[Autophagy 4:7, 1-4; 1 October 2008]; ©2008 Landes Bioscience

Brief Report

The effects of p53 on whole organism longevity are mediated by autophagy

Nektarios Tavernarakis,[1,*] Angela Pasparaki,[1] Ezgi Tasdemir,[2-4] Maria Chiara Maiuri[2-5] and Guido Kroemer[2-4,*]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology; Crete, Greece; [2]INSERM, U848; Villejuif, France; [3]Institut Gustave Roussy; Villejuif, France; [4]Université Paris-Sud XI; Villejuif, France; [5]Università degli Studi di Napoli "Federico II", Facoltà di Scienze Biotecnologiche; Napoli, Italy

Key words: aging, beclin 1, *Caenorhabditis elegans*, nematode, senescence

The tumor suppressor protein p53 has a major impact on organismal aging. Recently it has become clear that p53 not only controls DNA damage responses, senescence and apoptosis but also plays a major role in the control of autophagy. Thus, deletion, depletion, or inhibition of p53 induces autophagy in human, mouse and nematode cells. We therefore tested the hypothesis that the mutation of the p53 orthologue CEP-1 might increase the life span of *Caenorhabditis elegans* through an increase in baseline autophagy. For this, we evaluated the survival of nematodes lacking *cep-1*, alone or in combination with RNA inference with the autophagy gene *bec-1* (which encodes the orthologue of Atg6/Beclin 1). *cep-1* mutants exhibited a prolonged life span. While BEC-1 depletion during adult life did not cause significant modification of the life expectancy of wild type controls, it did reduce the increased life span of *cep-1* mutants down to approximately normal levels. These results indicate that the life span-extending effect of the *cep-1* mutation is mediated by autophagy. These results lend support to the hypothesis that autophagy has a broad positive impact on organismal aging.

Introduction

The tumor suppressor protein p53 has an essential role as a guardian of the genome and links DNA damage by exogenous mutagens as well as DNA damage resulting from oncogene activation to DNA repair, cell cycle arrest, senescence and apoptosis.[1] Mutation, inactivation, or loss of p53 therefore is one of the most frequent oncogenic events in human cancer. Nonetheless, p53 has also negative effects in the sense that it favors organismal aging.[2-5] Thus, gain of function mutations of p53 have been linked to a reduced frequency of tumors but also to accelerated aging and premature death in mice[6] and humans.[7-9] A dominant-negative *p53* transgene can enhance the longevity of *Drosophila melanogaster*,[10] and loss of function mutations of *cep1*, the *p53* orthologue in *Caenorhabditis elegans* can extend life span, at least in standard laboratory conditions.[11]

The effects of p53 on organismal aging has been linked to its cellular effects, in particular its contribution to permanent cell cycle arrest (senescence) and pro-apoptotic signal transduction pathways. Moreover, p53 reportedly may affect the insulin receptor signaling pathway,[10] whose inhibition has a major life span-extending effect that mimics that obtained by caloric restriction.[12] Recently, p53 has also been found to be involved in the regulation of autophagy. Thus, nuclear p53 can transactivate genes that increase autophagy in mammalian cells.[13,14] In addition, a cytoplasmic pool of p53 acts as a negative regulator of autophagy, and knockout, knockdown, or pharmacological inhibition of p53 stimulates autophagy in human, mouse and nematode cells.[15,16]

An ever-expanding body of literature suggests that autophagy constitutes one of the most potent anti-aging mechanisms.[17,18] Knockout of essential autophagy genes causes degenerative features in a variety of different organs and species as if inhibited autophagy accelerated the aging process.[19] Beth Levine and colleagues were the first to show that the increased longevity of *C. elegans* mutants with defective DAF-2 protein, i.e., an insulin receptor analog, involves increased autophagy.[20] Thus knockout of *bec-1* (which encodes the *C. elegans* orthologue of Atg6/Beclin 1) abolishes the life-span extension conferred by the *daf-2* mutation.[20] The knockout or knockdown of other essential autophagy genes such as *atg-1, atg-7, atg-8, atg-10* and *atg-12* similarly neutralizes the beneficial effects of the *daf-2* mutation,[20,21] in spite of the fact that autophagy on its own is not sufficient to mediate life-span extension.[22] Caloric restriction, which is the physiological inducer of autophagy, also mediates its life span-extending effects through the induction of autophagy. Thus, knockdown of essential autophagy genes including *bec-1, vps-34* or *atg7* reduces longevity induced by feeding-defective mutants or a mutant with low TOR activity.[22-24]

