Caenorhabditis elegans, An Invertebrate Model Organism for Biomedical Research

Chanatip Metheetrairut, Ph.D., Wichit Suthammarak, M.D., Ph.D.
Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Limitations in using human samples or performing human experimentation prompt us to seek other organisms to be used as a model in biomedical study. *Caenorhabditis elegans (C. elegans)* is one of the most powerful model organisms. For the past few decades, many studies in *C. elegans* have laid a foundation of knowledge regarding numerous biological processes and underlying mechanism of human diseases. In Thailand, however, *C. elegans* is quite new and not well known in research. This article aims to introduce this small yet tremendously useful invertebrate as a study model for biomedical research to the Thai research community.

Keywords: *Caenorhabditis elegans*, nematode, animal model

Siriraj Med J 2015;67:149-154
E-journal: http://www.sirirajmedj.com

INTRODUCTION

In 1974, the free-living, non-pathogenic, soil nematode *Caenorhabditis elegans* was introduced by Sydney Brenner as a model organism to study development, neuroscience, and other biological processes.[1] Since its introduction, *C. elegans* has become a widely used animal model. Nowadays, approximately a thousand laboratories worldwide exploit *C. elegans* as a model organism. Despite being an invertebrate, this nematode's powerful genetics and its physical characteristics have facilitated many key discoveries in biology. The most recognized research works involving *C. elegans* include three Nobel prizes – the study of programmed cell death by Sydney Brenner, John E. Sulston, and H. Robert Horvitz in 2002; the discovery of RNA interference (RNAi) by Andrew Z. Fire and Craig C. Mello in 2006; and the development of green fluorescent protein (GFP) by Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien in 2008. Undoubtedly, *C. elegans* has been widely regarded as one of the major model organisms for biomedical research.

A simple invertebrate as a model organism

Anatomy- C. elegans is a small roundworm, approximately 1 mm in length.[2] Its simple body plan is made up of 959 cells in an adult hermaphrodite (excluding germ cells) or 1,031 cells in an adult male.[2] Each cell division, programmed cell death, and terminal differentiation during development has been well documented.[3,4] In addition, the complete wiring of *C. elegans'* 302 neurons (381 in males) has been mapped.[5] The invariant nature of cell numbers, cell lineages, and connections between cells in *C. elegans* is immensely useful to biological research, especially in developmental biology and neuroscience. That is because it is possible to identify and follow each specific cell throughout the animal's life and discern any deviation from the normal pattern. The *C. elegans* research community also benefits from having

Correspondence to: Wichit Suthammarak
E-mail: wichit.sut@mahidol.ac.th
Received 5 February 2015
Revised 23 March 2015
Accepted 23 March 2015

these anatomical data collected and organized in a curated online database.[2]

Genetics- The genome of *C. elegans* contains 97 Mb (x10^6 base pairs) of DNAs, arranged into 5 pairs of autosomes (chr I-V) and a pair of sex chromosomes (XX) for hermaphrodites or a single sex chromosome for males. These chromosomes host approximately 19,000 genes in total with median gene length of approximately1.9 kilobase pairs.[6] *C. elegans* is the first multicellular organism that has had its whole genome sequenced. Comparative genomics between humans and nematodes reveals significant similarities in genomic sequence and expression patterns of both protein-coding and non-coding genes, suggesting conservation of fundamental processes throughout evolution.[6,7] The conservation of biological pathways and genes across phyla underpins the use of *C. elegans* as a model organism in research related to human health.

Life cycle- Generally, *C. elegans* goes through 6 distinct developmental stages during its lifetime: hermaphrodite mothers lay eggs that undergo embryonic development. Soon after, these eggs hatch and enter the first larval stage (L1); and specific developmental events occur at a specific time during each of the four larval stages (L1-L4), which are separated from each other by a molt (i.e. shedding and synthesis of new cuticles). Finally, animals complete their post-embryonic development and reach adult stage.[8] Adult animals then lay ~100-300 eggs, completing *C. elegans'* life cycle in approximately 2-3 days.[9] This rapid reproduction of a great number of progeny is advantageous for research. At any given temperature, all wild-type animals develop synchronously, facilitating time-sensitive or developmental stage-specific study.

