Systems Biology in Aging Research

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Abstract

Systems biology is an approach to collect high-dimensional data and analyze in an integrated manner. As aging is a complicated physiological functional decline in biological system, the methods in systems biology could be utilized in aging studies. Here we reviewed recent advances in systems biology in aging research and divide them into two major parts. One is the data resource, which includes omics data from DNA, RNA, proteins, epigenetic changes, metabolisms, and recently single-cell-level variations. The other is the data analysis methods consisting of network and modeling approaches. With all the data and the tools to analyze them, we could further promote our understanding of the systematic aging.

Keywords

Aging · Systems biology · Network · Omics · Single cell

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1.1   Introduction

Aging is the time-dependent physiological functional decline in all aspects of a biological system, which ultimately leads to death. Systems biology combines computational modeling and simulation, with large-scale experiments, to explore dynamic behavior in biological systems (Cassman 2005), which is an ideal approach to study a systems-level problem like aging. In this chapter we will discuss the data resource and analysis approaches in aging systems biology.

1.2   Data Resource for Systems Biology in Aging Research

The omics data are the basic building blocks for constructing a global view of a tissue or organism in the aging process through systems biology approaches. The following will highlight different omics data source in the aging research and their findings.

1.2.1   Genomics

The genome-wide association studies in aging research are based on genetic variants measured by single nucleotide polymorphism (SNP) chips or high-throughput genome sequencing and phenotypes such as the chronological age- or healthy aging-related phenotypes. A series of twin studies (Paneni et al. 2017; Ljungquist et al. 1998; Skytthe et al. 2003) have shown that 20–30% of the overall variation of human lifespan can be attributed to genetic factors, indicating that lifespan is not genetically determined to a large extent, although the genetic influences on lifespan increase after age 60 (Hjelmborg et al. 2006). With that being said, there are apparently some genetic determinants for longevity. The SNPs on APOE (Gerdes et al. 2000; Ewbank 2007; Deelen et al. 2011; Joshi et al. 2017; Sebastiani et al. 2017) and FOXO3A (Joshi et al. 2017; Willcox et al. 2008; Pawlikowska et al. 2009; Flachsbart et al. 2009; Anselmi et al. 2009) are repeatedly found to be associated with longevity in studies of centenarians versus younger controls. In contrast, a recent GWAS research on healthy aging reveals that healthy aging (in this study defined as people >80 years without chronic diseases and not taking chronic medications) shares no SNP loci with exceptional longevity, suggesting they are very divergent phenomena, although they are intuitively expected to share some common features (Erikson et al. 2016). Instead this study found no major single contributor to healthy aging (Erikson et al. 2016).

1.2.2   Epigenomics

Genome-wide DNA methylation can be measured by chip (Illumnia 450 K or 850 K chip) or sequencing (whole-genome bisulfite sequencing, reduced-representation

bisulfite sequencing, methylated DNA immunoprecipitation sequencing, or methyl-CpG binding domain enriched sequencing) (Bock et al. 2010; Harris et al. 2010). The global pattern of DNA methylation during aging is hypo-methylation in repetitive sequences, hyper-methylation in promoter regions, and higher intercell variability (Bacalini et al. 2014; Cevenini et al. 2008). A study using DNA methylation to estimate the state of aging in blood found that only three CpG sites could predict age with a mean absolute deviation from chronological age of less than 5 years (Weidner et al. 2014), providing a DNA methylation-based aging biomarker. A cross-sectional study that evaluated DNA methylation in boys aged 3–17 years found that >88% pediatric age-associated loci trend in the same direction as in adulthood, suggesting that some of the methylation changes with age take place in early life stages (Alisch et al. 2012). Aging-associated DNA methylation is shared across different tissues within the same individuals, as indicated by one research which found that differentially methylated regions in whole blood can be replicated in buccal cells (Rakyan et al. 2010), and another research found that age-methylation correlations are well preserved between the brain and blood (Horvath et al. 2012).

1.2.3 Transcriptomics

Transcriptome is also measured by either microarrays or RNA sequencing methods. Changes in the aging transcriptome are found to be tissue-specific, as most of the changes from the brain (Lu et al. 2004; Berchtold et al. 2008), skin (Glass et al. 2013), adipose tissue (Glass et al. 2013), kidney (Rodwell et al. 2004), and blood (Peters et al. 2015) did not overlap with other tissues. And the change also shows species specificity, because a cross-species analysis found only 73 genes consistently associated with age (de Magalhães et al. 2009). The repeated biological functions that change in the aging process include increased inflammation and decreased energy metabolism especially mitochondrial functions (Zierer et al. 2015).

1.2.4 Proteomics

Current proteomic techniques based on immunoassays, protein arrays, or mass spectrometry can measure only a small fraction of the proteome (up to 1000 proteins per a sample). The most comprehensive description of the human proteome across various human tissues, cell lines, and body fluids to date consists of 18,097 proteins collected from 16,857 liquid chromatography tandem-mass spectrometry (LCMS/MS) experiments (Wilhelm et al. 2014). Recent research using quantitative middle-down proteomics found that a histone variant H3.3 is accumulated during aging (Tvardovskiy et al. 2017), and another research in *Drosophila* showed that tissue-specific proteome in long-lived mutant strains new insights on the insulin/IGF signaling pathway (Tain et al. 2017). A proteomics study of young and old B cells found that protein related to stress management in mitochondria and DNA repair is under significant regulation during aging (Mayer et al. 2017). Besides the identification of proteins from proteomics data, a distinctive value of such data

source is the posttranslational modification (PTM) information, which cannot be directly measured by any other omics but can alter biochemical properties of proteins. PTM is significantly changed during aging, for example, levels of N-glycosylation correlate with familial longevity and healthy aging (Ruhaak et al. 2011) and linear combination of only three IgG glycans explained up to 58% of variance in age in a research of four European populations (Krištić et al. 2014). As mass spectrometry (MS)-based proteomics fields are more open to data sharing practice, it is the golden age to analyze public proteomics data (Martens and Vizcaíno 2017). OpenMS (Röst et al. 2016) is an open-source tool available to assist such analyses.