Based on these premises, we decided to investigate whether the increase in longevity conferred by p53/CEP-1 lesions in *C. elegans* would be mediated by enhanced autophagy.

Results and Discussion

Under standard laboratory conditions, wild-type animals and *cep-1(gk138) C. elegans* mutants grown on bacteria that carry a control

*Correspondence to: Nektarios Tavernarakis; Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology; Vasilika Vouton; P.O. Box 1385; Heraklion 71110; Crete, Greece; Tel.: +30.2810.391066, Fax: +30.2810.391067; Email: tavernarakis@imbb.forth.gr/ Guido Kroemer; INSERM Unit "Apoptosis, Cancer & Immunity"; Institut Gustave Roussy; PR1; 39 rue Camille Desmoulins; Villejuif F-94805 France; Tel.: 33.1.42.11.60.46; Fax: 33.1.42.11.60.47; Email: kroemer@igr.fr

Submitted: 07/01/08; Revised: 07/21/08; Accepted: 08/04/08

Previously published online as an *Autophagy* E-publication:
http://www.landesbioscience.com/journals/autophagy/article/6730

plasmid which directs the production of dsRNA targeting GFP (control RNAi), exhibit a mean life span of 17.2 ± 0.8 and 20.4 ± 0.9 days and a maximum life span (defined as the top 90% percentile) of 25.3 ± 1.4 and 31.8 ± 1.6 days, respectively (Table 1, Fig. 1). Hence, p53 deficiency in the nematode significantly ($p < 0.001$) enhances mean life span by 3.2 days and maximum life span by 6.5 days, a finding that is consistent with previous studies.[11] Under such standard culture conditions, *cep-1* mutant animals exhibit a significantly enhanced autophagy, as we have reported recently.[15,16]

To determine whether increased autophagy accounts for the increased life span of the *cep-1(gk138)* mutant, both *cep-1(gk138)* mutant animals and the wild-type controls were subjected to knockdown of the worm orthologue of Atg6/Beclin 1, BEC-1, using bacteria harboring a plasmid, which directs synthesis of dsRNA corresponding to the *bec-1* gene. It is well established that this knockdown diminishes autophagy induced by multiple different stimuli[20-25] including knockout or knockdown of *cep-1* (reviewed in refs. 15 and 16; and data not shown). Depletion of BEC-1 did not significantly modify the life span of wild-type nematodes, yet did reduce the longevity of *cep-1* mutant animals, down to approximately wild-type levels (Table 1, Fig. 1). This result indicates that BEC-1 is required for the life-prolonging effect of CEP-1 deficiency. Although BEC-1 might have autophagy-unrelated functions, these data strongly suggest that autophagy is involved in the modulation of life span by the worm ortholog of p53.

p53 and its nematode orthologue *CEP-1* act as tonic inhibitors of autophagy, meaning that their removal stimulates a continuous state of autophagy, at least during the G_1 and S phases of the cell cycle.[27] As indicated by an abundant literature, p53 allows for the adaptation of and response to genotoxic stress[28] in diverse organisms including *C. elegans*[29] and it has been implicitly speculated that this function would account for the impact of p53 on life-span regulation. Autophagy is considered as a mechanism to cope with metabolic stress and organellar damage.[19,30,31] Inactivation of *cep-1* induces a maximum level of baseline autophagy that is barely enhanced by starvation.[15,16]

