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SYMPOSIUM: Neuronal Apoptosis

Energy Requirement for Caspase Activation and Neuronal Cell Death

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Recent work has shown that execution of the apoptotic program involves a relatively limited number of pathways. According to a general view, these would converge to activate the caspase family of proteases. However, there is increasing evidence that apoptotic-like features can be found also when cells are treated with inhibitors of caspases as the cell permeable tripeptide, Z-Val-Ala-Asp-fluoromethyl-ketone (Z-VAD-fmk), or analogous compounds. This has posed the question as to whether apoptosis may occur in a caspase independent way, and whether caspase inhibitors may then be used to treat diseases characterised by an excess apoptosis. It is also becoming clear, that ATP depletion during the early phases of apoptosis can preclude caspase activation, and consequently switch execution of cell death towards necrosis. In vivo, a block or partial inhibition of the typical apoptotic demise may have profound implications, as persistence of damaged but "undead" cells within the nervous system, followed by delayed lysis may favour neuroinflammatory reactions. In this review, we discuss some recent findings, which suggest that cells may use diverging execution pathways, with different implications in neuropathology and therapy.

Introduction

Various genetically encoded programmes involved in signalling or in the initiation of cell death may decide the fate of individual cells or organs during development. The term "programmed cell death" has therefore been used primarily to describe the co-ordinated series of events leading to controlled cell demise in developing organisms (43). Because of this early definition, the term "physiological cell death" has been linked to the notion of "programmed cell death." Nevertheless, cells execute a biochemical programme of cell death also under pathological conditions, and the consequences for the individual cell may be practically indistinguishable from those observed during developmental demise. In many circumstances, developmental cell death as well as death under pathological conditions have the morphological characteristics described by Kerr, Wyllie and Currie and termed apoptosis (18). The concept of a death programme is, however, not necessarily linked to a morphological appearance. For example, in non-vertebrate systems, programmed cell death does not always display an apoptotic-like morphology (44). This is the reason why early work in *C. elegans* never described cell death as apoptotic (57). The discovery that both developmental and non-developmental death can share similar execution systems and controlling proteins has then finally blurred the boundary between developmental cell death and cell demise in adult tissues. In particular the characterisation of the main executioners of apoptosis, the caspases has linked the physiological type of death encountered in development with that observed in pathological situations through the whole life span.

Caspases and the link to cell death.

The finding that one class of death-related genes, those expressing caspases (cysteine aspartases) (58) are involved in cell death in genetically distant organisms has directed the attention on the central role of this protease family as the main effector of apoptotic cell demise. Consequently, there is a tendency to conceptually identify apoptosis with its main execution system (37). Caspases are constitutively expressed in mammals, similar to ced-3 in C. elegans (45, 54). To date more than 14 mammalian caspases have been identified. They are expressed as inactive pro-enzymes and proteolytically activated to form active tetramers. A recent classi-

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fication, divides them in three different groups based on the tetrapeptide recognition sequence (DExDases, WEHDases, (IVL)ExDases). Caspases participate in the signalling and execution of apoptosis (34) with the exception of WEHDases, which are implicated in inflammatory processes. Substrates include cytoskeletal proteins, nuclear structural proteins and enzymes, and some controllers of caspase activation (i.e, Bcl-2) (34). However, some caspases may eventually have death-unrelated, and perhaps vital functions and their activation may not always underlie cell death. Caspase multiplicity likely reflects their diverse roles in pathophysiological conditions. For example, caspases degrade amyloid precursor protein (APP), presenilins (PS1, PS2), tau, huntingtin, atrophin-1, ataxin-3, and the androgen receptor (8, 11, 20, 52, 54). On the other hand, cleavage of relevant substrates does not necessarily imply that caspase activation has a predominant pathogenetic role in neurodegeneration (48), and definitely it does not prove that apoptosis is at the basis of neurodegenerative diseases.

It is indeed unlikely that a single execution system, even as diversified as the one involving caspases, is the sole responsible for death execution. Should this be the case, viruses and transformed cells could have easily escaped or shut-down a programme converging on a single execution pathway. Also, it is difficult to conceive how the plethora of signals causing mammalian cell death would converge on a single linear pathway. Thus, in higher organisms, additional interrelated or independent pathways may have developed to regulate death.