Maintenance- While *C. elegans'* natural habitat is in the soil, they have been successfully maintained in a laboratory setting on simple solid agar-based media with *E. coli* bacteria as food source.[1] A large number of animals can also be reared in liquid media for larger-scale experiments.[1] Starved *C. elegans* generally survives as a 'dauer' larva – an alternative to the L2 larval stage induced under harsh environments including starvation – when kept at ~20°C for several months. In addition, at least a small fraction of them will survive and recover from freezing condition (-80°C freezer or -196°C liquid nitrogen tank) even after many years, thus enabling long-term storage. The majority of *C. elegans* population comprises self-fertilizing hermaphrodites; therefore each strain can easily be maintained in an isogenic manner.[1] Conversely, a small percentage of males found in *C. elegans* population can be crossed with hermaphrodites when research questions require progeny from two different transgenic or mutant strains. *C. elegans* is easy to maintain both on a short-term and long-term basis, making it an ideal model organism.

Availability of research tools- Not only does the advancement in microscopy allow single cell observation, but *C. elegans'* transparent body means that any proteins with fluorescent tags are also visible. Additionally, inexpensive genetic techniques allow manipulation of genes of interest, for example, tagged gene expression or overexpression by microinjecting transgenes; gene silencing via ingested RNAi constructs; or deletion of DNA segments by chemical mutagenesis. Furthermore, cellular tools such as single cell ablation are useful in studying cell-cell interaction or its role within an organ. Many research tools are now available for *C. elegans* work to aid in answering many different biological questions.

Conservation between *C. elegans* and humans

Comparison of human genome to those of model organisms allows the identification of homologous/orthologous genes. According to OrthoDisease,[10] an online database of human disease gene orthologs, 5,632 *C. elegans* genes are human homologs – of those, 1,186 genes are orthologous to human disease genes. This impressive feature makes *C. elegans* a very useful model to study biological processes in humans as well as molecular pathologies of human diseases. Several genetic manipulation techniques have been used to study phenotypic alterations, which have helped

to build up the understanding of molecular mechanism of many diseases in human.

Relevance of the *C. elegans* model to human diseases

As *C. elegans* is a non-vertebrate model with no circulatory system, hesitation for using *C. elegans* in biomedical research remains as to how relevant this model can be. It is unavoidably challenging to identify *C. elegans* phenotypes that are directly comparable to disease manifestation, although it can be one of the best disease models if the disease is characterized on a molecular level. No animal models are perfect human disease models, not even mammalian model organisms. Non-mammalian models such as *C. elegans* have generally been used in the early phase of a study to deliver a quick clue to the answer. *C. elegans* is also one of the fastest and most cost-effective tools for high-throughput screening for gene functions by RNAi screening. In addition, *C. elegans* allows us to approach each question in the context of a whole organism in which a complex interplay among tissue types or organ systems plays a role.

Deciphering molecular pathogenesis of human diseases using *C. elegans*

We have collected some significant biomedical discoveries using *C. elegans* as follows:

Diabetes mellitus-In humans and other vertebrates, insulin plays an important role in regulating plasma glucose. Impairment of insulin production, secretion, or signaling ultimately results in diabetes mellitus. Intriguingly, the insulin-signaling pathway, or so-called insulin-like signaling pathway, is also conserved in *C. elegans*. It controls metabolism, growth, and longevity of the nematode. *daf-2* encodes the homolog of mammalian insulin receptor family in *C. elegans* whereas INS-1, an insulin-like peptide, has been shown to be its ligand.[11,12] *daf-16* was identified as one of the negative regulators of the insulin-signaling pathway in *C. elegans*.[13] Further analysis revealed that DAF-16 is an ortholog of the forkhead transcription factor FOXO. Afterwards, a loss-of-function allele of FOXO was shown to restore insulin sensitivity and reversed the diabetic phenotypes of insulin-resistant mice.[14]

Recently, De Haes *et al*,[15] suggested that metformin, the most commonly used antidiabetic drug, increased longevity of *C. elegans* through peroxiredoxin PRDX-2 signaling. Interestingly, this pathway is evolutionarily conserved.[16] It may then be plausible that metformin also elicits this effect in humans, resulting in an increased overall life expectancy.