Unexpectedly, our results hence reveal that the two effects of *cep-1* mutation—(i) increase in life span and (ii) increase in autophagy—are functionally linked. Increased autophagy has been shown to be indispensable for the life-span increasing effects of feeding mutants (which reduce caloric uptake) and mutants affecting the insulin receptor pathway including loss of function mutations of target of rapamycin (TOR).[20-24] In mammalian cells, knockout or knockdown of p53 causes an inhibition of mammalian TOR (mTOR),[15] suggesting that defective CEP-1/p53 function might increase longevity through the inhibition of downstream signals that are normally activated by the insulin receptor pathway. However, the exact molecular mechanism by which p53 affects mTOR activity remains to be determined. In Drosophila, dominant-negative (DN) p53 can enhance life span if it is only expressed in the brain (but not in the fat body or muscle)[32] or even only in the insulin-producing brain cells (as opposed to dopaminergic or serotoninergic neurons), thus impairing the release of insulin-like peptides and interfering with PI3K activity in peripheral organs.[10] These results point to the possibility that p53 may (also) affect aging by endocrine signaling, a possibility that has not been investigated in mammals or nematodes thus far. In mammalian cells, p53 undoubtedly has cell-autonomous effects on autophagy,[15,16] and it remains an open conundrum whether the ablation of p53 in specific cell types

Table 1 Life-span data

Strain	Mean ± SEM (days)	Max* ± SEM (days)	Deaths/total†	p value
N2 (wild type)	17.2 ± 0.8	25.3 ± 1.4	115/127 (3)	-
cep-1(gk138)	20.4 ± 0.9	31.8 ± 1.6	116/134 (3)	<0.001#
N2; *bec-1(RNAi)*	16.8 ± 1.2	23.3 ± 1.8	107/122 (3)	ns#
cep-1(gk138); bec-1(RNAi)	18.0 ± 1.3	26.1 ± 1.9	112/132 (3)	<0.001‡

All aging experiments were performed on plates seeded with HT115(DE3) *E. coli* bacteria, carrying appropriate RNAi plasmid constructs (SEM: standard error of the mean; p values were calculated using the log-rank test, as described in Materials and Methods). *Maximum life span shown is the median life span of the longest-lived 10 percent of the animals assayed. †The number of confirmed death events, divided by the total number of animals included in life-span assays is shown. Total equals the number of animals that died plus the number of animals that were censored (see Materials and Methods). The number of independent life-span assays for each strain is shown in parentheses. The smallest number of animals followed for any strain tested was 134. #Compared with wild type animals subjected to control RNAi, assayed at the same temperature. ‡Compared with the corresponding mutant subjected to control RNAi. ns: no significant difference compared to control ($p > 0.5$).