1.2.5 Metabolomics

Metabolomics profiles the low-molecular-weight molecules in a biological sample. Similar to proteomics, this profiling is based on either mass spectrometry or nuclear magnetic resonance. To date, there is no analytical method available to determine and quantify all metabolites in a single experiment (Adamski and Suhre 2013). From 2008 till today, a series of metabolomics studies in human aging have been done (Gonzalez-Covarrubias et al. 2013; Lawton et al. 2008; Menni et al. 2013; Yu et al. 2012) in small to large cohorts. A lipidomics study in middle-aged offspring of nonagenarians found that improved antioxidant capacity and more efficient β-oxidation function might be responsible for increased lifespan in women (Gonzalez-Covarrubias et al. 2013), and another study found that C-linked glycosylated tryptophan was highly correlated with age and aging traits, such as lung function, bone mineral density, and blood pressure (Menni et al. 2013). Now metabolomics are often conducted with other layers of omics to facilitate the study, such as in the proteomics study mentioned before, metabolomics are used to verify their conclusions (Mayer et al. 2017).

1.2.6 Metagenomics

The human metagenomics refers to the collective genome of microbial species hosted by the human body. Metagenomics of fecal samples found that the separation of microbiota composition significantly correlated with measures of frailty, markers of inflammation and nutritional status in older people, as well as their residential situation (Claesson et al. 2012).

1.2.7 Phenomics

Phenomics refers to the clinical and lifestyle traits, ranging from anthropometric measures to health and lifestyle questionnaires (Moayyeri et al. 2013). As aging is tightly linked to lifestyle, for example, calorie restriction and exercise are

repeatedly found to slow aging (Green et al. 2017), phenomics is especially valuable in aging research. The Rockwood frailty index, which is composed of symptoms, signs, diseases, and disabilities, could be used as a measure of biological age (Rockwood and Mitnitski 2007). The phenomics could be interdependent on each other, such as faster telomere attrition, and higher inflammaging burden (measured by interleukin-1β) was associated with lower grip strength (Baylis et al. 2014). Recently, the human 3D face was also profiled for the aging study, and features extracted from the 3D such as eye slopes were found to be tightly associated with age, while physical age predicted from the 3D face was found to be more consistent with health indicators than chronological age (Chen et al. 2015).

1.2.8   Single-Cell/Organism Measurement

Although not necessarily through omics approaches, single-cell/organism measurement could also be informative for aging research and suit the need for systems biology as such experiments often generate big dataset for the downstream integrative analysis. The aging-related immune system changes have been investigated via 15-color flow cytometry panel (measures 14 proteins) in 28 T cell subpopulations in human (Lu et al. 2016) and single-cell RNA-seq in naïve and effector memory CD4+ T cells in mice from two divergent species (Martinez-Jimenez et al. 2017). The latter found that aging increases cell-to-cell variation on transcriptome level, which suggests that transcriptomic switch driven by immunological activation is no longer controlled as tight as in young mice (Martinez-Jimenez et al. 2017). Another single-cell RNA-seq study in human pancreas of 2544 single cells from 8 donors spanning six decades of life found that older donors display increased levels of transcriptional noise and potential fate drift (Enge et al. 2017). With the development of micro-fluid technology in model animals such as yeast *S. cerevisiae* (Chen et al. 2017) and worm *C. elegans* (Xian et al. 2013) or other equivalent culture techniques utilizing a polyethylene glycol hydrogel and a silicone elastomer (Pittman et al. 2017), there have been significant efforts to delineate the long-time puzzle about how aging differs among genetically identical individuals within the same species, which reflects the stochastic nature of the aging process.

1.3   Data Analysis for Systems Biology in Aging Research

The data analysis for aging systems biology generally could be separated into two parts: the network approach and the mathematical modeling approach. The following will briefly discuss the advance of the application of such approaches in the aging research and their conclusions.

1.3.1   Network Construction

One way to integrate the result of an omics study in a systems biology context is to project the variables of interest onto known reference networks, such as protein-protein interaction (PPI) networks, gene regulatory networks (GRN), or metabolic networks. PPI can be obtained from the Human Protein Reference Database (Keshava Prasad et al. 2009), the MIPS mammalian protein-protein interaction database (Pagel et al. 2005), the Reactome database (Fabregat et al. 2017), and the STRING database (Szklarczyk et al. 2017). Metabolic networks are mainly from Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2017).

On such predefined network, aging-associated proteins are found to be highly connected hubs in the PPI network (Bell et al. 2009), and type I diabetes is more tightly related to aging than type II diabetes using an asymmetric closeness based on the PPI network (Wang et al. 2009). Through integration of DNA methylation and PPI, tissue-independent age-associated hotspots were found to target stem cell differentiation pathways (West et al. 2013). By restricting the PPI to age-specific highly expressed genes, although the global network topologies did not change, the centrality of several genes correlated with age (Faisal and Milenković 2014). A study from our laboratory analyzed the topology of aging-related PPI subnetwork in which interacting gene pairs are transcriptionally co-expressed or anti-expressed during human brain aging and found that the PPIs connecting anti-expressed genes are enriched for lifespan regulators and transcriptional and epigenetic regulators (Xia et al. 2006).

Another way of network inference is through data-driven approaches, which can be separated into five major classes according to the Dialogue on Reverse Engineering Assessment and Methods (DREAM) project: regression, mutual information, correlation, Bayesian networks, and others (Marbach et al. 2012). One should keep in mind that network inference is at best an indication of association and experimental validations are always needed to demonstrate causality. The following are some examples of network construction efforts in aging research.

The weighted gene co-expression network analysis is a method to infer the gene-gene interaction networks from transcriptomics data (Zhang and Horvath 2005), and by applying the method to gene expression data from 30 adult human frontal cortex samples of different ages and comparing the resulting network to a network derived from AD transcriptome, Miller and colleagues found that healthy aging of the brain and AD share features in the decline or mitochondrial activity and synaptic plasticity (Miller et al. 2008). Such co-expression- or correlation-based network can be also used to integrate multiple layers of data, for example, in a recent effort to profile young and old adults' vaccinal responses, a multiscale, multifactorial response network spanning transcriptomic and metabolomics signatures, cell populations, and cytokine levels was built and reveals striking associations between orthogonal datasets (Li et al. 2017). Similar idea could be generalized to single-cell

transcriptome analysis, as has been done in the SCENIC computational tool, which could simultaneously reconstruct gene regulatory network and identify cellular states (Aibar et al. 2017).