Alzheimer's disease (AD)-The nervous system of *C. elegans* is far less complicated than that of humans, allowing simpler genetic dissection of the nervous system. Presenilin, the protein that plays an important role in pathogenesis of AD, was first discovered in *C. elegans*.[17] A few years afterwards, mutations in human presenilin-1 were implicated in early-onset familial AD.[18,19] Wittenburg *et al*,[20] showed that expression of human presenilin-1 in *C. elegans* could rescue the morphological and functional defects in cholinergic interneurons of *C. elegans* presinilin mutants, suggesting functional conservation of presenilin between in *C. elegans* and in humans.[21]

Autosomal dominant polycystic kidney disease (ADPKD)-Cystic kidney is a distinct characteristic of ADPKD in humans. This pathology results in gradual deterioration of kidney function, which ultimately leads to end-stage kidney failure. Although *C. elegans* has only a very primitive kidney-like organ, i.e. a single secretory cell, this invertebrate in fact provided the first evidence about the underlying molecular defect of ADPKD. Knockdown of the *C. elegans* genes *lov-1* and *pkd-2*, which are orthologs of human disease genes *PKD1* and *PKD2*, led to abnormal mating behavior.[22] This phenotype results from malfunctioning of the ciliated mechanosensory neurons in male tails, which are essential for proper mating behavior.[23] These findings suggest that ciliated cells require human polycystins (encoded by *PKD-1* and *PKD-2*) to sense pressure or fluid flow.[24] It was later confirmed in mice that polycystins contribute to fluid-flow sensing by the primary cilia in renal epithelium.[25] It has been suggested that dysfunctional polycystins may lead to cystic formation in kidneys due to the inability of renal cells to sense fluid flow and pressure which normally regulate tissue morphogenesis.[25]

Mitochondrial diseases-Dysfunction of mitochondria leads to a myriad of diseases that particularly involve organs with high-energy requirement. *C. elegans* is a useful model for mitochondrial disorders for several reasons. For instance, respiratory subunits of *C. elegans* mitochondria share extensive homology with their human counterparts.[26] There are classic mitochondrial mutants available, which include *gas-1* and *nuo-6* (complex I mutants), *clk-1* (ubiquinone deficiency mutant), *mev-1* (complex II mutant), and *isp-1* and *isp-1; ctb-1* (complex III mutants).

It has been demonstrated that mitochondrial supercomplexes, the functional entity of the electron transport chain that are conserved in many species including humans, also exist in *C. elegans*.[27] RNAi knockdown of subunits of the respiratory complex IV resulted in combined respiratory complex I and IV deficiencies in *C. elegans* despite normal level of complex I [27]. Evidently, low levels of complex IV shift the ratio of complex I from supercomplex I:III:IV to I:III. Since supercomplex I:III:IV catalyzes the NADH-Q reductase activity (complex I) 3 times faster than complex I in supercomplex I:III.[28,29] It was then proposed that this shift in supercomplex formation leads to a significant loss of enzymatic activity of complex I.[27] Evidence from studies in *C. elegans* shed light on a mechanism of combined respiratory complex I and IV deficiencies, which are cited as the most common form of combined deficiencies of respiratory complex in humans.[30-32]

Recently, Chen *et al*, used *C. elegans* mitochondria in quality assessment of respiratory complex assays, which are designed to be used as a diagnostic test for mitochondrial disorder in humans. The study emphasized the necessity of comparative testing of mitochondrial enzyme assays between laboratories.[33] Clearly, *C. elegans* can be an inexhaustible source of mitochondria with defined defects for assay development and as a potential source of control specimens.