These considerations may help to understand why different sets of caspases are recruited in different paradigms of mammalian cell death, and also why deletion of single caspases has only localised and partial effects on cell death (21, 22). Moreover, several forms of demise seem to be caspase-independent (15, 39, 56) or even be accelerated by caspase inhibitors (49). Indeed, other protease families have been implicated in apoptosis (1, 13), while caspases can be activated without causing cell death (16). In some cases, caspase inhibition does not alter the extent of death, but rather the shape of demise (15, 25).

The degree of caspase activation may also be of importance. Caspase activation varies quantitatively from system to system and it is unclear whether a threshold has to be reached to turn caspase activation into a lethal reaction. Recent observations have also suggested that a moderate degree of caspase activation does not always reflect in cell demise (16).

The major evidence for a central role of caspases in

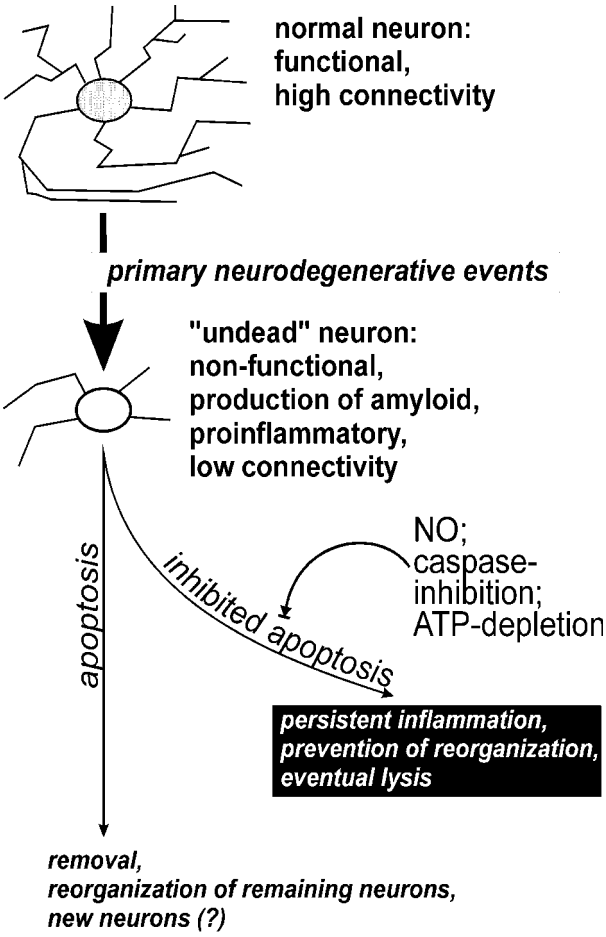

Figure 1. *Control of neuronal death by proteases and ATP.* In slow-developing neurodegenerative diseases, neuronal damage involves loss of connectivity and function, but not necessarily the loss of entire neurons at an early stage. Once a damage threshold is surpassed, neurons may die by apoptosis and quickly be removed. This would facilitate reorganisation of surrounding neurons or replacement by newly formed neurons. Inhibition of apoptosis by local mediators, energy loss or caspase inhibitors would delay removal of severely damaged neurons without reconstitution of function. In the worst case, neuroinflammatory events would be facilitated or enhanced, and further neuronal damage would ensue.

apoptosis has come from experiments where natural caspase inhibitors, or small peptide inhibitors such as Z-VAD-fmk block apoptosis. However, using the same caspase inhibitors, other studies have suggested that apoptosis can occur in a caspase-independent manner (for review see 3). Part of the problem may be due to the fact that caspase inhibitors can prevent the appearance of certain, but not all features of apoptosis in certain model systems (31). In this context, caution should be

exerted, because of possible non-specific effects of caspase inhibitors. Although generally considered specific for the caspase family, these inhibitory peptides may in fact block only some, but not all caspases. It is also clear that caspase inhibitors can also inhibit unrelated proteases, whose activity may be necessary for survival.