Probabilistic graphical models are an important class of networks that can be built with high-throughput data (Friedman 2004). In a study of metabolomics data, a Gaussian graphical model (GGM) was applied to infer association networks (Krumsiek et al. 2011). GGM is also applied in aging research to reconstruct networks from metabolic data and identify modules (Murphy et al. 2017). A Bayesian network is a directed acyclic graph inferred from data which could extract biological meaningful associations without prior knowledge (Friedman et al. 2000). Recently our laboratory developed an algorithm that could combine the public intervention data to infer a Bayesian network (Li et al. 2013) and applied it to transcriptomic data of *C. elegans* during normal aging and dietary restriction (DR), which led to the finding that there are extensive feedback controls which exist among three modules mediating DR-induced longevity and validated them by lifespan assay (Hou et al. 2016).

1.3.2   Model Aging Systems

The ultimate goal of systems biology is to quantitatively model an organism, conduct in silico experiments, and generate hypotheses and predictions. While whole-organism models have been attempted in yeast (Karr et al. 2012), modeling a subsystem of an organism based on prior knowledge also allows mechanistic insights on the biological process such as aging. A stochastic network model of cell senescence based on telomere reduction, mitochondria damage, and nuclear somatic mutations was built, and the simulation from this model was consistent with published data on intra-clonal variability in cell-doubling potential (Sozou and Kirkwood 2001). The same group also developed a mathematical model to describe the heat shock system and to describe the influence of chaperones and accumulation of misfolded proteins on aging (Proctor et al. 2005). Another modeling work focused on the mitochondrial fission and fusion events and found that the simulation from their model was consistent with two experimental findings so that this model could provide evidences for age-related accumulation of mitochondrial deletion mutants (Kowald et al. 2005). An in silico model of the chronic effects of elevated cortisol on hippocampal atrophy was developed, and simulations using ordinary differential equations suggested that chronic increase in cortisol levels leads to faster decline in hippocampal output than acute bursts (McAuley et al. 2009). The epigenetic changes in aging stem cells were also modeled to explain why increased stem cell proliferation can lead to progeroid phenotypes (Przybilla et al. 2014). One interesting effort besides the researches in the biological side of aging is the facial aging modeling,

which is useful in looking for lost children or wanted fugitives, utilizing four types of approaches: physical model-based approaches, prototyping, function-based approaches, and evaluation targeted approach, and the results were impressive (Suo et al. 2012).

1.4 Conclusions

With the rapid development of various omics mapping methods, and accumulating big data, studying aging at systems biology level is now not only feasible but becoming a necessity to complement traditional one-gene-at-a-time approaches. Aging systems biology (data sources and analysis are summarized in Fig. 1.1) will bring new insights to aging both macroscopically at the network level and microcosmically using mathematical models. Single-cell technology will further fuel the aging systems biology study toward single-cell levels, and linked with big data generated at the cellular, tissue, and whole-organism levels, the time is ripe for aging systems biology to take off and reap fruits.
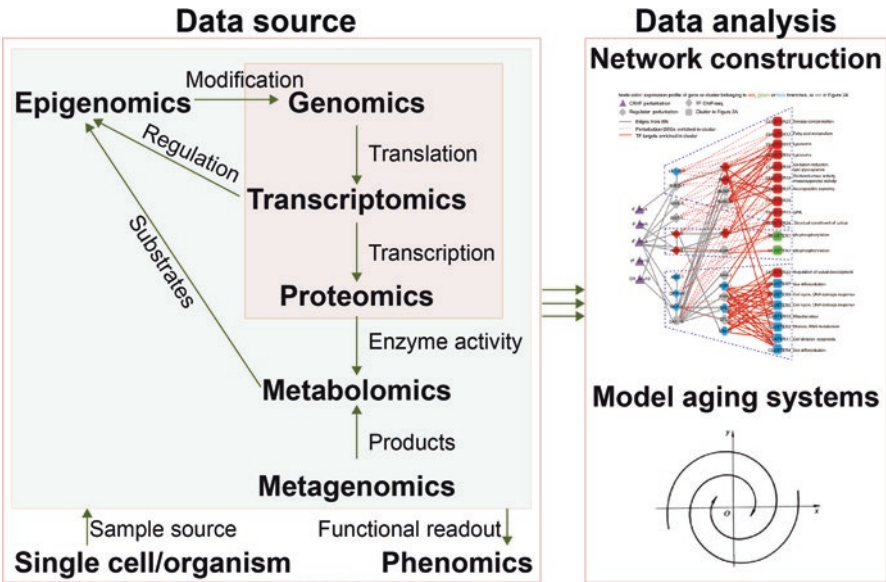

Fig. 1.1 Intervention of data source and analysis in aging systems biology. In this concise sketch map, all the types of data sources and analysis methods are nested in the network to show their interdependency to each other. The network is obtained from Hou et al. (2016)

References

Adamski J, Suhre K (2013) Metabolomics platforms for genome wide association studies – linking the genome to the metabolome. Curr Opin Biotechnol 24:39–47. Published online Epub2013/02//. https://doi.org/10.1016/j.copbio.2012.10.003

Aibar S, González-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, Hulselmans G, Rambow F, Marine J-C, Geurts P, Aerts J, J. v. d. Oord, Atak ZK, Wouters J, Aerts S (2017) SCENIC: single-cell regulatory network inference and clustering. Nat Methods 14:1083. Published online Epub2017/11//. https://doi.org/10.1038/nmeth.4463

Alisch RS, Barwick BG, Chopra P, Myrick LK, Satten GA, Conneely KN, Warren ST (2012) Age-associated DNA methylation in pediatric populations. Genome Res 22:623–632. Published online Epub2012/01/04/. https://doi.org/10.1101/gr.125187.111

Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, Condorelli G, Bellazzi R, Puca AA (2009) Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. Rejuvenation Res 12:95–104. Published online Epub2009/04//. https://doi.org/10.1089/rej.2008.0827

Bacalini MG, Friso S, Olivieri F, Pirazzini C, Giuliani C, Capri M, Santoro A, Franceschi C, Garagnani P (2014) Present and future of anti-ageing epigenetic diets. Mech Ageing Dev:136, 101–137, 115. Published online Epub2014/04//Mar- undefined. https://doi.org/10.1016/j.mad.2013.12.006

Baylis D, Ntani G, Edwards MH, Syddall HE, Bartlett DB, Dennison EM, Martin-Ruiz C, von Zglinicki T, Kuh D, Lord JM, Aihie Sayer A, Cooper C (2014) Inflammation, telomere length, and grip strength: a 10-year longitudinal study. Calcif Tissue Int 95:54–63. Published online Epub2014/07//. https://doi.org/10.1007/s00223-014-9862-7

Bell R, Hubbard A, Chettier R, Chen D, Miller JP, Kapahi P, Tarnopolsky M, Sahasrabuhde S, Melov S, Hughes RE (2009) A human protein interaction network shows conservation of aging processes between human and invertebrate species. PLoS Genet 5:e1000414. Published online Epub2009/03//. https://doi.org/10.1371/journal.pgen.1000414

Berchtold NC, Cribbs DH, Coleman PD, Rogers J, Head E, Kim R, Beach T, Miller C, Troncoso J, Trojanowski JQ, Zielke HR, Cotman CW (2008) Gene expression changes in the course of normal brain aging are sexually dimorphic. Proc Natl Acad Sci U S A 105:15605–15610. Published online Epub2008/10/07/. https://doi.org/10.1073/pnas.0806883105

Bock C, Tomazou EM, Brinkman AB, Müller F, Simmer F, Gu H, Jäger N, Gnirke A, Stunnenberg HG, Meissner A (2010) Quantitative comparison of genome-wide DNA methylation mapping technologies. Nat Biotechnol 28:1106–1114. Published online Epub2010/10//. https://doi.org/10.1038/nbt.1681

Cassman M (2005) Barriers to progress in systems biology. Nature 438:1079. Published online Epub2005/12/22/. https://doi.org/10.1038/4381079a

Cevenini E, Invidia L, Lescai F, Salvioli S, Tieri P, Castellani G, Franceschi C (2008) Human models of aging and longevity. Expert Opin Biol Ther 8:1393–1405. Published online Epub2008/09//. https://doi.org/10.1517/14712598.8.9.1393

Chen W, Qian W, Wu G, Chen W, Xian B, Chen X, Cao Y, Green CD, Zhao F, Tang K, Han JD (2015) Three-dimensional human facial morphologies as robust aging markers. Cell Res 25:574–587. Published online EpubMay

Chen KL, Crane MM, Kaeberlein M (2017) Microfluidic technologies for yeast replicative lifespan studies. Mech Ageing Dev 161:262–269. Published online Epub2017/01//. https://doi.org/10.1016/j.mad.2016.03.009

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HMB, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, Sinderen D v, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW (2012) Gut microbiota composition correlates with diet and health in the elderly. Nature 488:178. Published online Epub2012/08//. https://doi.org/10.1038/nature11319

Deelen J, Beekman M, Uh H-W, Helmer Q, Kuningas M, Christiansen L, Kremer D, van der Breggen R, Suchiman HED, Lakenberg N, van den Akker EB, Passtoors WM, Tiemeier H, van Heemst D, de Craen AJ, Rivadeneira F, de Geus EJ, Perola M, van der Ouderaa FJ, Gunn DA, Boomsma DI, Uitterlinden AG, Christensen K, van Duijn CM, Heijmans BT, Houwing-Duistermaat JJ, Westendorp RGJ, Slagboom PE (2011) Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. Aging Cell 10:686–698. Published online Epub2011/08//. https://doi.org/10.1111/j.1474-9726.2011.00705.x

de Magalhães JP, Curado J, Church GM (2009) Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics (Oxford, England) 25:875–881. Published online Epub2009/04/01/. https://doi.org/10.1093/bioinformatics/btp073

Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, Kim SK, Quake SR (2017) Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. Cell 171:321–330.e314. Published online Epub2017/10/05/. https://doi.org/10.1016/j.cell.2017.09.004

Erikson GA, Bodian DL, Rueda M, Molparia B, Scott ER, Scott-Van Zeeland AA, Topol SE, Wineinger NE, Niederhuber JE, Topol EJ, Torkamani A (2016) Whole-genome sequencing of a healthy aging cohort. Cell 0. Published online Epub2016/04/21/. https://doi.org/10.1016/j.cell.2016.03.022

Ewbank DC (2007) Differences in the association between apolipoprotein E genotype and mortality across populations. J Gerontol A Biol Sci Med Sci 62:899–907. Published online Epub2007/08//

Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, Haw R, Jassal B, Korninger F, May B, Milacic M, Roca CD, Rothfels K, Sevilla C, Shamovsky V, Shorser S, Varusai T, Viteri G, Weiser J, Wu G, Stein L, Hermjakob H, P. D'Eustachio (2017) The Reactome pathway knowledgebase. Nucleic Acids Res. Published online Epub2017/11/14/. https://doi.org/10.1093/nar/gkx1132

Faisal FE, Milenković T (2014) Dynamic networks reveal key players in aging. Bioinformatics (Oxford, England) 30:1721–1729. Published online Epub2014/06/15/. https://doi.org/10.1093/bioinformatics/btu089

Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A (2009) Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc Natl Acad Sci U S A 106:2700–2705. Published online Epub2009/02/24/. https://doi.org/10.1073/pnas.0809594106

Friedman N (2004) Inferring cellular networks using probabilistic graphical models. Science 303:799–805. Published online Epub2004/02/06/. https://doi.org/10.1126/science.1094068

Friedman J, Hastie T, Tibshirani R (2000) Additive logistic regression: a statistical view of boosting. Ann Stat 28:337–374. Published online Epub2000/04//. https://doi.org/10.1214/aos/1016218223

Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW (2000) Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a "frailty gene," not a "longevity gene". Genet Epidemiol 19:202–210. Published online Epub2000/10//. https://doi.org/10.1002/1098-2272(200010)19:3<202::AID-GEPI2>3.0.CO;2-Q

Glass D, Viñuela A, Davies MN, Ramasamy A, Parts L, Knowles D, Brown AA, Hedman AK, Small KS, Buil A, Grundberg E, Nica AC, Di Meglio P, Nestle FO, Ryten M, U. K. B. E. Consortium, Mu TC, Durbin R, McCarthy MI, Deloukas P, Dermitzakis ET, Weale ME, Bataille V, Spector TD (2013) Gene expression changes with age in skin, adipose tissue, blood and brain. Genome Biol 14:R75. Published online Epub2013/07/26/. https://doi.org/10.1186/gb-2013-14-7-r75

Gonzalez-Covarrubias V, Beekman M, Uh H-W, Dane A, Troost J, Paliukhovich I, van der Kloet FM, Houwing-Duistermaat J, Vreeken RJ, Hankemeier T, Slagboom EP (2013) Lipidomics of familial longevity. Aging Cell 12:426–434. Published online Epub2013/06//. https://doi.org/10.1111/acel.12064

Green CD, Huang Y, Dou X, Yang L, Liu Y, Han J-DJ (2017) Impact of dietary interventions on noncoding RNA networks and mRNAs encoding chromatin-related factors. Cell Rep 18:2957–2968. Published online Epub2017/03//. https://doi.org/10.1016/j.celrep.2017.03.001

Harris RA, Wang T, Coarfa C, Nagarajan RP, Hong C, Downey SL, Johnson BE, Fouse SD, Delaney A, Zhao Y, Olshen A, Ballinger T, Zhou X, Forsberg KJ, Gu J, Echipare L, O'Geen H, Lister R, Pelizzola M, Xi Y, Epstein CB, Bernstein BE, Hawkins RD, Ren B, Chung W-Y, Gu H, Bock C, Gnirke A, Zhang MQ, Haussler D, Ecker JR, Li W, Farnham PJ, Waterland RA, Meissner A, Marra MA, Hirst M, Milosavljevic A, Costello JF (2010) Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications. Nat Biotechnol 28:1097–1105. Published online Epub2010/10//. https://doi.org/10.1038/nbt.1682

Hjelmborg JV, Iachine I, Skytthe A, Vaupel JW, McGue M, Koskenvuo M, Kaprio J, Pedersen NL, Christensen K (2006) Genetic influence on human lifespan and longevity. Hum Genet 119:312. Published online Epub2006/04/01/. https://doi.org/10.1007/s00439-006-0144-y

Horvath S, Zhang Y, Langfelder P, Kahn RS, Boks MPM, van Eijk K, van den Berg LH, Ophoff RA (2012) Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol 13:R97. Published online Epub2012/10/03/. https://doi.org/10.1186/gb-2012-13-10-r97

Hou L, Wang D, Chen D, Liu Y, Zhang Y, Cheng H, Xu C, Sun N, McDermott J, Mair WB, Han J-DJ A Systems Approach (2016) To reverse engineer lifespan extension by dietary restriction. Cell Metab 23:529–540. Published online Epub2016/03//. https://doi.org/10.1016/j.cmet.2016.02.002

Joshi PK, Pirastu N, Kentistou KA, Fischer K, Hofer E, Schraut KE, Clark DW, Nutile T, Barnes CLK, Timmers PRHJ, Shen X, Gandin I, McDaid AF, Hansen TF, Gordon SD, Giulianini F, Boutin TS, Abdellaoui A, Zhao W, Medina-Gomez C, Bartz TM, Trompet S, Lange LA, Raffield L, Spek A, Galesloot TE, Proitsi P, Yanek LR, Bielak LF, Payton A, Murgia F, Concas MP, Biino G, Tajuddin SM, Seppälä I, Amin N, Boerwinkle E, Børglum AD, Campbell A, Demerath EW, Demuth I, Faul JD, Ford I, Gialluisi A, Gögele M, Graff M, Hingorani A, Hottenga J-J, Hougaard DM, Hurme MA, Ikram MA, Jylhä M, Kuh D, Ligthart L, Lill CM, Lindenberger U, Lumley T, Mägi R, Marques-Vidal P, Medland SE, Milani L, Nagy R, Ollier WER, Peyser PA, Pramstaller PP, Ridker PM, Rivadeneira F, Ruggiero D, Saba Y, Schmidt R, Schmidt H, Slagboom PE, Smith BH, Smith JA, Sotoodehnia N, Steinhagen-Thiessen E, Rooij FJA, Verbeek AL, Vermeulen SH, Vollenweider P, Wang Y, Werge T, Whitfield JB, Zonderman AB, Lehtimäki T, Evans MK, Pirastu M, Fuchsberger C, Bertram L, Pendleton N, Kardia SLR, Ciullo M, Becker DM, Wong A, Psaty BM, Duijn CM, Wilson JG, Jukema JW, Kiemeney L, Uitterlinden AG, Franceschini N, North KE, Weir DR, Metspalu A, Boomsma DI, Hayward C, Chasman D, Martin NG, Sattar N, Campbell H, Esko T, Kutalik Z, Wilson JF (2017) Genome-wide meta-analysis associates HLA-DQA1/DRB1 and LPA and lifestyle factors with human longevity. Nat Commun 8:910. Published online Epub2017/10/13/. https://doi.org/10.1038/s41467-017-00934-5

Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45:D353–D361. Published online Epub2017/01/04/. https://doi.org/10.1093/nar/gkw1092

Karr JR, Sanghvi JC, Macklin DN, Gutschow MV, Jacobs JM, Bolival B, Assad-Garcia N, Glass JI, Covert MW (2012) A whole-cell computational model predicts phenotype from genotype. Cell 150:389–401. Published online Epub2012/07/20/. https://doi.org/10.1016/j.cell.2012.05.044

