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Autophagy and senescence

A partnership in search of definition

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Autophagy and senescence share a number of characteristics, which suggests that both responses could serve to collaterally protect the cell from the toxicity of external stress such as radiation and chemotherapy and internal forms of stress such as telomere shortening and oncogene activation. Studies of oncogene activation in normal fibroblasts as well as exposure of tumor cells to chemotherapy have indicated that autophagy and senescence are closely related but not necessarily interdependent responses; specifically, interference with autophagy delays but does not abrogate senescence. The literature relating to this topic is inconclusive, with some reports appearing to be consistent with a direct relationship between autophagy and senescence and others indicative of an inverse relationship. Before this question can be resolved, additional studies will be necessary where autophagy is clearly inhibited by genetic silencing and where the temporal responses of both autophagy and senescence are monitored, preferably in cells that are intrinsically incapable of apoptosis or where apoptosis is suppressed. Understanding the nature of this relationship may provide needed insights relating to cytoprotective as well as potential cytotoxic functions of both autophagy and senescence.

Keywords: autophagy, senescence

Abbreviations: AMPK, AMP-activated protein kinase; ATG, autophagy related; ATM, Ataxia telangiectasia mutated; BECN1, Beclin 1, autophagy related; CDK, cyclin dependent kinase; CDKN1A/p21, cyclin-dependent kinase inhibitor 1A; CTSB, cathepsin B; IGF1, insulin-like growth factor 1; MAP1LC3/LC3, microtubule-associated protein 1 light chain 3; MTORC, MTOR complex; Rb, retinoblastoma; TP53, tumor protein p53

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Autophagy, a catabolic process involving the degradation of a cell's own components through the lysosomal machinery[1] serves as a protective response under conditions of nutrient deprivation and is also frequently observed in tumor cells exposed to chemotherapy or radiation.[2,3] Cellular senescence, defined as a biological state in which cells have lost the ability to divide, but remain metabolically active[4,5] is likewise a frequent response to chemotherapy or radiation.[5]

Conceptually, autophagy and senescence share a number of common characteristics that suggest that these processes could serve similar ends in the cell. Autophagy and senescence both represent responses to stress that have either cytoprotective or cytotoxic functions. The protective function of autophagy is perceived as allowing prolonged survival of the cell under conditions of externally imposed stress; conversely, there is also extensive evidence for autophagy associated with cell death.[6] Although studies from Kroemer's group have presented compelling evidence against this latter interpretation of autophagic function,[7] it nevertheless appears logical that excessive autophagy, which is essentially a form of cellular self-cannibalism, must ultimately lead to loss of cell viability. Finally, there is evidence, also from Kroemer's group, that autophagy may be necessary for an effective immune response in tumor cells exposed to chemotherapy.[8]

Senescence may reflect an effort by the cell to evade the toxic impact of stress, whereby the cell enters a prolonged growth arrested state but does not die. Although senescence has historically been considered to be irreversible, this is not strictly the case since, e.g., for replicative senescence, activation of telomerase has the capacity to confer proliferative immortality. Furthermore, evidence that a subpopulation of tumor cells can ultimately recover and proliferate subsequent

to senescence induced by chemotherapy or radiation[5] is consistent with the premise that senescence could be permissive for tumor survival in the face of stress, which is similar to the fundamental characterization of cytoprotective autophagy. In this context, we have previously suggested[9] that senescence may also be a central component of tumor dormancy. Conversely, senescence may represent one pathway for elimination of the cell's reproductive capacity, abrogating recovery of self-renewal capacity either directly or via activation of an immune response.[10]

While crosstalk between apoptosis and autophagy is well-established,[11,12] the potential relationship(s) between autophagy and senescence remain poorly defined. A number of papers have provided indirect or circumstantial evidence for the collateral induction of autophagy and senescence. An increase of autophagic vacuoles and senescence-associated β-galactosidase activity has been observed in aging fibroblasts[13] while markers of autophagy and senescence have been collaterally observed in bile duct cells of patients with primary biliary cirrhosis as well as in biliary epithelial cells isolated from mice and treated with either hydrogen peroxide or etoposide.[14,15] Autophagic vesicles are also evident in dying senescent keratinocytes[16,17] where cell death is proposed to occur through the generation of reactive oxygen species; autophagy markers have been observed in senescent endothelial cells[18] as well as in senescent human dental pulp cells.[19] However, these studies do not address whether autophagy and senescence are linked or interdependent responses.