While the existence of alternative pathways may be conceivable, to date, the evidence for effective alternative execution systems is limited. One candidate protein is the apoptosis-inducing factor (AIF) (47). This 57 kDa protein can directly cause some of the apoptotic features. Apoptosis may also involve activation of other protease families including serine proteases, cathepsin and calpains. This raises the question as to whether every protease (i.e, even proteinase K) may indeed trigger apoptotic-like changes. This was the conclusion of an early study (55). Dysregulation of proteolysis may indeed be a general mechanism to dispose of ageing cells, where intracellular deposition of misfolded proteins is a powerful stimulus for protease activation (17). Interestingly, the concept that oxidatively-modified proteins can be degraded more effectively has been around for quite a while (26) along with the notion that oxidative stress may eliminate cells by activating proteases (35).

Energy requirement for the shape of cell death

While the occurrence of a caspase-independent apoptosis is strongly debated, it is instead widely accepted that, in some cases, caspase inhibitors only delay cell demise. Cells die eventually with morphologically different characteristics (15, 25). Evidence that cells triggered to undergo apoptosis are instead forced to die by necrosis when energy levels are rapidly compromised has been recently provided (25).

In vivo, under pathological conditions, apoptosis and necrosis may often coexist (24) and previous work in our laboratory has shown that intracellular energy levels are rapidly dissipated in necrosis, but not in apoptosis of cultured neurons (2).

To examine the events that determine the mode of execution of cell death (apoptosis or necrosis) following exposure to a single insult, individual parts of the death programme can be blocked by manipulating the intracellular ATP level. With this approach it has been possible to determine that when intracellular ATP was lowered, typical apoptotic stimuli caused instead necrosis (25). ATP could be either depleted or repleted to defined levels and for defined periods of time. Therefore, it has been possible to identify a defined period of time after the exposure of lymphoid cells to apoptogenic stimuli (staurosporine or an agonistic anti-CD95 monoclonal antibody) during which energy-dependent steps are required to complete the apoptotic program. If ATP concentrations are markedly reduced during this period, activation of downstream caspases and all typical apoptotic features are blocked. Stimulated cells die nonetheless. However, death has a necrotic appearance. These findings provide direct evidence that the complete apoptotic programme involves energy-requiring steps, one of which may be at the level of the formation of the protein complex between Apaf-1, cyt-c and procaspases (29). Lack of ATP at this step would prevent the resulting downstream degradative processes including caspase-3 activation, poly-(ADP-ribose)-polymerase cleavage and lamin cleavage, and exposure of phosphatidylserine (PS) on the outer membrane.

The implications of energy deprivation for the final outcome, apoptosis or necrosis, may be particularly relevant in the nervous system. An increased rate of apoptosis has been suggested to be a feature of several neurodegenerative diseases, although its role in the manifestation and progression of disease is still unclear. Apoptotic features are elicited in cultured neurons by β-amyloid and prion proteins, or by expressing a mutated Huntingtin protein (9, 10, 19, 40). Nevertheless, it is unclear whether the onset of the pathological manifestations *in vivo* is due to neuronal loss by apoptosis, or with functional neuronal damage. A common feature of neurodegenerative disorders is the accumulation of intracellular inclusions mostly formed by protein aggregates that are usually difficult to unfold or degrade (17). The potentially pathogenic consequence of accumulation of misfolded proteins include alterations of axonal transport, cytoskeletal damage and finally loss of connectivity with target cells. Thus, it appears reasonable that apoptosis would be triggered to dispose of these dysfunctional neurons. However, if apoptosis is blocked, for example by a concomitant defect in energy metabolism injured cells may persist and later lyse. A defect in energy metabolism may derive either from mitochondrial genetic alterations, as suggested for some neuropathological syndromes (46), or from generation of mediators in injured areas.

