patterns with (a) best cleavage site patterns, (b) other amino acids in the significant section of the cleavage site patterns, and (c) other amino acids in the insignificant section of the cleavage site patterns. The 25 cleavage sites from 20 caspase-1 substrates have been predicted with the minimal 4 amino acid matches with the amino acid residues in the significant section of the cleavage site patterns, which are presented in the upper portion of the table. The cleavage sites from three caspase-1 substrates have not been predicted with the current threshold of two amino acid matches with the amino acid residues in the significant section of the cleavage site patterns, which are presented in the lower portion of the table. **B.** Summary of predicted caspase-1 substrate cleavage site. The prediction is classified into two groups: no match and matched, based on a criterion that whether or not the numbers of the amino acid matches between the target sequences and the best cleavage site patterns are larger or equal to two. The summary presented in the Table 3B includes four columns: (1) group; (2) criterion; (3) match hit; and (4) percentage of the hits in the groups.

A. Prediction of the cleavage site of caspase-1 substrates

Substrate	NCBI/Protein Database (GI) Accession No.	Cleavage Position (P1 amino acid)	Best Sequence Matches	Other Significant Matches	Total Matches (X/20)
β-tubulin	4580988	355	2	2	15
		31	4	2	16
ARP3	5031573	172	2	4	20
F-actin α-1	5453597	114	7	2	17
		10	3	3	16
		125	2	1	16
Aldolase A	28614	141	2	3	17
TIP	136066	56	2	1	14
		36	2	0	17
		225	3	1	15
α-enolase	4503571	265	4	2	19
		91	2	1	16
		383	3	2	18
Pyruvate kinase	35505	369	3	2	14
L-Lactate Dehydrogenase	13786848	166	2	1	14
ATP synthase	32189394	365	3	1	16

A. Prediction of the cleavage site of caspase-1 substrates

Substrate	NCBI/Protein Database (GI) Accession No.	Cleavage Position (P1 amino acid)	Best Sequence Matches	Other Significant Matches	Total Matches (X/20)
Malate dehydrogenase	5174539	177	2	0	14
		314	3	1	16
		33	3	2	16
Adenylate kinase 2	7524346	132	5	0	18
Adenyl-cyclase	5453595	33	9	1	17
Catalase	179950	52	3	2	17
Peroxiredoxin 6	3318842	123	4	4	19
Carbonic anhydrase II	179780	110	4	2	16
Glyoxalase I	6573422	23	4	1	15
Rnh1	15029922	182	4	2	18
		390	2	1	14
EEF1A1	48735185	368	4	1	18
Tu elongation factor	704416	126	3	2	17
		132	3	4	19
Ribosomal protein S9	550023	26	4	0	13
		95	3	1	16
Proteosome α7 subunit	4092058	185	4	2	19
SET translocation	4506891	263	6	2	20
		150	3	2	16
		202	4	3	18
		260	4	4	18
PPPIA	190281	255	3	2	15
GRB2	4504111	33	2	0	13
		14	3	1	17
Rac2	4506381	65	2	2	16
		150	4	1	18
		124	5	0	17
Rab GDI	285975	183	4	1	19
Rho GDI beta	56676393	19	6	3	18
		182	3	2	12

A. Prediction of the cleavage site of caspase-1 substrates

Substrate	NCBI/Protein Database (GI) Accession No.	Cleavage Position (P1 amino acid)	Best Sequence Matches	Other Significant Matches	Total Matches (X/20)
Calreticulin precursor	4757900	397	7	2	20
		237	3	0	16
		362	3	0	17
Cyclophilin B	118090	183	4	1	16
		117	5	0	14
Chloride ch 1	4588526	226	2	1	18
Annexin IV	189617	284	4	2	17
		304	3	2	14
		65	2	3	17
		163	3	1	14
		176	3	3	18
PHAP1/April	1498227	182	7	2	19
		159	3	1	17
		171	4	2	20
RAB7	1174149	-	-	-	-
HSP27	662841	-	-	-	-
ER protein 29	5803013	-	-	-	-

B. Summary of predicted caspase-1 substrate cleavage site

	Number of:	Hits	Percentage
No Match	0–1	3	4.8%
Matched	2+	60	95.2%