Tumor-While it is impossible to recreate a full imitation of a tumor in a nematode, some fundamental aspects of it can be studied in isolation. The most important one is programmed cell death. In *C. elegans*, programmed cell death is studied in the context of development – certain cells are destined to die at a specific stage in all wild-type animals. Therefore, it is feasible to identify abnormal cell fates, which allow researchers to examine the genetic components and influence of neighboring cells on programmed cell death. Some important apoptotic genes identified in *C. elegans* are: ced-3, a member of the caspase protein family which executes cell killing; *ced-1* and *ced-2*, homologs of human CD91 and CRKII, respectively, which function in cell corpse engulfment following cell death; and *ced-9*, an inhibitor of apoptosis, whose human homolog was later identified to be the tumor-suppressor Bcl-1.[34]

Aging-Aging is a natural phenomenon for all species including humans. The lifespan of individuals may seem like a straightforward phenotype, but it results from the complex interplay between the accumulation of random damages throughout life and a myriad of genetic factors.[35-36] Model organisms such as *C. elegans* offer scientists the opportunity to dissect these interactions in a more manageable manner due to their simple genetics and short lifespan of approximately 2-3 weeks.[36]

One of the main pathways that influences aging in *C. elegans* is the insulin/insulin-like growth factor signaling (IIS) pathway.[36] This IIS pathway includes *age-1*, *daf-2*, and other genes that interact with or function upstream or downstream of these two genes.[13,36,37] In general, overall decrease of insulin-like signaling increases lifespan of an organism, no matter if that decrease is due to changes in levels of expression or activity of any factors. This is true not only in *C. elegans*, but also in other species such as *Drosophila* and mice.[38-41] In humans, longevity is associated with FOXO3A, a transcription factor in the IIS pathway; FOXO3's homologu, *daf-16*, likewise affects lifespan in *C. elegans*.[42-45]

While the IIS pathway responds to both internal and external stimuli including nutrient availability, diet also affects lifespan through the nutrient-sensing TOR (target of rapamycin) signaling pathway and mitochondrial metabolism (see review).[46] Not only does dietary restriction extends lifespan in *C. elegans* and several other species, but diet is also associated with mortality rate in humans (see review).[47] Several studies revealed the effect of diet on mitochondrial metabolism and energy generation: mild inhibition of mitochondrial respiratory chain activity lengthens

lifespan of yeast, *C. elegans*, *Drosophila*, and mice (see reviews)[35,48]. Furthermore, mitochondria are also a source of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It was originally believed that increased oxygen and increased production of ROS and RNS cause oxidative stress and thus shorten lifespan, whereas other studies showed that modest increase in ROS production has positive, if not neutral, impact on lifespan.[35,49,50] In addition, mutations of several other components of the mitochondrial respiratory chain either shorten or lengthen lifespan.[51,52] Further research is needed to reveal how the mutations that affect mitochondrial function in a similar manner produce seemingly opposite lifespan phenotypes as they may involve different downstream effectors we have yet to elucidate.

CONCLUSION

A plethora of human homologs and conserved biological processes have made *C. elegans* one of the most useful model organisms for biological study. Several important discoveries in biomedical research which stemmed from work done in *C. elegans* signify the success in applying this invertebrate for a disease model. Despite many advantages, research in *C. elegans* also has some limitations that preclude studies in certain aspects of human pathology. Yet, for many diseases, doing research in a simpler model could yield new insights that may lead to further development in disease prevention and treatment. Successful discovery depends upon defining specific research aims and being aware of the limitations of using *C. elegans* in a study.

