Title: Autophagic cell death exists.

Authors: Clarke PG, Puyal J

Journal: Autophagy

Year: 2012 Jun

Volume: 8

Issue: 6

Pages: 867-9

DOI: 10.4161/auto.20380

Autophagic cell death exists.

By Peter G.H. Clarke[1] and Julien Puyal[1]

[1] Département de Biologie Cellulaire et de Morphologie, University of Lausanne, Switzerland.

***Corresponding author:**

Peter G.H. Clarke

Département de Biologie Cellulaire et de Morphologie (DBCM),

University of Lausanne,

Rue du Bugnon 9,

CH-1005 Lausanne,

Switzerland.

Tel: +41 21 692 5120

Fax: +41 21 692 5105

e-mail: peter.clarke@unil.ch

Key words: Apoptosis, autophagy, autophagic cell death, cell death, necrosis

Abstract

The term autophagic cell death (ACD) initially referred to cell death with greatly enhanced autophagy, but is increasingly used to imply a death-mediating role of the autophagy, as shown by a protective effect of autophagy inhibition. In addition, many authors require that autophagic cell death must not involve apoptosis or necrosis. Adopting these new and restrictive criteria, and emphasizing their own failure to protect human osteosarcoma cells by autophagy inhibition, the authors of a recent Editor's Corner article in this journal argue for the extreme rarety or nonexistence of autophagic cell death. We here maintain that, even with the more stringent recent criteria, autophagic cell death exists in several situations, some of which were ignored by the Editor's Corner authors. We reject their additional criterion that the autophagy in ACD must be the agent of ultimate cell dismantlement. And we argue that rapidly dividing mammalian cells such as cancer cells are not the most likely situation for finding pure ACD.

In their Editor's Corner article "The end of autophagic cell death?"[1], S. Shen, Kepp and Kroemer deplore the fact that 486 entries in Medline, almost 7% of articles on the subject of autophagy, refer to *autophagic cell death* or *autophagic death* (henceforth ACD). In the light of their own group's recent failure to find ACD in human osteosarcoma cells despite the testing of as many as 1'400 anti-cancer agents[2], the Editor's Corner article launches a trenchant critique of ACD, and argues that it may not exist at all. We think the authors overstate their case, adopting an unrealistically strict definition of ACD and neglecting some of the best demonstrated cases.

The original definition of ACD was morphological. The term was introduced in the 1980s to describe dying cells that contained numerous autolysosomes and a few autophagosomes and lacked the characteristics of other types of cell death[3]. The fact that the autolysosomes sometimes contained most of the cytoplasm and part of the nucleus, in dying cells showing no morphological signs of apoptosis or necrosis, was sometimes argued to imply a role of autophagy in the death mechanism (either in cell killing or in cell dismantlement), but this was not part of the definition[3]. As recently as 2009, a paper summarizing the recommendations of a cell death nomenclature committee[4] favored the initial purely morphological definition, but a still more recent (2012) set of recommendations[5] proposed a functional definition according to which autophagy must not only occur in ACD, but must mediate the death and be suppressed by inhibition of the autophagic pathway. Shen et al. insist that this rarely happens, but admit that it sometimes does. In fact, numerous studies report that the blockade of autophagy (by pharmacological inhibitors, or by RNAi knock-down or conditional knock-out or mutation of autophagy genes) can prevent or delay the death of cells manifesting enhanced autophagy[6-18]. Doubts can be raised about the specificity of the inhibitors, and the possibility that autophagy proteins may have additional

functions other than in autophagy[19], but the convergent results from multiple approaches have convinced most specialists that autophagy can promote the death of cells[5,8,10,19,20].

But the recent Editor's Corner article goes beyond the new recommendations, in requiring two additional criteria. The first of these may have merits, but the second seems to us excessive.