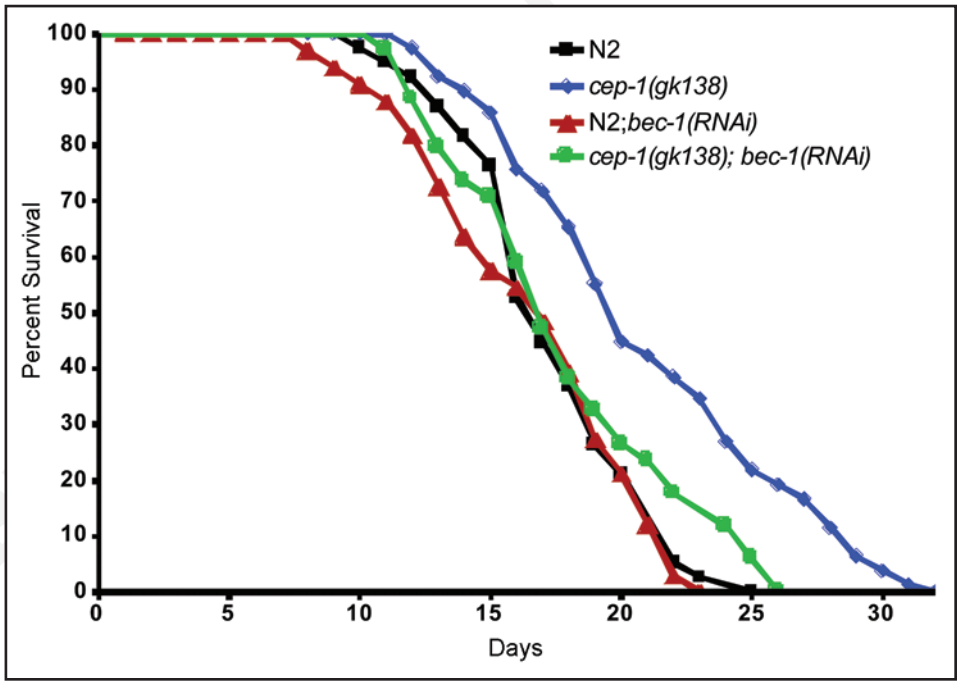

Figure 1. Knockdown of *bec-1* suppresses life-span extension by p53 deficiency in *C. elegans*. Survival curves of wild type (N2) and *cep-1(gk138)* mutant animal populations subjected to *bec-1* RNAi. The percentage of animals remaining alive is plotted against animal age. Assays were carried out at 20°C. Combined life-span data from independent experiments are given in Table 1.

might have a beneficial effect on organismal aging. Nonetheless, the exact mechanisms through which an increase in autophagy may inhibit organismal aging remain a matter of speculation (and this study admittedly does not contribute any mechanistic insights into this question, either). Thus, an increase in autophagy might reduce signs of cellular aging (such as accumulation of misfolded proteins and lipofuscin), retard cellular senescence (defined as an irreversible arrest in the cell cycle), reduce the propensity of cells to die, or even mediate noncell-autonomous metabolic effects, by the mobilization of metabolites from intracellular stores in conditions of starvation. Moreover, our data show that autophagy is required for the increase in life span induced by the loss of *cep-1*, yet do not prove that enhanced autophagy suffices to prolong longevity.

As a further caveat, the relationship between p53, autophagy and aging likewise is not simply linear. Thus, genetic models of premature aging (such as the *zmpste24* or the *klotho* knockouts) manifest enhanced autophagy in multiple tissues,[33,34] perhaps as a result of compensatory mechanisms. Thus far, no attempts have been undertaken to investigate whether inhibition of autophagy would accelerate the phenotypic manifestation of progeria. According to some reports, p53 function decreases with aging, at least in the context of DNA damage responses,[35] while autophagy tends to decline with old age.[17] This is ostensibly at odds with the postulate that a reduction in p53 levels should stimulate autophagy.[15] The exploration of the precise post-translational p53 modifications that affect the regulation of autophagy as well as the comprehension of the mechanisms though which the function of p53 is lost in aging tissues may resolve this apparent contradiction in the future.

Materials and Methods

Strains and genetics. We followed standard procedures for *C. elegans* strain maintenance. Nematode rearing temperature was kept at 20°C, unless noted otherwise. The following strains were used in this study: N2: wild-type Bristol isolate and *cep-1(gk138)*. The *cep-1(gk138)* mutant strain (TJ1) was generously provided by the Caenorhabditis Genetics Center.

RNAi and plasmid constructs. For RNAi experiments, we constructed a plasmid that directs the synthesis of a dsRNA corresponding to the *bec-1* gene in *E. coli* bacteria, which were then fed to animals, according to a previously described methodology.[25] To augment RNAi, animals were reared for two generations on dsRNA-producing *E. coli* bacteria before examination. To construct the *bec-1* RNAi plasmid, a *bec-1* gene-specific fragment was obtained by PCR amplification directly from *C. elegans* genomic DNA using appropriate primer sets (5'GCTCTAGAGTTATCACAGAAGCTCTG3' and 5'CGGGATCCGTCCATACAATGCGTACG3'). The PCR-generated fragment was sub-cloned into the pL4440 plasmid vector and resulting constructs were transformed into HT115(DE3) *E. coli* bacteria, deficient for RNase-E.[26]

