Role of caspases and non-caspase proteases in cell death

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Abstract

Undoubtedly, caspases are the major driving force for apoptosis execution and mechanisms of their activation and inhibition have been largely unveiled. Recent progress has been made with regard to the exact intracellular ordering of caspases, monitoring their activities *in vivo* and unveiling their substrate degradomes. Moreover, non-caspase proteases seem to assist caspases in the completion of the death execution program. Here we will consider some very recent data dealing with these aspects. We will also provide novel insights into the mechanisms that dictate apoptotic variability within a cell population.

Introduction and context

Caspases coordinate, in a proteolytic cascade and with the help of other proteases, the rapid and efficient elimination of a cell by apoptosis. They are classified into monomeric initiator caspases (caspase-8 and -9) with long prodomains (death effector domain [DED], and caspase recruitment domain [CARD]), which are activated by recruitment to and dimerization on protein platforms, and already dimerized effector caspases (caspase-3, -6 and -7), which require for their activation the cleavage (e.g., by initiator caspases) of an intersubunit linker that binds to the dimer interface [1].

In the extrinsic signaling pathway, the recruitment platform is the death-inducing signaling complex (DISC) consisting of the activated death receptor, the adapter FADD (Fas-associated protein with death domain) and initiator caspase-8 and -10. In the intrinsic mitochondrial pathway, the platform is the apoptosome, a complex of the adapter Apaf-1 (apoptotic protease activating factor 1), mitochondrially-released cytochrome c and initiator caspase-9 [1]. In both cases, the main role of caspase-8 and -9 is to cleave and activate effector caspase-3 and -7. Caspase-6 is an effector caspase

with a limited substrate specificity whose exact role in apoptosis remains ill-defined [2]. Caspase-2 is recruited to another platform, the PIDDosome (PIDD is the p53-induced protein with death domain), which is involved in translating a danger signal, such as DNA damage, into either a repair or death response depending on the cellular or environmental context [3]. Similarly, caspases-1, -4 and -5 in humans and caspase-11 in mice are part of inflammasome platforms that assemble in response to external, pathogenic stimuli and guide the production and secretion of pro-inflammatory cytokines such as interleukin (IL)-1 and IL-18 [4]. Under certain circumstances, such as salmonella-infected macrophages, the antimicrobial, inflammatory response may result in a caspase-1-mediated form of programmed cell death, called pyroptosis [5]. Caspase-12 is an inhibitor of caspase-1 [4], and caspase-14 is probably the only truly non-apoptotic human caspase mediating keratinocyte differentiation [1].

In the caspase field, three major issues are still under scrutiny: why are three effector caspases needed? Are non-caspase proteases important to support or even replace caspase-mediated signaling? And why is effector

caspase activation and apoptosis not synchronized, even in a clonal cell population?

Major recent advances

Redundancy, substrate specificity and amplification loops in caspase signaling

Two recent papers reported on the caspase substrate repertoire in apoptotic cells [6,7]. In one case, the proteomes from control and apoptosis-stimulated systems were separated on one-dimensional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) after gel elution [6]. In the other approach a gel-free technology was used by selectively biotinylating free protein amino termini that are generated in apoptotic cells upon caspase-mediated cleavages to enrich for LC-MS/MS [7]. Both groups identified hundreds of new substrates and support the concept that most of them are cleaved into domain-containing fragments that may either gain or lose function. The cleaved substrates are often stable, suggesting that dismantling the apoptotic cell is more like folding a tent after careful removal of pegs than disposing debris after an explosion. Walsh *et al.* [8] tested some of these substrates for caspase-3 or -7 specificity. They found that caspase-3 was more promiscuous than caspase-7 because 12 out of 20 substrates were preferentially cleaved by caspase-3 whereas only one was more susceptible to processing by caspase-7. Thus, caspase-3 and -7 may, in part, be functionally distinct, explaining why caspase-3 and caspase-7 null mice show distinct phenotypes on some genetic backgrounds [9]. In this regard, a recent study published for the first time a non-redundant role of caspase-7 in mediating lipopolysaccharide-induced lymphocyte apoptosis and mortality in mice [10]. This might be due to the fact that caspase-7, unlike caspase-3, can be processed and activated by caspase-1 [11]. Thus, under certain conditions caspase-7 may be crucial for inflammatory events triggered by the caspase-1 inflammasome.