The first of these additional criteria, in conformity with some recent usage[21,22] but not all[20], is that ACD must be a distinct death mechanism, *independent of apoptosis or necrosis*. Thus, situations where autophagy triggers apoptosis[14,17,23,24] or necrosis, or occurs in parallel with them, are excluded even when the autophagy has been clearly shown to promote cell death. This criterion was recommended in a recent critical review in this journal by H.M. Shen and Codogno[8], except that the definition of necrosis was widened to include autophagic cell death, and the conclusion was that, even with this strict definition, ACD does exist in several situations (they cited about ten). Shen and Codogno argued that the physiological role of ACD (e.g. in development) may be limited mainly to lower eukaryotes[25] and invertebrates[15,16], but that it occurred even in mammalian cells in artificial situations, including hippocampal neural stem cells following insulin withdrawal[12]. The Editor's Corner authors admit that ACD (even in this restricted sense) may be involved in "model organisms such as drosophila", but cite only one case, and focus their discussion on mammalian cells, especially cancer cells. They seem to be unconcerned by the fact that the research on what they call "model organisms" is sufficient to prove the existence of ACD.

But they also introduce a second definitional criterion, which appears to us excessive. It maintains that for cell death to be ACD the autophagy must "…be itself responsible for the final dismantling of cellular content and hence execute a lethal pathway" (legend of their Fig. 2). We think the "final dismantling of cellular content" would be not so much lethal as post-lethal, and it seems arbitrarily restrictive to require that ACD fulfil both this criterion and the

criterion that inhibition of autophagy must protect. It is reasonable to postulate a role of autophagy *either* in the induction of cell death *or* in the final dismantlement of cells, but not to require both in the same cell. In the former case autophagy inhibition should promote survival, in the latter it might delay the clearance of cell debris[26,27], but would hardly be expected to promote survival. It would be a remarkable coincidence if autophagy played both roles in a single cell.

We do however appreciate that the Editor's Corner authors required that ACD involve a role of autophagy in cell dismantlement because there is no currently accepted alternative mechanism of death execution and cell destruction apart from apoptosis and necrosis. Wholesale cell dismantlement is indeed a possibility, as one of us once suggested[3]. Alternatively, autophagy might initiate some other nonapoptotic and non-necrotic death mechanism that is currently unknown. Its clarification might one day justify a change in terminology, but for the moment we need the term ACD. The abundance of detection protocols for apoptosis probably cause its prevalence to be overestimated as compared to ACD (and also to necrosis). Abandoning the term would exacerbate this problem and the imposition of restrictive theory-laden nomenclature might inhibit some scientists from making discoveries that would refute current opinion.

Finally, it may be inappropriate to use mammalian cancer cells as a test case for the existence of ACD, for three reasons. First, pure cases of ACD seem to be relatively rare in mammals. A review of cell death in development covering the literature up to 1989 concluded that ACD (morphologically defined) was the predominant type of cell death in metamorphosing insects and amphibians, but found only one case in mammals[3]; and more recent evidence indicates that, even though autophagy does contribute to cell death in mammals[7,8,14], this often involves a complex interaction between multiple death pathways rather than pure ACD[8,21]. Second, dividing and recently post-mitotic cells tend to be so

sensitive to apoptosis that pure, nonapoptotic ACD may be unable to occur in most cases. For example, studies on neurons indicate that it takes several days of post-mitotic development before the autophagic death mechanism begins to predominate over the apoptotic one[28]. Third, cancer cell lines are hardly representative of what happens in normal animals, and they have multiple mutations, so that death-mediating functions of autophagy might be selected against. For these three reasons, even though ACD probably does occur in mammalian cancer cells[22], focusing on them may give an exaggerated impression of its rarety.

In conclusion, even with the recent tendency to include death-promotion by autophagy and independence from apoptosis and necrosis in the definition of ACD, it does occur. The additional requirement of Shen et al. that ACD must involve a role for autophagy in cell dismantlement seems excessive. And mammalian cancer cells may not provide a good model for testing whether ACD exists.