The first direct studies of the potential relationship between autophagy and senescence are provided in a seminal paper by Young et al.[20] in fibroblasts. This work suggests that oncogene-induced senescence could well be dependent on prior induction of autophagy, as pharmacologic and genetic approaches that interfered with autophagy also suppress senescence. However, the actual conclusion derived from this work is that autophagy *delays* but does not abrogate the senescence response. Specifically, genetic silencing of autophagy only attenuates the development of senescence and, as is evident from Figure 4 which shows a delay from day 4 to day 6 in the production of the senescence associated cytokines IL-6 and IL-8, and Figure S6 where the extent of senescence is identical by day 6 in vector control and ATG5 silenced cells,[20] senescence in the autophagy-compromised cells eventually achieves essentially identical levels as in the autophagy-competent cells. Furthermore, senescence could not be reversed when autophagy was compromised, again supporting the conclusion that even if autophagy accelerates senescence, once initiated, senescence is essentially autophagy independent.

A recent publication from our laboratory, where autophagy and senescence were induced by either adriamycin or camptothecin in MCF-7 breast tumor cells and HCT-116 colon carcinoma cells,[21] confirms the findings of Young et al.,[20] albeit for chemotherapy. We reported that autophagy and senescence appear to be regulated by overlapping signaling pathways involving the generation of reactive oxygen species, activation of ATM, induction of TP53 and CDKN1A/p21 and dephosphorylation of Rb. As with studies of oncogene-induced senescence,[20] when autophagy is suppressed using pharmacological or genetic approaches, senescence is initially delayed but is restored in the autophagy-inhibited cells. Our studies[21] coupled with those of Young et al.[20] suggest that while autophagy clearly can accelerate its onset, induction of senescence in response to external or internal "stress" such as DNA damage or oncogene activation does not obligatorily require prior autophagy. The basis for the capacity of autophagy to influence senescence in our experimental system is currently under investigation but could reflect the cell's efforts to generate energy in anticipation of the prolonged arrest associated with the senescent state.

While subsequent studies that have examined the putative relationship between autophagy and senescence may appear to support a similar association, these responses have generally been assessed at only a single time point and without addressing whether the senescent phenotype is eventually restored in the face of autophagy suppression. In a very detailed series of studies that evaluated both autophagy and senescence induced by Karposi's sarcoma-associated herpesvirus (KSHV) in primary human foreskin fibroblasts, Leidal et al.[22] report the opposing action of the viral proteins, v-cyclin and v-FLIP on collateral regulation of autophagy and senescence. Whereas v-FLIP (ortholog of human CFLAR/FLIP) is shown to be antagonistic to their induction, v-cyclin promotes both autophagy and senescence, where the triggering of autophagy is apparently a consequence of negative feedback on MTORC signaling through AMPK. However, these studies also tend to suggest that autophagy and senescence are likely to be dissociable since in cells transduced with v-cyclin, knockdown of either of the autophagy regulator proteins, ATG5 or ATG7, produces only a modest and essentially transient suppression of IL6 and IL8, cytokines that are associated with the senescence secretory phenotype.[23]

Singh et al.[24] have reported collateral suppression of both autophagy and senescence in H1299 non-small cell lung cancer cells expressing a proteolytic CCNE/cyclin E fragment (p18-cyclin E), 6 d post-radiation. This work shows a clear and pronounced impact of autophagy inhibition through genetic silencing of ATG7 in reducing the senescent population, although the actual percentage of the cell population that enters the senescent state was not evaluated. Silencing of autophagy also served to increase the sub G_1, presumably apoptotic, cell population (shown at day 1 post irradiation), as is frequently reported in the literature.[11,12] However, subversion of the cytoprotective function of autophagy through apoptosis complicates an analysis of these findings, as the collateral interference with autophagy and senescence is likely to simply reflect the inability of cells that are dying by apoptosis to escape death by entering a state of senescence.