Keshava Prasad TS, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan DS, Sebastian A, Rani S, Ray S, Harrys Kishore CJ, Kanth S, Ahmed M, Kashyap MK, Mohmood R, Ramachandra YL, Krishna V, Rahiman BA, Mohan S, Ranganathan P, Ramabadran S, Chaerkady R, Pandey A (2009) Human protein reference database – 2009 update. Nucleic Acids Res 37:D767–D772. Published online Epub2009/01//. https://doi.org/10.1093/nar/gkn892

Kowald A, Jendrach M, Pohl S, Bereiter-Hahn J, Hammerstein P (2005) On the relevance of mitochondrial fusions for the accumulation of mitochondrial deletion mutants: a

modelling study. Aging Cell 4:273–283. Published online Epub2005/10//. https://doi.org/10.1111/j.1474-9726.2005.00169.x

Krištić J, Vučković F, Menni C, Klarić L, Keser T, Beceheli I, Pučić-Baković M, Novokmet M, Mangino M, Thaqi K, Rudan P, Novokmet N, Sarac J, Missoni S, Kolčić I, Polašek O, Rudan I, Campbell H, Hayward C, Aulchenko Y, Valdes A, Wilson JF, Gornik O, Primorac D, Zoldoš V, Spector T, Lauc G (2014) Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci 69:779–789. Published online Epub2014/07//. https://doi.org/10.1093/gerona/glt190

Krumsiek J, Suhre K, Illig T, Adamski J, Theis FJ (2011) Gaussian graphical modeling reconstructs pathway reactions from high-throughput metabolomics data. BMC Syst Biol 5:21. Published online Epub2011/01/31/. https://doi.org/10.1186/1752-0509-5-21

Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, Hanson RW, Kalhan SC, Ryals JA, Milburn MV (2008) Analysis of the adult human plasma metabolome. Pharmacogenomics 9:383–397. Published online Epub2008/04//. https://doi.org/10.2217/14622416.9.4.383

Li JE, Liu Y, Liu M, Han J-DJ (2013) Functional dissection of regulatory models using gene expression data of deletion mutants. PLoS Genet 9:e1003757. Published online Epub2013. https://doi.org/10.1371/journal.pgen.1003757

Li S, Sullivan NL, Rouphael N, Yu T, Banton S, Maddur MS, McCausland M, Chiu C, Canniff J, Dubey S, Liu K, Tran V, Hagan T, Duraisingham S, Wieland A, Mehta AK, Whitaker JA, Subramaniam S, Jones DP, Sette A, Vora K, Weinberg A, Mulligan MJ, Nakaya HI, Levin M, Ahmed R, Pulendran B (2017) Metabolic phenotypes of response to vaccination in humans. Cell 169:862–877.e817. Published online Epub2017/05/18/. https://doi.org/10.1016/j.cell.2017.04.026

Ljungquist B, Berg S, Lanke J, McClearn GE, Pedersen NL (1998) The effect of genetic factors for longevity: a comparison of identical and fraternal twins in the Swedish Twin Registry. J Gerontol A Biol Sci Med Sci 53:M441–M446. Published online Epub1998/11//

Lu T, Pan Y, Kao S-Y, Li C, Kohane I, Chan J, Yankner BA (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429:883–891. Published online Epub2004/06/24/. https://doi.org/10.1038/nature02661

Lu Y, Biancotto A, Cheung F, Remmers E, Shah N, McCoy JP, Tsang JS (2016) Systematic analysis of cell-to-cell expression variation of T lymphocytes in a human cohort identifies aging and genetic associations. Immunity 45:1162–1175. Published online Epub2016/11/15/. https://doi.org/10.1016/j.immuni.2016.10.025

Marbach D, Costello JC, Küffner R, Vega N, Prill RJ, Camacho DM, Allison KR, Kellis M, Collins JJ, Stolovitzky G (2012) Wisdom of crowds for robust gene network inference. Nat Methods 9:796–804. Published online Epub2012/07/15/. https://doi.org/10.1038/nmeth.2016

Martens L, Vizcaíno JA (2017) A golden age for working with public proteomics data. Trends Biochem Sci 42:333–341. Published online Epub2017/05/01/. https://doi.org/10.1016/j.tibs.2017.01.001

Martinez-Jimenez CP, Eling N, Chen H-C, Vallejos CA, Kolodziejczyk AA, Connor F, Stojic L, Rayner TF, Stubbington MJT, Teichmann SA, Roche Mdl, Marioni JC, Odom DT (2017) Aging increases cell-to-cell transcriptional variability upon immune stimulation. Science 355:1433–1436. Published online Epub2017/03/31/. https://doi.org/10.1126/science.aah4115

Mayer RL, Schwarzmeier JD, Gerner MC, Bileck A, Mader JC, Meier-Menches SM, Gerner SM, Schmetterer KG, Pukrop T, Reichle A, Slany A, Gerner C (2017) Proteomics and metabolomics identify molecular mechanisms of aging potentially predisposing for chronic lymphocytic leukemia. Mol Cell Proteomics. Published online Epub2017/12/01/. https://doi.org/10.1074/mcp.RA117.000425

Menni C, Kastenmüller G, Petersen AK, Bell JT, Psatha M, Tsai P-C, Gieger C, Schulz H, Erte I, John S, Brosnan MJ, Wilson SG, Tsaprouni L, Lim EM, Stuckey B, Deloukas P, Mohney R, Suhre K, Spector TD, Valdes AM (2013) Metabolomic markers reveal novel pathways of ageing and early development in human populations. Int J Epidemiol 42:1111–1119. Published online Epub2013/08//. https://doi.org/10.1093/ije/dyt094

Miller JA, Oldham MC, Geschwind DH (2008) A systems level analysis of transcriptional changes in Alzheimer's disease and normal aging. J Neurosci: Off J Soc Neurosci 28:1410–1420. Published online Epub2008/02/06/. https://doi.org/10.1523/JNEUROSCI.4098-07.2008

Moayyeri A, Hammond CJ, Valdes AM, Spector TD (2013) Cohort profile: Twins UK and healthy ageing twin study. Int J Epidemiol 42:76–85. Published online Epub2013/02//. https://doi.org/10.1093/ije/dyr207