To address the role of ATP in neuronal apoptosis, and the possible role of signalling molecules such as nitric oxide (NO) in modulating apoptosis, we recently performed a set of experiments where cerebellar granule neurons (CGC) were depleted of ATP. Neurons were treated with the microtubule-disassembling agent, colchicine to model the cytoskeletal damage and axonal loss seen in neurodegenerative conditions (51). This

treatment induced activation of caspases and classical apoptosis. However, if ATP was depleted by mitochondrial poisons, the execution of apoptosis was blocked. Lowering of neuronal ATP could also be elicited by NO, suggesting that local production of NO can interfere with the execution of apoptosis, by impairing energy metabolism. ATP depletion prevented both the activation of caspases and the exposure of phagocytosis-recognition molecules. However, caspase inhibition did not prevent the initial cytoskeletal damage and neurite loss, whereas recognition molecules for phagocytosis, such as phosphatidylserines (PS) were not displayed on the neuronal surface. Notably, when caspase inhibitors such as Z-VAD-fmk were used to block apoptosis, neurons with a damaged cytoskeleton went on to die, with slowed-down kinetics, but still exhibiting some morphological apoptotic features (own unpublished observations). Consequently, we may speculate that *in vivo*, a partial execution of apoptosis, lacking phagocyte recognition molecules would likely result in the persistence of damaged cells within the tissue. On the other hand, there may also be situations in which pro-apoptotic signals such as CD95 stimulation can activate a caspase-dependent pro-inflammatory reaction preceding cell death and dissolving the dogma that apoptosis is always dissociated from inflammatory reactions (32)

Therapeutic implications of a branched death program

The observation that caspase inhibition hindered PS exposure, but did not prevent late lysis in colchicine-treated neurons raises the question as to whether inhibition of caspases is always desirable. Caspase inhibitors have been shown to be effective in acute liver injury (32) and in models of stroke (8, 42). The efficacy of caspase inhibitors to treat slow-progressing neurodegenerative diseases may be more problematic. The pathogenesis of Alzheimer's, Huntington's and Parkinson's disease may be independent from neuronal loss at least in early stages. For example, motor alterations are observed in transgenic mice overexpressing the mutated Huntingtin protein, prior to any major pathological evidence of death (4). Similarly, deficits in synaptic activity are accompanied by minimal loss of presynaptic or postsynaptic elements or cell death, in mice overexpressing the amyloid precursor protein (5). Notably, mice expressing exon 1 of the human huntingtin gene with an expanded CAG/polyglutamine repeat exhibit a significant decrease in striatal volume without any detectable neuronal loss or the appearance of any disease sign (14). This suggests that loss of connectivity or changes in extracellular matrix occur before cell death.

Nevertheless, during disease progression, apoptosis would be activated in affected neurons (i.e, in neurons expressing long-polyglutamine stretches for Huntington's disease) as shown in several models systems (19, 33, 38). Then, while pharmacological inhibition of caspases would prolong neuronal survival (38), it may also allow the persistence of functionally damaged neurons. Unless strategies to promote regeneration and re-establishment of connectivity are implemented concomitantly, caspase-inhibited neurons or neurons unable of completing the apoptotic execution would eventually lyse and paradoxically, promote the onset of inflammatory responses, with further progression of disease. This potential vicious loop may be interrupted more efficiently, by interfering with activation of the pro-inflammatory caspases, rather than inhibiting those involved in the execution of apoptosis. This may account, at least in part, for the protective effect of dominant-negative caspase-1 mutants in mice expressing exon 1 of the human huntingtin gene with an expanded CAG/polyglutamine repeat (36).

Clearly, caspase-based therapeutic strategies alone or in combination with other agents may be useful in stroke. Observations in stroke models suggest that apoptosis occurs mainly in the border regions (penumbra), while necrosis dominates in the more severely stressed areas of the ischemic core (6). Apoptosis of penumbral neurons may be due both to a mild direct excitotoxic-ischemic insult, but also to secondary mediators such as oxygen radicals, cytokines and lipid peroxidation products from the necrotic core (28, 30). Intervention, a few hours after the ischemic insults, is normally aimed to reduce spreading of the lesion and to inhibit delayed cell death in the border areas. If the level of injury decides the activation of different pathways for the execution of cell death, it is apparent that caspase inhibitors may be most effective in areas where the intensity of the excitotoxic insult is low, and positive feedback loops between different execution subroutines are not fully established. In the regions where the stress is more intense, inhibition of caspases alone may not prevent cell death (12). Thus, strategies that combine agents to reduce the overall intensity of the insult and the overall lesion size (i.e., N-methyl-D-aspartate (NMDA)- antagonists and other ion channel blockers or selective bNOS inhibitors), with agents that block execution of apoptosis (caspase inhibitors) has proven more successful than individual treatments (42). Finally, caspase inhibition has been recently proven to be effective in improving survival of nigral transplants in hemiparkinsonian rats and thereby improving functional recovery (41).