Studies by Patschan et al. in endothelial cells exposed to glycated collagen I[18] demonstrate that a blockade to autophagy using 3-methyladenine suppresses senescence. However, it is again difficult to evaluate whether autophagy is actually a precondition for senescence given both the relative lack of specificity of 3-methyladenine for autophagy inhibition as

well as the approximately 3-fold increase in the extent of apoptosis when the cells are cotreated with the glycated collagen and 3-methyladenine. Interpretation of these findings is further complicated by the fact that the autophagy induced by glycated collagen is undetectable by 72 h posttreatment.

A recent paper by Mosieniak et al.[25] in which MCF-7 and U2OS cells were treated with curcumin also suggests that autophagy inhibition by knocking down of ATG5 delays senescence. In these studies, the autophagy inhibition is transient and therefore recovery of senescence might have been expected if the two responses were linked; unfortunately, the temporal recovery of senescence does not appear to have been examined.

Maddodi et al.[26] also have published work that supports the capacity of the BRAF oncogene to promote both autophagy and senescence in melanoma cells, where autophagy is associated with inhibition of MTORC. Interestingly, in this study, autophagy appears to mediate tumor cell death in culture while overexpression of the BRAF oncogene in tumor xenografts is associated with suppression of tumor growth. However, while it is clear that both autophagy and senescence are induced by BRAF, again it cannot be discerned whether senescence is dependent on prior autophagy or alternatively if the two responses occur collaterally but independently.

Finally, Capparelli et al.[27] report that CDK inhibitors induce both senescence and upregulation of autophagy-associated genes such as BECN1, CTSB and MAP1LC3/LC3 in hTERT immortalized fibroblasts; however, in the absence of any direct assessment of autophagy induction, it cannot be determined whether the two responses are collaterally induced let alone functionally connected.

In contrast to these findings suggesting at the very least a close linkage between autophagy and senescence, a number of reports have appeared in the literature that support an inverse relationship, where *inhibition* of autophagy promotes development of the senescent phenotype. Intuitively, this type of relationship is entirely logical if both autophagy and senescence act in a cytoprotective manner, as senescence might serve as a "backup" response in the event that autophagy induction fails to provide effective protection to the injured cells.

Studies by Kang et al.,[28] that were, like those of Young et al.[20] also performed in fibroblasts, present quite convincing evidence that suppression of autophagy is in fact permissive for senescence, presumably relating to the increased generation of reactive oxygen species by dysfunctional mitochondria when autophagy is compromised. A fundamental difference between the experimental conditions in this work and the studies by Young et al.[20] as well as our own findings[21] is that the inverse relationship between autophagy and senescence is observed in the absence of external or internally imposed stress and reflects basal autophagy as opposed to oncogene or chemotherapy induced autophagy and senescence.

Studies by Wang et al.[29] in mouse embryonic fibroblasts as well as HCT-116 colon carcinoma cells present relatively convincing data that also appear to be in contradiction to the conclusions of Young et al. relating to oncogene-induced senescence.[20] More specifically, TP53BP2 (tumor protein p53 binding protein, 2)/ASPP2 is shown to mediate RAS-induced senescence through suppression of autophagy, apparently by blocking formation of a complex between ATG16L1, ATG5 and ATG12. These conclusions are supported by experimental data demonstrating that RAS-induced senescence is suppressed by the expression of ATG5 and, conversely, facilitated by inducible ATG3 deletion.

Another study, by Fujii et al.[30] in a model of chronic obstructive pulmonary disease exposed to cigarette smoke extract, also presents evidence in support of the premise that autophagy inhibition *promotes* senescence. However, changes in the extent of senescence upon the silencing of autophagy related genes, when observed, are relatively small. Specifically, silencing of SQSTM1/p62 has *no* effect on senescence whereas silencing of ATG5 only increases the senescent population from ~4 to ~8%. At low concentrations of the cigarette smoke extract, silencing of LC3 does increase the percentage of β-gal positive (senescent) cells (from 12% to 18%) while at a higher concentration of the extract, senescence is increased from 14% to 21% of the cell population. However, clearly the bulk of the cell population is not undergoing senescence arrest in the face of autophagy silencing.