McAuley MT, Kenny RA, Kirkwood TBL, Wilkinson DJ, Jones JJL, Miller VM (2009) A mathematical model of aging-related and cortisol induced hippocampal dysfunction. BMC Neurosci 10:26. Published online Epub2009/03/25/. https://doi.org/10.1186/1471-2202-10-26

Murphy RA, Moore SC, Playdon M, Meirelles O, Newman AB, Milijkovic I, Kritchevsky SB, Schwartz A, Goodpaster BH, Sampson J, Cawthon P, Simonsick EM, Gerszten RE, Clish CB, Harris TB, A. B. C. S. Health (2017) Metabolites associated with lean mass and adiposity in older black men. J Gerontol A Biol Sci Med Sci 72:1352–1359. Published online Epub2017/10/01/. https://doi.org/10.1093/gerona/glw245

Pagel P, Kovac S, Oesterheld M, Brauner B, Dunger-Kaltenbach I, Frishman G, Montrone C, Mark P, Stümpflen V, Mewes H-W, Ruepp A, Frishman D (2005) The MIPS mammalian protein-protein interaction database. Bioinformatics (Oxford, England) 21:832–834. Published online Epub2005/03//. https://doi.org/10.1093/bioinformatics/bti115

Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG (2017) The aging cardiovascular system: understanding it at the cellular and clinical levels. J Am Coll Cardiol 69:1952–1967. Published online Epub2017/04/18/. https://doi.org/10.1016/j.jacc.2017.01.064

Pawlikowska L, Hu D, Huntsman S, Sung A, Chu C, Chen J, Joyner AH, Schork NJ, Hsueh W-C, Reiner AP, Psaty BM, Atzmon G, Barzilai N, Cummings SR, Browner WS, Kwok P-Y, Ziv E, F. Study of Osteoporotic (2009) Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. Aging Cell 8:460–472. Published online Epub2009/08//. https://doi.org/10.1111/j.1474-9726.2009.00493.x

Peters MJ, Joehanes R, Pilling LC, Schurmann C, Conneely KN, Powell J, Reinmaa E, Sutphin GL, Zhernakova A, Schramm K, Wilson YA, Kobes S, Tukiainen T, C. Nabec/Ukbec, Ramos YF, Göring HHH, Fornage M, Liu Y, Gharib SA, Stranger BE, De Jager PL, Aviv A, Levy D, Murabito JM, Munson PJ, Huan T, Hofman A, Uitterlinden AG, Rivadeneira F, van Rooij J, Stolk L, Broer L, Verbiest MMPJ, Jhamai M, Arp P, Metspalu A, Tserel L, Milani L, Samani NJ, Peterson P, Kasela S, Codd V, Peters A, Ward-Caviness CK, Herder C, Waldenberger M, Roden M, Singmann P, Zeilinger S, Illig T, Homuth G, Grabe H-J, Völzke H, Steil L, Kocher T, Murray A, Melzer D, Yaghootkar H, Bandinelli S, Moses EK, Kent JW, Curran JE, Johnson MP, Williams-Blangero S, Westra H-J, McRae AF, Smith JA, Kardia SLR, Hovatta I, Perola M, Ripatti S, Salomaa V, Henders AK, Martin NG, Smith AK, Mehta D, Binder EB, Nylocks KM, Kennedy EM, Klengel T, Ding J, Suchy-Dicey AM, Enquobahrie DA, Brody J, Rotter JI, Chen Y-DI, Houwing-Duistermaat J, Kloppenburg M, Slagboom PE, Helmer Q, den Hollander W, Bean S, Raj T, Bakhshi N, Wang QP, Oyston LJ, Psaty BM, Tracy RP, Montgomery GW, Turner ST, Blangero J, Meulenbelt I, Ressler KJ, Yang J, Franke L, Kettunen J, Visscher PM, Neely GG, Korstanje R, Hanson RL, Prokisch H, Ferrucci L, Esko T, Teumer A, van Meurs JBJ, Johnson AD (2015) The transcriptional landscape of age in human peripheral blood. Nat Commun 6:8570. Published online Epub2015/10/22/. https://doi.org/10.1038/ncomms9570

Pittman WE, Sinha DB, Zhang WB, Kinser HE, Pincus Z (2017) A simple culture system for long-term imaging of individual *C. elegans*. Lab Chip 17:3909–3920. Published online Epub2017/11/07/. https://doi.org/10.1039/C7LC00916J

Proctor CJ, Soti C, Boys RJ, Gillespie CS, Shanley DP, Wilkinson DJ, Kirkwood TBL (2005) Modelling the actions of chaperones and their role in ageing. Mech Ageing Dev 126:119–131. Published online Epub2005/01//. https://doi.org/10.1016/j.mad.2004.09.031

Przybilla J, Rohlf T, Loeffler M, Galle J (2014) Understanding epigenetic changes in aging stem cells – a computational model approach. Aging Cell 13:320–328. Published online Epub2014/04//. https://doi.org/10.1111/acel.12177

Rakyan VK, Down TA, Maslau S, Andrew T, Yang T-P, Beyan H, Whittaker P, McCann OT, Finer S, Valdes AM, Leslie RD, Deloukas P, Spector TD (2010) Human aging-associated DNA

hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20:434–439. Published online Epub2010/04//. https://doi.org/10.1101/gr.103101.109

Rockwood K, Mitnitski A (2007) Frailty in relation to the accumulation of deficits. J Gerontol A Biol Sci Med Sci 62:722–727. Published online Epub2007/07//

Rodwell GEJ, Sonu R, Zahn JM, Lund J, Wilhelmy J, Wang L, Xiao W, Mindrinos M, Crane E, Segal E, Myers BD, Brooks JD, Davis RW, Higgins J, Owen AB, Kim SK (2004) A transcriptional profile of aging in the human kidney. PLoS Biol 2:e427. Published online Epub2004/12//. https://doi.org/10.1371/journal.pbio.0020427

Röst HL, Sachsenberg T, Aiche S, Bielow C, Weisser H, Aicheler F, Andreotti S, Ehrlich H-C, Gutenbrunner P, Kenar E, Liang X, Nahnsen S, Nilse L, Pfeuffer J, Rosenberger G, Rurik M, Schmitt U, Veit J, Walzer M, Wojnar D, Wolski WE, Schilling O, Choudhary JS, Malmström L, Aebersold R, Reinert K, Kohlbacher O (2016) OpenMS: a flexible open-source software platform for mass spectrometry data analysis. Nat Methods 13:741–748. Published online Epub2016/08/30/. https://doi.org/10.1038/nmeth.3959