Conclusions

It is not surprising that initially simple death programs, developed early during phylogeny, undergo complex modifications in mammalian cells. Large gene families have evolved to provide a more intricate control of cell death in higher organisms, in part perhaps to accommodate the need of individual organ differentiation. Some characteristics of the original cell death machinery that would affect predominantly the shape of death may have become more significant or predominant in some subsets of mammalian cells. A further consequence of the increased complexity may be that an increasing number of feed-back loops gives rise to multiple possibilities of initiation, control and execution.

In our view, stimulation of self-feeding loops, which maintain both the activation of executioners and the neutralisation of anti-apoptotic defence systems, is necessary for the completion of most death programs. The main implication of this standpoint is the exclusion of a single, predominant and molecular-defined commitment step. It seems likely that accumulation of damage incompatible with cell survival would require disruption of several vital functions. Once such a threshold is trespassed, multiple positive feed-back loops would ensure the progression of the death programme to the end, and the safe disposal of the injured cell. This also implies that the morphological appearance of cell death (apoptosis or necrosis) is not linked to a single commitment point, but rather is the result of a more or less complete execution of subroutines deciding on the shape of dying cells.

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References

1. Adjei PN, Kaufmann SH, Leung W-Y, Mao F, Gores GJ (1996) Selective induction of apoptosis in Hep 3B cells by toposiomerase I inhibitors: evidence for a protease-dependent pathway that does not activate cysteine protease P32. *J Clin Invest* 98: 2588-2596
2. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P (1995) Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* 15: 961-973
3. Borner C, Monney L (1999) Apoptosis without caspases: an inefficient molecular guillotine? *Cell Death & Diff* 6:497-507
4. Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *J Neurosci* 19: 3248-3257
5. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TV, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci* 2: 271-276
6. Charriaut-Marlangue C, Aggoun-Zouaoui D, Represa A, Ben-Ari Y (1996) Apoptotic features of selective neuronal death in ischemia, epilepsy and gp120 toxicity. *Trends Neurosci* 19: 109-114
7. Ellerby LM, Andrusiak RL, Wellington CL, Hackam AS, Propp SS, Wood JD, Sharp AH, Margolis RL, Ross CA, Salvesen GS, Hayden MR, Bredesen DE (1999) Cleavage of atrophin-1 at caspase site aspartic acid 109 modulates cytotoxicity. *J Biol Chem* 274: 8730-8736
8. Fink K, Zhu J, Namura S, Shimizu-Sasamata M, Endres M, Ma J, Dalkara T, Yuan J, Moskowitz MA (1998) Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. *J Cereb Blood Flow Metab* 18: 1071-1076
9. Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, Bugiani O, Tagliavini F (1993) Neurotoxicity of a prion protein fragment. *Nature* 362: 543-546
10. Forloni G, Bugiani O, Tagliavini F, Salmona M (1996) Apoptosis-mediated neurotoxicity induced by beta-amyloid and PrP fragments. *Mol Chem Neuropathol* 28: 163-171
11. Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang J, LeBlanc A, Smith D, Rigby M, Shearman MS, Clarke EE, Zheng H, Van Der Ploeg LH, Ruffolo SC, Thornberry NA, Xanthoudakis S, Zamboni RJ, Roy S, Nicholson DW (1999) Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. *Cell* 97: 395-406
12. Green D, Kroemer G (1998) The central executioners of apoptosis: caspases or mitochondria? *Trends Cell Biol* 8: 267-271
13. Grimm LM, Goldberg AL, Poirier GG, Schwartz LM, Osborne BA (1996) Proteasomes play an essential role in thymocyte apoptosis. *EMBO J* 15: 3835-3844
14. Hansson O, Petersen A, Leist M, Nicotera P, Castilho RF, Brundin P (1999) Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. *Proc Natl Acad Sci USA* 96: 8727-8732
15. Hirsch T, Marchetti P, Susin SA, Dallaporta B, Zamzami N, Marzo I, Geuskens M, Kroemer G (1997) The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. *Oncogene* 15: 1573-1581
16. Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M (1998) Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO J* 17: 6124-6134

17. Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. *J Cell Biol* 143: 1883-1898

18. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. *Br J Cancer* 26: 239-247

19. Kim M, Lee HS, LaForet G, McIntyre C, Martin EJ, Chang P, Kim TW, Williams M, Reddy PH, Tagle D, Boyce FM, Won L, Heller A, Aronin N, DiFiglia M (1999) Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J Neurosci* 19: 964-973

20. Kobayashi Y, Miwa S, Merry DE, Kume A, Mei L, Doyu M, Sobue G (1998) Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine repeat length-dependent manner. *Biochem Biophys Res Commun* 252: 145-50

21. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1beta converting enzyme. *Science* 267: 2000-2003

22. Kuida K, Zheng TS, Na S, Kuan C-Y, Yang D, Karasuyama H, Rakic P, Flavell RA (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384: 368-372

23. Künstle G, Leist M, Uhlig S, Revesz L, Feifel R, MacKenzie A, Wendel A (1997) ICE-protease inhibitors block murine liver injury and apoptosis caused by CD95 or by TNF-α. *Immunol Lett* 55: 5-10

24. Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG, Wendel A (1995) Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. *Am J Pathol* 146: 1220-1234

25. Leist M, Single B, Castoldi AF, Kühnle S, Nicotera P (1997) Intracellular ATP concentration: a switch deciding between apoptosis and necrosis. *J Exp Med* 185: 1481-1486

26. Levine RL, Oliver CN, Fulks RM, Stadtman ER (1981) Turnover of bacterial gluatmine synthase: oxidative inactivation precedes proteolysis. *Proc Natl Acad Sci USA* 78: 2120-2125

27. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X (1997) Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479-489

28. Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. *New Engl J Med* 330: 613-622

29. Liu X, Kim CN, Yang J, Jemmerson R, Wang X (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86: 147-157

30. Mattson MP (1998) Modification of ion homeostasis by lipid peroxidation: roles in neuronal degerneation and adaptive plasticity. *TINS* 21: 53-57

31. McCarthy NJ, Whyte MKB, Gilbert CS, Evan GI (1997) Inhibition of Ced-3/ICE-related proteases does not prevent cell death induced by oncogenes, DNA damage, or the bcl-2 homologue bak. *J Cell Biol* 136: 215-227

32. Miwa K, Asano M, Horai R, Iwakura Y, Nagata S, Suda T (1998) Caspase 1-independent IL-1beta release and inflammation induced by the apoptosis inducer Fas ligand. *Nat Med* 11:1287-1292

33. Miyashita T, Matsui J, Ohtsuka Y, Mami U, Fujishima S, Okamura-Oho Y, Inoue T, Yamada M (1999) Expression of extended polyglutamine sequentially activates initiator and effector caspases. *Biochem Biophys Res Commun* 257: 724-730

34. Nicholson D, Thornberry NA, (1997) Caspases: killer proteases. *Trends Biochem* 22: 299-306

35. Nicotera P, Hartzell,P, Baldi C, Svensson S-A, Bellomo G, Orrenius S (1986) Cystamine induces toxicity in hepatocytes through the elevation of cytosolic Ca^{2+} and the stimulation of a nonlysosomal proteolytic system. *J Biol Chem* 261: 14628-14635

36. Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, Chung WM, Frey AS, Menon AS, Li XJ, Stieg PE, Yuan J, Penney JB, Young AB, Cha JH, Friedlander RM (1999) Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 399: 263-267

37. Samali A, Zhivotovsky B, Jones D, Nagata S, Orrenius S (1999) Apoptosis: Cell death defined by caspase activation. *Cell Death & Diff* 6: 495-496