Caspase-3 can also process other caspases, such as caspase-6 and -2. In turn, caspase-6 processes caspase-8 and -10 [2,12]. Using a specific inhibitor of caspase-3, and small interfering RNA-mediated ablation of specific caspases, Inoue *et al.* [13] recently validated this caspase ordering pathway not only for caspase-3 but also for caspase-7. This positions caspase-6 in an important amplification loop downstream of caspase-3 and -7. The role of caspase-2 in this loop still remains to be determined. Caspase-2 was previously suggested to function as an initiator caspase for DNA damage and heat-shock-induced apoptosis upstream of mitochondria. Meanwhile, this has been largely questioned [3]. It seems that caspase-2 may amplify apoptotic responses

by being activated downstream of caspase-9/-3 [14] or, alternatively, to drive a DNA damage response that bypasses p53, B-cell lymphoma protein-2 (Bcl-2) and caspase-3 [15]. Moreover, a non-apoptotic role of caspase-2 was proposed in the context of DNA damage-associated cell cycle arrest through the recruitment and activation on a cytosolic and a nuclear PIDDosome platform [3,16] because caspase-2 deficient cells failed to show G2/M arrest, leading to more mitotic cells whose DNA was not repaired. In accordance with such a mechanism, Ho *et al.* [17] recently suggested a tumor-suppressive role of caspase-2 by controlling cell cycle checkpoints.

Monitoring caspases in vivo and ways to activate them therapeutically

Since the number of therapies associated with increased or decreased apoptosis continue to grow, it is crucial to directly visualize and quantify apoptosis *in vivo*. Edgington *et al.* [18] developed fluorescently-labeled activity-based probes (ABPs) against caspases that provided direct readouts of kinetics of apoptosis in thymi and tumors in live mice. In contrast to the already-used polymer-based quenched fluorescent substrates that are cleaved in multiple locations to produce fluorescent products, the ABPs are retained at the site of proteolysis by forming a covalent bond with the active-site cysteine of caspases [19]. Thus, the fluorescent ABPs show more rapid and selective uptake into tumors and brighter signals compared to substrate probes.

Human diseases characterized by high apoptosis resistance, such as cancer, may be treated in the future with small-molecule drugs that can activate effector caspase proenzymes. Wolan *et al.* [20] used high-throughput screening to identify a compound that promoted selective autoproteolytic activation of pro-caspase-3 at physiological concentrations by stabilizing the on-state conformation of the proenzyme. Moreover, Walters *et al.* [21] recently reported on a caspase-3 mutation that prevented the intersubunit linker from binding in the dimer interface, allowing the active site to form in the procaspase in the absence of proteolysis. This pseudo-activated caspase-3 was not inhibited by XIAP (X-linked inhibitor of apoptosis protein) and rapidly killed cells at low concentrations.

With a little help from your friends: non-caspase proteases assisting in cell death

In mammalian cells, cell death often continues when caspases are inhibited or deleted. This form of cell death seems to be mediated by other proteases, such as calpains, serine proteases or lysosomal proteases, which act in parallel with caspases to amplify apoptosis

signaling or induce other forms of cell death such as necrosis [22].

Calpains mainly play a pro-death role when calcium signaling is involved. In 2005, Nicotera's group showed that the plasma membrane $\text{Na}^+/\text{Ca}^{2+}$ exchanger is cleaved by calpain in the ischemic brain and in cerebellar granule neurons exposed to glutamate leading to a Ca^{2+} -mediated neuronal demise [23]. Moreover, calpains were recently shown to redistribute to the nuclear envelope, where they degraded nuclear pore complexes and increased nuclear leakiness in response to a Ca^{2+} overload [24]. Furthermore, calpain-1 was found to be activated in spontaneous and Fas-mediated apoptosis of neutrophils by a caspase-induced cleavage of its inhibitor calpastatin. This resulted in the truncation of pro-apoptotic Bax (Bcl-2-associated X protein) [25] and the autophagy-related 5 (Atg5) protein [26], which both enhanced cytochrome c release and most likely deviated the cells from autophagy to apoptosis.