Life-span analysis. Life-span assays were performed at 20°C. Synchronous animal populations were generated by hypochlorite treatment of gravid adults to obtain tightly synchronized embryos that were allowed to develop into adulthood on standard, OP50-seeded, NGM plates before transfer to RNAi plates. For RNAi life-span experiments, worms were placed on NGM plates containing 0.5–1 mM IPTG and seeded with HT115(DE3) bacteria transformed with either the pL4440 vector or the test RNAi construct. Progeny were grown at 20°C unless otherwise noted, through the L4 larval stage and then transferred to fresh RNAi plates following the L4 moult, at groups of 10–20 worms per plate for a total of 100–150 individuals per experiment. The day of egg harvest was used as t = 0. Animals were transferred to fresh plates every two to four days thereafter and were examined every day for touch-provoked movement and pharyngeal pumping, until death. Worms that died due to internally hatched eggs, an extruded gonad, or desiccation due to crawling on the edge of the plates, were censored and incorporated as such into the data set. Each survival assay was repeated at least three times and figures represent typical assays. Survival curves were created using the product-limit method of Kaplan and Meier. The log-rank (Mantel-Cox) test was used to evaluate differences between survivals and determine p values. We used the Prism software package (GraphPad Software Inc., San Diego, California) to carry out statistical analysis and to determine life-span values.

Statistical analysis. Statistical analyses were carried out using the Prism software package (GraphPad Software Inc., San Diego, California) and the Microsoft Office 2003 Excel software package (Microsoft Corporation, Redmond, Washington). Mean values were compared using unpaired t tests. For multiple comparisons, we used the one-factor (ANOVA) variance analysis corrected by the posthoc Bonferroni test.

Acknowledgements

We are indebted to Lorenzo Galluzzi for comments and critical reading of the manuscript. The *cep-1(gk138)* mutant strain (TJ1) used in this work was provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). We thank A. Fire for RNAi plasmid vectors. This study was supported by funds from the EU 6th Framework Program (to N.T. and G.K.), the EU 7th Framework Program, as well as grants from Ligue contre le Cancer, Agence Nationale pour la Recherche and Institut National sur le Cancer (to G.K.).

References

1. Vicencio JM, Galluzzi L, Tajeddine N, Ortiz C, Criollo A, Tasdemir E, Morselli E, Ben Younes A, Maiuri MC, Lavandero S, Kroemer G. Senescence, apoptosis or autophagy? When a damaged cell must decide its path—a mini-review. Gerontology 2008; 54:92-9.
2. Campisi J. Cancer and ageing: rival demons? Nat Rev Cancer 2003; 3:339-49.
3. Donehower LA. p53: guardian AND suppressor of longevity? Exp Gerontol 2005; 40:7-9.
4. Finkel T, Serrano M, Blasco MA. The common biology of cancer and ageing. Nature 2007; 448:767-74.
5. Serrano M, Blasco MA. Cancer and ageing: convergent and divergent mechanisms. Nat Rev Mol Cell Biol 2007; 8:715-22.
6. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A, Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002; 415:45-53.
7. van Heemst D, Mooijaart SP, Beekman M, Schreuder J, de Craen AJ, Brandt BW, Slagboom PE, Westendorp RG; Long Life study group. Variation in the human TP53 gene affects old age survival and cancer mortality. Exp Gerontol 2005; 40:11-5.
8. Orsted DD, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Tumor suppressor p53 Arg72Pro polymorphism and longevity, cancer survival and risk of cancer in the general population. J Exp Med 2007; 204:1295-301.
9. Bojesen SE, Nordestgaard BG. The common germline Arg72Pro polymorphism of p53 and increased longevity in humans. Cell Cycle 2008; 7:158-63.
10. Bauer JH, Chang C, Morris SN, Hozier S, Andersen S, Waitzman JS, Helfand SL. Expression of dominant-negative Dmp53 in the adult fly brain inhibits insulin signaling. Proc Natl Acad Sci USA 2007; 104:13355-60.
11. Arum O, Johnson TE. Reduced expression of the *Caenorhabditis elegans* p53 ortholog cep-1 results in increased longevity. J Gerontol A Biol Sci Med Sci 2007; 62:951-9.
12. Bergamini E, Cavallini G, Donati A, Gori Z. The role of autophagy in aging: its essential part in the anti-aging mechanism of caloric restriction. Ann NY Acad Sci 2007; 1114:69-78.

13. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, Ryan KM. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006; 126:121-34.
14. Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci USA 2005; 102:8204-9.
15. Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, Criollo A, Morselli E, Zhu C, Harper F, Nannmark U, Samara C, Pinton P, Vicencio JM, Carnuccio R, Moll UM, Madeo F, Paterlini-Brechot P, Rizzuto R, Szabadkai G, Pierron G, Blomgren K, Tavernarakis N, Codogno P, Cecconi F, Kroemer G. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 2008; 10:676-87.
16. Tasdemir E, Maiuri MC, Morselli E, Criollo A, D'Amelio M, Djavaheri-Mergny M, Cecconi F, Tavernarakis N, Kroemer G. A dual role of p53 in the control of autophagy. Autophagy 2008; 4:810-4.
17. Cuervo AM. Autophagy and aging—when "all you can eat" is yourself. Sci Aging Knowledge Environ 2003; 25.
18. Tóth ML, Sigmond T, Borsos E, Barna J, Erdélyi P, Takács-Vellai K, Orosz L, Kovács AL, Csikós G, Sass M, Vellai T. Longevity pathways converge on autophagy genes to regulate life span in *caenorhabditis elegans*. Autophagy 2008; 4:330-8.
19. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132:27-42.
20. Meléndez A, Tallóczy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. Science 2003; 301:1387-91.
21. Hars ES, Qi H, Ryazanov AG, Jin S, Cai L, Hu C, Liu LF. Autophagy regulates ageing in *C. elegans*. Autophagy 2007; 3:93-5.
22. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of life span by dietary restriction in *C. elegans*. PLoS Genet 2008; 4:24.
23. Jia K, Levine B. Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. Autophagy 2007; 3:597-9.
24. Morck C, Pilon M. Caloric restriction and autophagy in *Caenorhabditis elegans*. Autophagy 2007; 3:51-3.
25. Samara C, Syntichaki P, Tavernarakis N. Autophagy is required for necrotic cell death in *Caenorhabditis elegans*. Cell Death Differ 2008; 15:105-12.
26. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. Genome Biol 2001; 2.
27. Tasdemir E, Maiuri MC, Tajeddine N, Vitale I, Criollo A, Vicencio JM, Hickman JA, Geneste O, Kroemer G. Cell cycle-dependent induction of autophagy, mitophagy and reticulophagy. Cell Cycle 2007; 6:2263-7.
28. Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol 2007; 8:275-83.
29. Derry WB, Bierings R, van Iersel M, Satkunendran T, Reinke V, Rothman JH. Regulation of developmental rate and germ cell proliferation in *Caenorhabditis elegans* by the p53 gene network. Cell Death Differ 2007; 14:662-70.
30. Klionsky DJ. Cell biology: regulated self-cannibalism. Nature 2004; 431:31-2.
31. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451:1069-75.
32. Bauer JH, Poon PC, Glatt-Deeley H, Abrams JM, Helfand SL. Neuronal expression of p53 dominant-negative proteins in adult *Drosophila melanogaster* extends life span. Curr Biol 2005; 15:2063-8.
33. Mariño G, Ugalde AP, Salvador-Montoliu N, Varela I, Quirós PM, Cadiñanos J, van der Pluijm I, Freije JM, López-Otín C. Premature aging in mice activates a systemic metabolic response involving autophagy induction. Hum Mol Genet 2008; 15:2196-211.
34. Shiozaki M, Yoshimura K, Shibata M, Koike M, Matsuura N, Uchiyama Y, Gotow T. Morphological and biochemical signs of age-related neurodegenerative changes in klotho mutant mice. Neuroscience 2008; 152:924-41.
35. Feng Z, Hu W, Teresky AK, Hernando E, Cordon-Cardo C, Levine AJ. Declining p53 function in the aging process: a possible mechanism for the increased tumor incidence in older populations. Proc Natl Acad Sci USA 2007; 104:16633-8.