38. Sanchez I, Xu CJ, Juo P, Kakizaka A, Blenis J, Yuan J (1999) Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 22: 623-633

39. Sarin A, Williams MS, Alexander-Miller MA, Berzofsky JA, Zacharchuk CM, Henkart PA (1997) Target cell lysis by CTL granule exocytosis is independent of ICE/Ced-3 family proteases. *Immunity* 6: 209-215

40. Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95: 55-66

41. Schierle GS, Hansson O, Leist M, Nicotera P, Widner H, Brundin P (1999) Caspase inhibition reduces apoptosis and increases survival of nigral transplants. *Nat Med* 5: 97-100

42. Schulz JB, Weller M, Moskowitz MA (1999) Caspases as treatment targets in stroke and neurodegenerative diseases. *Ann Neurol* 45: 421-429

43. Schwartz LM, Osborne BA (1993) Programmed cell death, apoptosis and killer genes. *Immunol Today* 14: 582-590

44. Schwartz LM, Smith SW, Jones MEE, Osborne BA (1993) Do all programmed cell deaths occur via apoptosi? *Proc Natl Acad Sci USA* 90: 980-984

45. Shaham S, Horvitz HR (1996) Developing caenorhabditis elegans neurons may contain both cell-death protective and killer activities. *Genes & Develop* 10: 578-591

46. Shoubridge EA (1998) Mitochondrial encephalomyopathies. *Curr Opin Neurol* 11: 491-496

47. Susin SA, Zamzami N, Castedo M, Daugas E, Wang H-G, Geley S, Fassy F, Reed JC, Kroemer G (1997) The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. *J Exp Med* 186: 25-37

48. Van de Craen M, de Jonghe C, van den Brande I, Declercq W, van Gassen G, van Criekinge W, Vanderhoeven I, Fiers W, van Broeckhoven C, Hendriks L, Vandenabeele P (1999) Identification of caspases that cleave presenilin-1 and presenilin-2. Five presenilin-1 (PS1) mutations do not alter the sensitivity of PS1 to caspases. *FEBS Lett* 445: 149-54

49. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, Grooten J, Fiers W, Vandenabeele P (1998) Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J Exp Med* 187: 1477-1485

50. Villa P, Kauffmann SH, Earnshaw WC (1997) Caspases and caspase inhibitors. *Trends Biochem Sci* 22: 388-393

51. Volbracht C, Leist M, Nicotera P (1999) ATP controls neuronal apoptosis triggered by microtubule breakdown or potassium depravation. *Mol Med* 5: 477-489

52. Weidemann A, Paliga K, Dürrwang U, Reinhard FB, Schuckert O, Evin G, Masters CL (1999) Proteolytic processing of the Alzheimer's disease amyloid precursor protein within its cytoplasmic domain by caspase-like proteases. *J Biol Chem* 274: 5823-5829

53. Weil M, Jacobson MD, Coles HSR, Davies TJ, Gardner RL, Raff KD, Raff MC (1996) Constitutive expression of the machinery for programmed cell death. *J Cell Biol* 133: 1053-1059

54. Wellington CL, Ellerby LM, Hackam AS, Margolis RL, Trifiro MA, Singaraja R, McCutcheon K, Salvesen GS, Propp SS, Bromm M, Rowland KJ, Zhang T, Rasper D, Roy S, Thornberry N, Pinsky L, Kakizuka A, Ross CA, Nicholson DW, Bredesen DE, Hayden MR (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J Biol Chem* 273: 9158-9167

55. Williams MS, Henkart PA (1994) Apoptotic cell death induced by intracellular proteolysis. *J Immunol* 153: 4247-4255

56. Xiang J, Chao DT, Korsmeyer SJ (1997) BAX-induced cell death may not require interleukin 1 beta-converting enzyme-like proteases. *Proc Natl Acad Sci USA* 93: 14559-14563

57. Yuan J, Horvitz HR (1990) The caenorhabditis elegans genes ced-3 and ced-4 act cell autonomously to cause programmed cell death. *Develop Biol* 138: 33-41

58. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1beta-converting enzyme. *Cell* 75: 641-652