Masson and Tschopp [27] identified a family of serine proteases, the granzymes (Gzms), present in cytotoxic T cells and natural killer cells, which were capable of eliminating virally infected and malignant cells in cooperation with the FasL-Fas signaling system. Meanwhile, it has become clear that GzmB mediates caspase-dependent apoptosis by either cleaving Bid (BH3-interacting domain death agonist) and activating Bax/Bak-dependent cytochrome c release or directly processing caspase-3 [28]. By contrast, Metkar *et al.* [29] recently reported that GzmA contributes to anti-viral immunity, not via its pro-apoptotic action, but by triggering the processing of pro-IL-1 β to mature IL-1 β , thereby stimulating a pro-inflammatory response. Thus, Gzms resemble caspases in their capacity to influence both apoptosis and inflammation depending on the isoform. In this respect, the orphan human GzmM and GzmK may similarly act as pro-inflammatory proteases, explaining the observed ongoing anti-viral defence in *gzmAxB-/-* mice [30,31]. Moreover, it is conceivable that other cell types may also exploit a serine protease-based mechanism to regulate apoptosis. A few candidate serine proteases have so far been proposed, such as HtrA2 (high-temperature-regulated A2), AP24 (24 kDa apoptotic protease) and serine proteases implicated in endoplasmic reticulum stress-induced apoptosis [22].

Cathepsins encompass three classes of lysosomal proteases, the serine proteases cathepsin A and cathepsin G, the aspartic proteases cathepsin D and cathepsin E and the 11 so far known human cysteine proteases, cathepsins B, C, F, H, K, L, O, S, V, W and X/Z [22]. They have been implicated in apoptosis regulation because of their

cytoplasmic release due to increased lysosomal membrane permeabilization (LMP) [32]. Although they depend on acidic pH for optimal activity, they seem to retain some of their catalytic activity at physiological pH. Therefore, upon their release into the cytoplasm they can directly cleave caspases [22] or, as recently reported, pro- and anti-apoptotic members of the Bcl-2 family or XIAP [33]. Unfortunately, in most studies, LMP was artificially induced by lysosomotropic agents such as Leu-Leu-OMe (L-leucyl-L-leucine methyl ester), and it has therefore remained unclear if cathepsin release indeed plays an initiating role in apoptosis signaling. Now, Oberle *et al.* [34] report that cells deficient in Bax/Bak, apoptosomal components or caspase-3/-7 are resistant to LMP in response to various apoptotic stimuli, indicating that cathepsin release and action occurs downstream of mitochondria. Moreover, since some cathepsin knock-out cells (B, L but not D) exhibited delayed but not blocked apoptosis, this lysosomal signaling pathway seems to amplify rather than initiate apoptosis. This process could nevertheless be physiologically relevant because a lack of this amplification loop would lead to delayed cell death and/or inefficient removal of apoptotic bodies and may be the underlying cause of a lack of resolution of inflammation due to neutrophil accumulation in cathepsin D knock-outs [35], reduced keratinocyte apoptosis resulting in periodic hair loss in cathepsin L-deficient mice [36,37] and diminished liver damage upon tumor necrosis factor (TNF)-alpha challenge in mice deficient of cathepsin B [38]. More recently, Kirkegaard *et al.* [39] offered a possibility to stabilize lysosomes by delivering Hsp70 (heat-shock protein 70) via the endocytotic pathway. Through its interaction with an endolysosomal phospholipid, Hsp70 facilitates the activity of acid spingomyelinase and thereby stabilizes the lysosomal membrane. This could be exploited to correct Niemann-Pick disease-associated pathology (in which acid spingomyelinase is defective). Conversely, as tumor cells express high levels of lysosomal Hsp70, a reduction of this protein may sensitize these cells to LMP and cathepsin-mediated cell death.