REFERENCES

1. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974 May;77(1):71-94.
2. Altun ZF, Herndon LA, Crocker C, Lints R, Hall DH, eds. WormAtlas 2002-2015. Available from: http://www.wormatlas.org.
3. Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Developmental Biology. 1977 Mar;56(1):110-56.
4. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode Caenorhabditis elegans. Developmental biology. 1983 Nov;100(1):64-119.
5. Jarrell TA, Wang Y, Bloniarz AE, Brittin CA, Xu M, Thomson JN, et al. The connectome of a decision-making neural network. Science. 2012 Jul 27;337(6093):437-44.
6. Consortium CeS. Genome sequence of the nematode C. elegans: a platform for investigating biology. Science. 1998 Dec 11;282(5396):2012-8.
7. Gerstein MB, Rozowsky J, Yan KK, Wang D, Cheng C, Brown JB, et al. Comparative analysis of the transcriptome across distant species. Nature. 2014 Aug 28;512(7515):445-8.
8. Byerly L, Cassada RC, Russell RL. The life cycle of the nematode Caenorhabditis elegans. I. Wild-type growth and reproduction. Developmental biology. 1976 Jul 1;51(1):23-33.
9. Byerly L, Scherer S, Russell RL. The life cycle of the nematode Caenorhabditis elegans. II. A simplified method for mutant characterization. Developmental biology. 1976 Jul 1;51(1):34-48.
10. O'Brien KP, Westerlund I, Sonnhammer EL. Ortho Disease: a database of human disease orthologs. Human mutation. 2004 Aug;24(2):112-9.
11. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science. 1997 Aug 15;277(5328):942-6.
12. Ruvkun G, Hobert O. The taxonomy of developmental control in Caenorhabditis elegans. Science. 1998 Dec 11;282(5396):2033-41.
13. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, et al. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature. 1997 Oct 30;389(6654):994-9.
14. Nakae J, Biggs WH, 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, et al. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet. 2002 Oct;32(2):245-53.
15. De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, et al. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proceedings of the National Academy of Sciences of the United States of America. 2014 Jun 17;111(24):E2501-9.
16. Jarvis RM, Hughes SM, Ledgerwood EC. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free radical biology & medicine. 2012 Oct 1;53(7):1522-30.
17. Sundaram M, Greenwald I. Suppressors of a lin-12 hypomorph define genes that interact with both lin-12 and glp-1 in Caenorhabditis elegans. Genetics. 1993 Nov;135(3):765-83.
18. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature. 1995 Jun 29;375(6534):754-60.
19. Haass C, De Strooper B. The presenilins in Alzheimer's disease--proteolysis holds the key. Science. 1999 Oct 29;286(5441):916-9.
20. Wittenburg N, Eimer S, Lakowski B, Rohrig S, Rudolph C, Baumeister R. Presenilin is required for proper morphology and function of neurons in C. elegans. Nature. 2000 Jul 20;406(6793):306-9.
21. Levitan D, Doyle TG, Brousseau D, Lee MK, Thinakaran G, Slunt HH, et al. Assessment of normal and mutant human presenilin function in Caenorhabditis elegans. Proceedings