Ruhaak LR, Uh H-W, Beekman M, Hokke CH, Westendorp RGJ, Houwing-Duistermaat J, Wuhrer M, Deelder AM, Slagboom PE (2011) Plasma protein N-glycan profiles are associated with calendar age, familial longevity and health. J Proteome Res 10:1667–1674. Published online Epub2011/04/01/. https://doi.org/10.1021/pr1009959

Sebastiani P, Gurinovich A, Bae H, Andersen S, Malovini A, Atzmon G, Villa F, Kraja AT, Ben-Avraham D, Barzilai N, Puca A, Perls TT (2017) Four genome-wide association studies identify new extreme longevity variants. J Gerontology: Ser A 72:1453–1464. Published online Epub2017/10/12/. https://doi.org/10.1093/gerona/glx027

Skytthe A, Pedersen NL, Kaprio J, Stazi MA, Hjelmborg JVB, Iachine I, Vaupel JW, Christensen K (2003) Longevity studies in GenomEUtwin. Twin Res 6:448–454. Published online Epub2003/10//. https://doi.org/10.1375/136905203770326457

Sozou PD, Kirkwood TB (2001) A stochastic model of cell replicative senescence based on telomere shortening, oxidative stress, and somatic mutations in nuclear and mitochondrial DNA. J Theor Biol 213:573–586. Published online Epub2001/12/21/. https://doi.org/10.1006/jtbi.2001.2432

Suo J, Chen X, Shan S, Gao W, Dai Q (2012) A concatenational graph evolution aging model. IEEE Trans Pattern Anal Mach Intell 34:2083–2096. Published online Epub2012/11//. https://doi.org/10.1109/TPAMI.2012.22

Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C (2017) The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45:D362–D368. Published online Epub2017/01/04/. https://doi.org/10.1093/nar/gkw937

Tain LS, Sehlke R, Jain C, Chokkalingam M, Nagaraj N, Essers P, Rassner M, Grönke S, Froelich J, Dieterich C, Mann M, Alic N, Beyer A, Partridge L (2017) A proteomic atlas of insulin signalling reveals tissue-specific mechanisms of longevity assurance. Mol Syst Biol 13:939. Published online Epub2017/09/15/

Tvardovskiy A, Schwämmle V, Kempf SJ, Rogowska-Wrzesinska A, Jensen ON (2017) Accumulation of histone variant H3.3 with age is associated with profound changes in the histone methylation landscape. Nucleic Acids Res 45:9272–9289. Published online Epub2017/09/19/. https://doi.org/10.1093/nar/gkx696

Wang J, Zhang S, Wang Y, Chen L, Zhang X-S (2009) Disease-aging network reveals significant roles of aging genes in connecting genetic diseases. PLoS Comput Biol 5:e1000521. Published online Epub2009/09//. https://doi.org/10.1371/journal.pcbi.1000521

Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Bauerschlag DO, Jöckel K-H, Erbel R, Mühleisen TW, Zenke M, Brümmendorf TH, Wagner W (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol 15:R24. Published online Epub2014. https://doi.org/10.1186/gb-2014-15-2-r24

West J, Beck S, Wang X, Teschendorff AE (2013) An integrative network algorithm identifies age-associated differential methylation interactome hotspots targeting stem-cell differentia-

tion pathways. Sci Rep 3:1630. Published online Epub2013/04/09/. https://doi.org/10.1038/srep01630

Wilhelm M, Schlegl J, Hahne H, Gholami AM, Lieberenz M, Savitski MM, Ziegler E, Butzmann L, Gessulat S, Marx H, Mathieson T, Lemeer S, Schnatbaum K, Reimer U, Wenschuh H, Mollenhauer M, Slotta-Huspenina J, Boese J-H, Bantscheff M, Gerstmair A, Faerber F, Kuster B (2014) Mass-spectrometry-based draft of the human proteome. Nature 509:582. Published online Epub2014/05//. https://doi.org/10.1038/nature13319

Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD (2008) FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci U S A 105:13987–13992. Published online Epub2008/09/16/. https://doi.org/10.1073/pnas.0801030105

Xia K, Xue H, Dong D, Zhu S, Wang J, Zhang Q, Hou L, Chen H, Tao R, Huang Z, Fu Z, Chen Y-G, Han J-DJ (2006) Identification of the proliferation/differentiation switch in the cellular network of multicellular organisms. PLoS Comput Biol 2:e145. Published online Epub2006/11/24/. https://doi.org/10.1371/journal.pcbi.0020145

Xian B, Shen J, Chen W, Sun N, Qiao N, Jiang D, Yu T, Men Y, Han Z, Pang Y, Kaeberlein M, Huang Y, Han J-DJ (2013) WormFarm: a quantitative control and measurement device toward automated *Caenorhabditis elegans* aging analysis. Aging Cell 12:398–409. Published online Epub2013/06//. https://doi.org/10.1111/acel.12063

Yu Z, Zhai G, Singmann P, He Y, Xu T, Prehn C, Römisch-Margl W, Lattka E, Gieger C, Soranzo N, Heinrich J, Standl M, Thiering E, Mittelstraß K, Wichmann H-E, Peters A, Suhre K, Li Y, Adamski J, Spector TD, Illig T, Wang-Sattler R (2012) Human serum metabolic profiles are age dependent. Aging Cell 11:960–967. Published online Epub2012/12//. https://doi.org/10.1111/j.1474-9726.2012.00865.x

Zhang B, Horvath S (2005) A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol 4, Article17. Published online Epub2005. https://doi.org/10.2202/1544-6115.1128

Zierer J, Menni C, Kastenmüller G, Spector TD (2015) Integration of 'omics' data in aging research: from biomarkers to systems biology. Aging Cell 14:933–944. Published online Epub2015/12/01/. https://doi.org/10.1111/acel.12386