Together with you I die: the molecular basis for synchronous dying

It has remained enigmatic why cells, even in clonal populations, undergo apoptosis at different rates despite simultaneous addition of an apoptotic stimulus. Although caspase-3 activation occurs within minutes after cytochrome c release, the duration of events preceding cytochrome c release can vary from 1 to 24 hours. A number of factors could contribute to this population variability, including differences in cell cycle stages, metabolic rates, the local environment of single cells or

stochasticity in biochemical reactions triggered by apoptotic stimuli. Now, two recent papers reveal that the cell-cell variability of apoptosis times is due to the divergence of the molecular composition of the cells. Using a novel single fluorophore reporter to measure caspase-3/-7 activities inside cells, Bhola *et al.* [40] report that the onset of proteolytic activity is tightly synchronized amongst the most related sister cells and lost over successive generations. Similarly, Spencer *et al.* [41] found that variability in the apoptotic phenotype arises from cell-to-cell differences in protein levels of caspases, caspase inhibitors, Bcl-2 family members, and so on, that exist before TRAIL (TNF-related apoptosis-inducing ligand) exposure. A particular protein state of a single cell is then transmitted to the daughter cells, giving rise to transient non-genetic heritability in cell fate. Due to ongoing protein synthesis, the protein content of sister cells rapidly diverges so that soon these cells are no more similar to each other than pairs of cells chosen at random.

Future directions

Although we have learned much about the regulation of mammalian caspases, their ordering within signaling cascades and their specific roles in particular cell death regimens *in vitro* and *in vivo*, we do not know yet the full scope of action of the two effector caspases-7 and -6. Is caspase-7 mainly a downstream target of caspase-1 involved in inflammation? Is caspase-6 only an amplifier caspase or does it play a discrete role under particular stress responses? What about the large number of caspase substrates that have recently been identified by mass spectrometry [6,7]? Can they be validated *in vivo* (i.e., at physiologically relevant caspase concentrations of 1 nM in dying cells) and which of them, if any, exert a functional significance in mediating the execution of apoptosis? Another major task will be to determine if caspase-2 is only a checkpoint controller, or also an apoptotic caspase at specific cell cycle stages, for example, leading to mitotic catastrophe, a form of apoptosis that may depend on the molecular profile of the cell [42]. In this respect it will be exciting to continue the recent work on determining how the protein content of apoptosis signaling molecules sets the threshold for death induction in single cells, preferentially supported by systems biology approaches as shown by Spencer *et al.* [41]. This will have major implications for future anti-cancer therapies as resistance to chemotherapy is most likely due to such cell-to-cell variability within a given tumor cell population.

Another crucial aspect will certainly be the better detection of apoptotic cells in diseased tissues using specific fluorescent probes and the development of more drugs that activate the caspase-3 proenzyme. In

particular, a drug mimicking the caspase-3 mutant described by Walters *et al.* [21] holds great promise to activate caspase-3 without proteolytic processing and eventually to kill even tumor cells expressing high XIAP levels.

Finally, we desperately need to understand the exact role of non-caspase proteases in cell death regulation. The implication of calpains, serine proteases, cathepsins and other proteases in this process seems to be a relatively recent evolutionary response to the complexity of biological processes in humans. Thus, these enzymes will be promising targets for future therapeutic intervention – for example, to save neurons or immune cells from degeneration – irrespective of whether these proteases amplify caspase-dependent signaling or trigger alternative caspase-independent processes. Our future work will include the identification and molecular characterization of more apoptotic and pro-inflammatory serine proteases and the discovery of the signaling pathway leading from caspases to LMP and cathepsin release from lysosomes.

Abbreviations

ABP, activity-based probe; Bak, B-cell lymphoma protein-2-antagonist/killer; Bax, B-cell lymphoma protein-2-associated X protein; Bcl-2, B-cell lymphoma protein-2; Fas, F7-associated surface protein; FasL, Fas ligand; Gzm, granzyme; Hsp, heat-shock protein; IL, interleukin; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LMP, lysosomal membrane permeabilization; TNF, tumor necrosis factor; XIAP, X-linked inhibitor of apoptosis protein.

Competing interests

The authors declare that they have no competing interests.