Recent studies by Drullion et al.[31] also appear to support an inverse relationship between autophagy and senescence in K562 leukemia cells that are either untreated or exposed to imatinib in that silencing of ATG5 markedly increases the senescence-arrested cell population. However, unexpectedly, silencing of either ATG7 or BECN1 does not alter the extent of senescence, a finding that is somewhat confounding and that raises serious reservations as to the meaning of these data.

Evidence for an inverse relationship is further supported by a report from Bitto et al.,[32] where long-term exposure of fibroblasts to IGF1 promotes senescence while markers of autophagy such as puncta formation, SQSTM1/p62 degradation and protein degradation are suppressed. These studies, however, neglect to indicate the actual extent of senescence or the time frame during which senescence occurs.

Taking all of the available data into account, it would be premature to postulate the existence of an invariant relationship between autophagy and either oncogene-induced or stress-induced senescence. In this context, it is worth noting that, other than the reports of autophagy markers in aging fibroblasts,[13] senescent keratinocytes,[16,17] endothelial cells[18] and dental pulp cells,[19] to our knowledge no studies have been published the directly address the potential role of autophagy in *replicative* senescence. The studies by Young et al.[20] as well as those from our own laboratory[21] argue for a close linkage, but without interdependence. The studies by Wang et al.[29] also are indicative of collateral induction (by v-cyclin) or suppression (by v-FLIP) of autophagy and senescence but do not directly address their putative interdependence; data relating to cytokine secretion strongly suggests that the senescence response can recover in the face of autophagy suppression similar to our findings[21] as well as those by Young et al.[20] The studies by Singh et al.[24] and Patschan et al.[18] are somewhat inconclusive as a consequence of the fact that

the cells die by apoptosis when autophagy is inhibited.