of the National Academy of Sciences of the United States of America. 1996 Dec 10;93(25):14940-4.
22. Barr MM, Sternberg PW. A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. Nature. 1999 Sep 23;401(6751):386-9.
23. Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH, Sternberg PW. The Caenorhabditis elegans autosomal dominant polycystic kidney disease gene homologs lov-1 and pkd-2 act in the same pathway. Current biology : CB. 2001 Sep 4;11(17):1341-6.
24. Kaletta T, Van der Craen M, Van Geel A, Dewulf N, Bogaert T, Branden M, et al. Towards understanding the polycystins. Nephron Experimental nephrology. 2003 Jan;93(1):e9-17.
25. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet. 2003 Feb;33(2):129-37.
26. Falk MJ, Zhang Z, Rosenjack JR, Nissim I, Daikhin E, Nissim I, et al. Metabolic pathway profiling of mitochondrial respiratory chain mutants in C. elegans. Mol Genet Metab. 2008 Apr;93(4):388-97.
27. Suthammarak W, Yang YY, Morgan PG, Sedensky MM. Complex I function is defective in complex IV-deficient Caenorhabditis elegans. J Biol Chem. 2009 Mar 6;284 (10):6425-35.
28. Suthammarak W, Morgan PG, Sedensky MM. Mutations in mitochondrial complex III uniquely affect complex I in Caenorhabditis elegans. J Biol Chem. 2010 Dec 24;285 (52):40724-31.
29. Schafer E, Seelert H, Reifschneider NH, Krause F, Dencher NA, Vonck J. Architecture of active mammalian respiratory chain supercomplexes. J Biol Chem. 2006 Jun 2;281 (22):15370-5.
30. Korenke GC, Bentlage HA, Ruitenbeek W, Sengers RC, Sperl W, Trijbels JM, et al. Isolated and combined deficiencies of NADH dehydrogenase (complex I) in muscle tissue of children with mitochondrial myopathies. European journal of pediatrics. 1990 Dec;150(2):104-8.
31. Mizusawa H, Watanabe M, Kanazawa I, Nakanishi T, Kobayashi M, Tanaka M, et al. Familial mitochondrial myopathy associated with peripheral neuropathy: partial deficiencies of complex I and complex IV. Journal of the neurological sciences. 1988 Sep;86(2-3):171-84.
32. Tanaka M, Nishikimi M, Suzuki H, Ozawa T, Koga Y, Nonaka I. Partial deficiency of subunits in complex I or IV of patients with mitochondrial myopathies. Biochemistry international. 1987 Mar;14(3):525-30.
33. Chen X, Thorburn DR, Wong LJ, Vladutiu GD, Haas RH, Le T, et al. Quality improvement of mitochondrial respiratory chain complex enzyme assays using Caenorhabditis elegans. Genetics in medicine : official journal of the American College of Medical Genetics. 2011 Sep;13(9):794-9.
34. Horvitz HR. Worms, life, and death (Nobel lecture). Chembiochem : a European journal of chemical biology. 2003 Aug 4;4(8):697-711. PubMed PMID: 12898619.
35. Kenyon CJ. The genetics of ageing. Nature. 2010 Mar 25;464(7288):504-12.
36. Antebi A. Genetics of aging in Caenorhabditis elegans. PLoS genetics. 2007 Sep;3(9):1565-71.
37. Friedman DB, Johnson TE. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics. 1988 Jan;118(1):75-86.
38. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, et al. Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. Science. 2001 Apr 6;292(5514):104-6.
39. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science. 2001 Apr 6;292(5514):107-10.
40. Bluher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. Science. 2003 Jan 24;299(5606):572-4.
41. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature. 2003 Jan 9;421(6919):182-7.
42. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A C. elegans mutant that lives twice as long as wild type. Nature. 1993 Dec 2;366(6454):461-4.
43. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, et al. FOXO3A genotype is strongly associated with human longevity. Proceedings of the National Academy of Sciences of the United States of America. 2008 Sep 16;105(37): 13987-92.
44. Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, Nikolaus S, et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proceedings of the National Academy of Sciences of the United States of America. 2009 Feb 24;106(8):2700-5.
45. Soerensen M, Nygaard M, Dato S, Stevnsner T, Bohr VA, Christensen K, et al. Association study of FOXO3A SNPs and aging phenotypes in Danish oldest-old individuals. Aging cell. 2015 Feb;14(1):60-6.
46. Cerqueira FM, Kowaltowski AJ. Mitochondrial metabo lism in aging: effect of dietary interventions. Ageing research reviews. 2013 Jan;12(1):22-8.
47. Lorenzini A. How Much Should We Weigh for a Long and Healthy Life Span? The Need to Reconcile Caloric Restriction versus Longevity with Body Mass Index versus Mortality Data. Frontiers in endocrinology. 2014;5:121.
48. Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell. 2005 Feb 25;120(4):449-60.
49. Hwang AB, Ryu EA, Artan M, Chang HW, Kabir MH, Nam HJ, et al. Feedback regulation via AMPK and HIF-1 mediates ROS-dependent longevity in Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America. 2014 Oct 21;111(42): E4458-67.
50. Lee SJ, Hwang AB, Kenyon C. Inhibition of respiration extends C. elegans life span via reactive oxygen species that increase HIF-1 activity. Current biology : CB. 2010 Dec 7;20(23):2131-6.
51. Suthammarak W, Somerlot BH, Opheim E, Sedensky M, Morgan PG. Novel interactions between mitochondrial superoxide dismutases and the electron transport chain. Aging cell. 2013 Dec;12(6):1132-40.
52. Yang W, Hekimi S. A mitochondrial superoxide signal triggers increased longevity in Caenorhabditis elegans. PLoS biology. 2010;8(12):e1000556.