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References

1. Pop C, Salvesen GS: **Human caspases: activation, specificity, and regulation.** *J Biol Chem* **284**:21777-81.
2. Cullen SP, Martin SJ: **Caspase activation pathways: some recent progress.** *Cell Death Differ* 2009, **16**:935-8.

3. Krumschnabel G, Sohm B, Bock F, Manzl C, Villunger A: **The enigma of caspase-2: the laymen's view.** *Cell Death Differ* 2009, **16**:195-207.
 4. Bryant C, Fitzgerald KA: **Molecular mechanisms involved in inflammasome activation.** *Trends Cell Biol* 2009, **19**:455-64.
 5. Fernandes-Alnemri T, Wu J, Yu JW, Datta P, Miller B, Jankowski W, Rosenberg S, Zhang J, Alnemri ES: **The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation.** *Cell Death Differ* 2007, **14**:1590-604.
 6. Dix MM, Simon GM, Cravatt BF: **Global mapping of the topography and magnitude of proteolytic events in apoptosis.** *Cell* 2008, **134**:679-91.
- F1000 Factor 6.0 *Must Read*
Evaluated by Matthew Bogoy 20 Oct 2008
7. Mahrus S, Trinidad JC, Barkan DT, Sali A, Burlingame AL, Wells JA: **Global sequencing of proteolytic cleavage sites in apoptosis by specific labeling of protein N termini.** *Cell* 2008, **134**:1-11.
 8. Walsh JG, Cullen SP, Sheridan C, Lüthi AU, Gerner C, Martin SJ: **Executioner caspase-3 and caspase-7 are functionally distinct proteases.** *Proc Natl Acad Sci U S A* 2008, **105**:12815-9.
 9. Lakhani SA, Masud A, Kuida K, Porter GA Jr, Booth CJ, Mehal WZ, Inayat I, Flavell RA: **Caspases 3 and 7: key mediators of mitochondrial events of apoptosis.** *Science* 2006, **311**:847-51.
- F1000 Factor 4.8 *Must Read*
Evaluated by John Abrams 22 May 2006, Robert Sapolsky 03 Jul 2006
10. Lamkanfi M, Moreira LO, Makena P, Spierings DCJ, Boyd K, Murray PJ, Green DR, Kanneganti TD: **Caspase-7 deficiency protects from endotoxin-induced lymphocyte apoptosis and improves survival.** *Blood* 2009, **113**:2742-5.
 11. Lamkanfi M, Kanneganti TD, Van Damme P, Vanden Berghe T, Vanoverberghe I, Vandekerckhove J, Vandenebeele P, Gevaert K, Nunez G: **Targeted peptide-centric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes.** *Mol Cell Proteomics* 2008, **7**:2350-63.
 12. Cowling V, Downward J: **Caspase-6 is the direct activator of caspase-8 in the cytochrome c-induced apoptosis pathway: absolute requirement for removal of caspase-6 prodomain.** *Cell Death Differ* 2002, **9**:1046-56.
 13. Inoue S, Browne G, Melino G, Cohen GM: **Ordering of caspases in cells undergoing apoptosis by the intrinsic pathway.** *Cell Death Differ* 2009, **16**:1053-61.
- F1000 Factor 6.0 *Must Read*
Evaluated by Christoph Borner 06 Jul 2009
14. O'Reilly LA, Ekert P, Harvey N, Marsden V, Cullen L, Vaux DL, Hacker G, Magnusson C, Pakusch M, Cecconi F, Kuida K, Strasser A, Huang DC, Kumar S: **Caspase-2 is not required for thymocyte or neuronal apoptosis even though cleavage of caspase-2 is dependent on both Apaf-1 and caspase-9.** *Cell Death Differ* 2002, **9**:832-41.
 15. Sidi S, Sanda T, Kennedy RD, Hagen AT, Jette CA, Hoffmans R, Pascual J, Imamura S, Kishi S, Amatruda JF, Kanki JP, Green DR, D'Andrea AA, Look AT: **Chk1 suppresses a caspase-2 apoptotic response to DNA damage that bypasses p53, Bcl-2, and caspase-3.** *Cell* 2008, **133**:864-77.
- F1000 Factor 6.6 *Must Read*
Evaluated by John Abrams 03 Jun 2008, Sharad Kumar 06 Jun 2008, Matthew O'Connell 11 Jun 2008, Surender Kharbanda 16 Jun 2008