The studies by Kang et al.[28] clearly support an inverse relationship between autophagy and senescence; however these experiments were performed in the absence of any external stress stimulus such as chemotherapy or radiation or an internal stress stimulus such as telomere shortening or oncogene activation. The work of Fujii et al.,[30] Wang et al.[29] and Druillion et al.[31] generally support the premise that autophagy suppression rather than its induction is permissive for a senescence response. Consequently, as there are currently insufficient data upon which to base any all-encompassing conclusions, additional and extensive studies are necessary where: a) autophagy is unequivocally and "irreversibly" silenced during the course of the study; b) both autophagy and senescence are temporally monitored and rigorously quantified; and c) the studies are preferably performed in cells that are either intrinsically incapable of undergoing apoptosis or where apoptosis is suppressed through either pharmacological or genetic strategies, which would prevent the abrogation of senescence simply as a consequence of cell death. In addition, in the case of oncogene-induced senescence, it should prove useful to evaluate the autophagy and senescence responses to more than one oncogene in the same experimental model system (possibly RAS and RAF1, although there is evidently crosstalk between these oncogenic signaling pathways). In the case of stress (chemotherapy)-induced senescence, studies should likely be performed evaluating drugs with different mechanisms of action and in multiple tumor cell lines. One approach that might be considered to assure that all of the target cells are affected equally in terms of autophagy suppression would be a high titer adenovirus infection of cells with a floxed essential *ATG* gene. In conclusion, it is quite intriguing that at this relatively early stage of research, there is little consensus as to whether autophagy and senescence are, in fact, related and, if related, whether a direct or inverse relationship exists between these critical responses to external and internal forms of cellular stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol 2010; 221:3-12; PMID:20225336; http://dx.doi.org/10.1002/path.2697
2. Zhou S, Zhao L, Kuang M, Zhang B, Liang Z, Yi T, et al. Autophagy in tumorigenesis and cancer therapy: Dr. Jekyll or Mr. Hyde? Cancer Lett 2012; 323:115-27; PMID:22542808; http://dx.doi.org/10.1016/j.canlet.2012.02.017
3. Palumbo S, Comincini S. Autophagy and ionizing radiation in tumors: the "survive or not survive" dilemma. [Review]. J Cell Physiol 2013; 228:1-8; PMID:22585676; http://dx.doi.org/10.1002/jcp.24118
4. Evan GI, d'Adda di Fagagna F. Cellular senescence: hot or what? Curr Opin Genet Dev 2009; 19:25-31; PMID:19181515; http://dx.doi.org/10.1016/j.gde.2008.11.009
5. Gewirtz DA, Holt SE, Elmore LW. Accelerated senescence: an emerging role in tumor cell response to chemotherapy and radiation. Biochem Pharmacol 2008; 76:947-57; PMID:18657518; http://dx.doi.org/10.1016/j.bcp.2008.06.024
6. Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, et al. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif 2012; 45:487-98; PMID:23030059; http://dx.doi.org/10.1111/j.1365-2184.2012.00845.x
7. Shen S, Kepp O, Michaud M, Martins I, Minoux H, Métivier D, et al. Association and dissociation of autophagy, apoptosis and necrosis by systematic chemical study. Oncogene 2011; 30:4544-56; PMID:21577201; http://dx.doi.org/10.1038/onc.2011.168
8. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 2011; 334:1573-7; PMID:22174255; http://dx.doi.org/10.1126/science.1208347
9. Gewirtz DA. Autophagy, senescence and tumor dormancy in cancer therapy. Autophagy 2009; 5:1232-4; PMID:19770583; http://dx.doi.org/10.4161/auto.5.8.9896
10. Ewald JA, Desotelle JA, Wilding G, Jarrard DF. Therapy-induced senescence in cancer. J Natl Cancer Inst 2010; 102:1536-46; PMID:20858887; http://dx.doi.org/10.1093/jnci/djq364
11. Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis 2008; 13:1-9; PMID:17990121; http://dx.doi.org/10.1007/s10495-007-0154-9
12. Giansanti V, Torriglia A, Scovassi AI. Conversation between apoptosis and autophagy: "Is it your turn or mine?" Apoptosis 2011; 16:321-33; PMID:21404107; http://dx.doi.org/10.1007/s10495-011-0589-x
13. Gerland LM, Peyrol S, Lallemand C, Branche R, Magaud JP, French M. Association of increased autophagic inclusions labeled for beta-galactosidase with fibroblastic aging. Exp Gerontol 2003; 38:887-95; PMID:12915210; http://dx.doi.org/10.1016/S0531-5565(03)00132-3
14. Sasaki M, Miyakoshi M, Sato Y, Nakanuma Y. Autophagy may precede cellular senescence of bile ductular cells in ductular reaction in primary biliary cirrhosis. Dig Dis Sci 2012; 57:660-6; PMID:21989821; http://dx.doi.org/10.1007/s10620-011-1929-y
15. Sasaki M, Miyakoshi M, Sato Y, Nakanuma Y. Autophagy mediates the process of cellular senescence characterizing bile duct damages in primary biliary cirrhosis. Lab Invest 2010; 90:835-43; PMID:20212459; http://dx.doi.org/10.1038/labinvest.2010.56
16. Gosselin K, Deruy E, Martien S, Vercamer C, Bouali F, Dujardin T, et al. Senescent keratinocytes die by autophagic programmed cell death. Am J Pathol 2009; 174:423-35; PMID:19147823; http://dx.doi.org/10.2353/ajpath.2009.080332
17. Deruy E, Gosselin K, Vercamer C, Martien S, Bouali F, Slomianny C, et al. MnSOD upregulation induces autophagic programmed cell death in senescent keratinocytes. PLoS One 2010; 5:e12712; PMID:20856861; http://dx.doi.org/10.1371/journal.pone.0012712
18. Patschan S, Chen J, Polotskaia A, Mendelev N, Cheng J, Patschan D, et al. Lipid mediators of autophagy in stress-induced premature senescence of endothelial cells. Am J Physiol Heart Circ Physiol 2008; 294:H1119-29; PMID:18203850; http://dx.doi.org/10.1152/ajpheart.00713.2007
19. Li L, Zhu YQ, Jiang L, Peng W. Increased autophagic activity in senescent human dental pulp cells. Int Endod J 2012; 45:1074-9; PMID:22551517; http://dx.doi.org/10.1111/j.1365-2591.2012.02064.x
20. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, et al. Autophagy mediates the mitotic senescence transition. Genes Dev 2009; 23:798-803; PMID:19279323; http://dx.doi.org/10.1101/gad.519709
21. Goehe RW, Di X, Sharma K, Bristol ML, Henderson SC, Valerie K, et al. The autophagy-senescence connection in chemotherapy: must tumor cells (self) eat before they sleep? J Pharmacol Exp Ther 2012; 343:763-78; PMID:22927544; http://dx.doi.org/10.1124/jpet.112.197590
22. Leidal AM, Cyr DP, Hill RJ, Lee PWK, McCormick C. Subversion of autophagy by Kaposi's sarcoma-associated herpesvirus impairs oncogene-induced senescence. Cell Host Microbe 2012; 11:167-80; PMID:22341465; http://dx.doi.org/10.1016/j.chom.2012.01.005
23. Pazolli E, Alspach E, Milczarek A, Prior J, Piwnica-Worms D, Stewart SA. Chromatin remodeling underlies the senescence-associated secretory phenotype of tumor stromal fibroblasts that supports cancer progression. Cancer Res 2012; 72:2251-61; PMID:22422937; http://dx.doi.org/10.1158/0008-5472.CAN-11-3386
24. Singh K, Matsuyama S, Drazba JA, Almasan A. Autophagy-dependent senescence in response to DNA damage and chronic apoptotic stress. Autophagy 2012; 8:236-51; PMID:22240589; http://dx.doi.org/10.4161/auto.8.2.18600
25. Mosieniak G, Adamowicz M, Alster O, Jaskowiak H, Szczepankiewicz AA, Wilczynski GM, et al. Curcumin induces permanent growth arrest of human colon cancer cells: link between senescence and autophagy. Mech Ageing Dev 2012; 133:444-55; PMID:22613224; http://dx.doi.org/10.1016/j.mad.2012.05.004
26. Maddodi N, Huang W, Havighurst T, Kim K, Longley BJ, Setaluri V. Induction of autophagy and inhibition of melanoma growth in vitro and in vivo by hyperactivation of oncogenic BRAF. J Invest Dermatol 2010; 130:1657-67; PMID:20182446; http://dx.doi.org/10.1038/jid.2010.26
27. Capparelli C, Chiavarina B, Whitaker-Menezes D, Pestell TG, Pestell RG, Hulit J, et al. CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis. Cell Cycle 2012; 11:3599-610; PMID:22935696; http://dx.doi.org/10.4161/cc.21884