References

1. Shen SS, Kepp O, Kroemer G. The end of autophagic cell death? Autophagy 2012; 8:1-3

2. Shen S, Kepp O, Michaud M et al. Association and dissociation of autophagy, apoptosis and necrosis by systematic chemical study. Oncogene 2011; 30:4544-4556

3. Clarke PGH. Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol 1990; 181:195-213

4. Kroemer G, Galluzzi L, Vandenabeele P et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009; 16:3-11

5. Galluzzi L, Vitale I, Abrams JM et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ 2012; 19:107-120

6. Koike M, Shibata M, Tadakoshi M et al. Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. Am J Pathol 2008; 172:454-469

7. Puyal J, Vaslin A, Mottier V et al. Postischemic treatment of neonatal cerebral ischemia should target autophagy. Ann Neurol 2009; 66:378-389

8. Shen HM, Codogno P. Autophagic cell death Loch Ness monster or endangered species? Autophagy 2011; 7:457-465

9. Luciani MF, Giusti C, Harms B et al. Atg1 allows second-signaled autophagic cell death in Dictyostelium. Autophagy 2011; 7:501-508

10. Denton D, Nicolson S, Kumar S. Cell death by autophagy: facts and apparent artefacts. Cell Death Differ 2012; 19:87-95

11. Shimizu S, Kanaseki T, Mizushima N et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6:1221-1228

12. Yu SW, Baek SH, Brennan RT et al. Autophagic death of adult hippocampal neural stem cells following insulin withdrawal. Stem Cells 2008; 26:2602-2610

13. Yu L, Alva A, Su H et al. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004; 304:1500-1502

14. Grishchuk Y, Ginet V, Truttmann AC et al. Beclin 1-independent autophagy contributes to apoptosis in cortical neurons. Autophagy 2011; 7:1115-1131

15. Berry DL, Baehrecke EH. Growth arrest and autophagy are required for salivary gland cell degradation in Drosophila. Cell 2007; 131:1137-1148

16. Denton D, Shravage B, Simin R et al. Autophagy, Not Apoptosis, Is Essential for Midgut Cell Death in Drosophila. Current Biology 2009; 19:1741-1746

17. Nezis IP, Shravage BV, Sagona AP et al. Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late Drosophila melanogaster oogenesis. Journal of Cell Biology 2010; 190:523-531

18. Hou YCC, Chittaranjan S, Barbosa SG et al. Effector caspase Dcp-1 and IAP protein Bruce regulate starvation-induced autophagy during Drosophila melanogaster oogenesis. Journal of Cell Biology 2008; 182:1127-1139

19. Yousefi S, Simon HU. Autophagy in cancer and chemotherapy. Results Probl Cell Differ 2009; 49:183-190

20. Uchiyama Y, Koike M, Shibata M et al. Autophagic neuron death. Methods Enzymol 2009; 453:33-51

21. Clarke PGH, Puyal J, Vaslin A et al. Multiple Types of Programmed Cell Death and their Relevance to Perinatal Brain Damage. In: Ramenghi LA, Evrard P, Mercuri E, eds. Perinatal Brain Damage: from Pathogenesis to Neuroprotection. Montrouge, France: John Libbey Eurotext, 2008:23-36

22. Elgendy M, Sheridan C, Brumatti G et al. Oncogenic Ras-Induced Expression of Noxa and Beclin-1 Promotes Autophagic Cell Death and Limits Clonogenic Survival. Molecular Cell 2011; 42:23-35

23. Wang Y, Han R, Liang ZQ et al. An autophagic mechanism is involved in apoptotic death of rat striatal neurons induced by the non-N-methyl-D-aspartate receptor agonist kainic acid. Autophagy 2008; 4:214-226

24. Scott RC, Juhasz G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Current Biol 2007; 17:1-11

25. Luciani MF, Kubohara Y, Kikuchi H et al. Autophagic or necrotic cell death triggered by distinct motifs of the differentiation factor DIF-1. Cell Death and Differentiation 2009; 16:564-570

26. Mellen MA, de la Rosa EJ, Boya P. The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. Cell Death Differ 2008; 15:1279-1290

27. Qu XP, Zou ZJ, Sun QH et al. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 2007; 128:931-946

28. Decker RS. Retrograde responses of developing lateral motor column neurons. J Comp Neurol 1978; 180:635-660