16. Shi M, Vivian CJ, Lee KJ, Ge C, Morotomi-Yano K, Manzl C, Bock F, Sato S, Tomomori-Sato C, Zhu R, Haug JS, Swanson SK, Washburn MP, Chen DJ, Chen BP, Villunger A, Florens L, Du C: **DNA-PKcs-PIDDosome: a nuclear caspase-2-activating complex with role in G2/M checkpoint maintenance.** *Cell* 2009, **136**:508-20.
- F1000 Factor 4.9 *Must Read*
Evaluated by Sharad Kumar 26 Feb 2009, Christoph Borner 17 Mar 2009, Hao Wu 06 Apr 2009
17. Ho LH, Taylor R, Dorstyn L, Cakouros D, Bouillet P, Kumar S: **A tumor suppressor function for caspase-2.** *Proc Natl Acad Sci U S A* 2009, **106**:5336-41.
 18. Edgington LE, Berger AB, Blum A, Albrow VE, Paulick MG, Lineberry N, Bogoy M: **Noninvasive optical imaging of apoptosis by caspase-targeted activity-based probes.** *Nat Med* 2009, **15**:967-73.
- F1000 Factor 3.0 *Recommended*
Evaluated by Benjamin Cravatt 10 Sep 2009
19. Blum G, Weimer RM, Edgington LE, Adams W, Bogoy M: **Comparative assessment of substrates and activity based probes as tools for non-invasive optical imaging of cysteine protease activity.** *PLoS ONE* 2009, **4**:e6374.
 20. Wolan DW, Zorn JA, Gray DC, Wells JA: **Small-molecule activators of a proenzyme.** *Nature* 2009, **326**:853-8.
- F1000 Factor 9.0 *Exceptional*
Evaluated by Kristiina Vuori 20 Nov 2009
21. Walters J, Pop C, Scott FL, Drag M, Swartz P, Mattos C, Salvesen GS, Clark AC: **A constitutively active and uninhibitable caspase-3 zymogen efficiently induces apoptosis.** *Biochem J* 2009, **424**:335-45.
 22. Schrader K, Huai J, Jöckel L, Oberle C, Borner C: **Non-caspase proteases: triggers or amplifiers of apoptosis?** *Cell Mol Life Sci* 2010, **67**:1607-18.
 23. Bano D, Young KW, Guerin CJ, Lefevre R, Rothwell NJ, Naldini L, Rizzuto R, Carafoli E, Nicotera P: **Cleavage of the plasma membrane Na⁺/Ca²⁺ exchanger in excitotoxicity.** *Cell* 2005, **120**:275-85.
- F1000 Factor 8.2 *Exceptional*
Evaluated by Robert Sapolsky 23 Mar 2005, Anna Huttenlocher 06 Apr 2005, Christoph Borner 18 Apr 2005
24. Bano D, Dinsdale D, Cabrera-Socorro A, Maida S, Lambacher N, McColl B, Ferrando-May E, Hengartner MO, Nicotera P: **Alteration of the nuclear pore complex in Ca²⁺-mediated cell death.** *Cell Death Differ* 2010, **17**:119-33.
 25. Altnauer F, Conus S, Cavalli A, Folkers G, Simon HU: **Calpain-I regulates Bax and subsequent Smac-dependent caspase-3 activation in neutrophil apoptosis.** *J Biol Chem* 2004, **279**:5947-57.
 26. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, Brunner T, Simon HU: **Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis.** *Nat Cell Biol* 2006, **8**:1124-32.
- F1000 Factor 3.0 *Recommended*
Evaluated by Valina Dawson 30 Oct 2006
27. Masson D, Tschopp J: **A family of serine esterases in lytic granules of cytolytic T lymphocytes.** *Cell* 1987, **49**:679-85.
 28. Chowdhury D, Lieberman J: **Death by a thousand cuts: granzyme pathways of programmed cell death.** *Annu Rev Immunol* 2008, **26**:389-420.
 29. Metkar SS, Menaa C, Pardo J, Wang B, Wallich R, Freudenberg M, Kim S, Raja SM, Shi L, Simon MM, Froelich CJ: **Human and mouse granzyme A induce a pro-inflammatory cytokine response.** *Immunity* 2008, **29**:720-33.
 30. Jenkins MR, Trapani JA, Doherty PC, Turner SJ: **Granzyme K expressing cytotoxic T lymphocytes protects against influenza virus in granzyme AB-/- mice.** *Viral Immunol* 2009, **21**:341-6.
 31. Regner M, Pavlinovic L, Koskinen A, Young N, Trapani JA, Müllbacher A: **Cutting edge: rapid and efficient in vivo**