28. Kang HT, Lee KB, Kim SY, Choi HR, Park SC. Autophagy impairment induces premature senescence in primary human fibroblasts. PLoS One 2011; 6:e23367; PMID:21858089; http://dx.doi.org/10.1371/journal.pone.0023367
29. Wang Y, Wang XD, Lapi E, Sullivan A, Jia W, He YW, et al. Autophagic activity dictates the cellular response to oncogenic RAS. Proc Natl Acad Sci U S A 2012; 109:13325-30; PMID:22847423; http://dx.doi.org/10.1073/pnas.1120193109
30. Fujii S, Hara H, Araya J, Takasaka N, Kojima J, Ito S, et al. Insufficient autophagy promotes bronchial epithelial cell senescence in chronic obstructive pulmonary disease. Oncoimmunology 2012; 1:630-41; PMID:22934255; http://dx.doi.org/10.4161/onci.20297
31. Drullion C, Trégoat C, Lagarde V, Tan S, Gioia R, Priault M, et al. Apoptosis and autophagy have opposite roles on imatinib-induced K562 leukemia cell senescence. Cell Death Dis 2012; 3:e373; PMID:22898871; http://dx.doi.org/10.1038/cddis.2012.111
32. Bitto A, Lerner C, Torres C, Roell M, Malaguti M, Perez V, et al. Long-term IGF-I exposure decreases autophagy and cell viability. PLoS One 2010; 5:e12592; PMID:20830296; http://dx.doi.org/10.1371/journal.pone.0012592