cytotoxicity by cytotoxic T cells is independent of granzymes A and B. *J Immunol* 2009, **183**:37-40.

32. Conus S, Simon HU: **Cathepsins: key modulators of cell death and inflammatory responses.** *Biochem Pharmacol* 2008, **76**:1374-82.
33. Droga-Mazovec G, Bojić L, Petelin A, Ivanova S, Romih R, Repnik U, Salvesen GS, Stoka V, Turk V, Turk B: **Cysteine cathepsins trigger caspase-dependent cell death through cleavage of Bid and anti-apoptotic Bcl-2 homologues.** *J Biol Chem* 2008, **283**:19140-5.
34. Oberle C, Huai J, Reinheckel T, Tacke M, Rassner M, Ekert PG, Buellesbach J, Borner C: **Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocytes.** *Cell Death Differ* 2010, [Epub ahead of print].
35. Conus S, Perozzo R, Reinheckel T, Peters C, Scapozza L, Yousefi S, Simon HU: **Caspase-8 is activated by cathepsin D initiating neutrophil apoptosis during the resolution of inflammation.** *J Exp Med* 2008, **205**:685-98.

F1000 Factor 6.0 *Must Read*

Evaluated by Philipp M Lepper 07 May 2008

36. Tobin DJ, Foitzik K, Reinheckel T, Mecklenburg L, Botchkarev VA, Peters C, Paus R: **The lysosomal protease cathepsin L is an important regulator of keratinocyte and melanocyte differentiation during hair follicle morphogenesis and cycling.** *Am J Pathol* 2002, **160**:1807-21.
37. Reinheckel T, Hagemann S, Dollwet-Mack S, Martinez E, Lohmüller T, Zlatkovic G, Tobin DJ, Maas-Szabowski N, Peters C: **The lysosomal**

cysteine protease cathepsin L regulates keratinocyte proliferation by control of growth factor recycling. *J Cell Sci* 2005, **118**:3387-95.

38. Guicciardi ME, Deussing J, Miyoshi H, Bronk SF, Svingen PA, Peters C, Kaufmann SH, Gores GJ: **Cathepsin B contributes to TNF-alpha-mediated hepatocyte apoptosis by promoting mitochondrial release of cytochrome c.** *J Clin Invest* 2000, **106**:1127-37.
39. Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, Zylicz A, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jäättelä M: **Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology.** *Nature* 2010, **463**:549-53.

F1000 Factor 6.0 *Must Read*

Evaluated by Robert Abraham 02 Feb 2010

40. Bhola PD, Simon SM: **Determinism and divergence of apoptosis susceptibility in mammalian cells.** *J Cell Sci* 2009, **122**:4296-302.
41. Spencer SL, Gaudet S, Albeck JG, Burke JM, Sorger PK: **Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis.** *Nature* 2009, **459**:428-32.

F1000 Factor 9.8 *Exceptional*

Evaluated by David Fisher 08 May 2009, Yutaka Tagaya 15 Jun 2009, John Lazo 12 Mar 2010

42. Vakifahmetoglu H, Olsson M, Zhivotovsky B: **Death through a tragedy: mitotic catastrophe.** *Cell Death Differ* 2008, **15**:1153-62.