Review

Mechanisms Underlying the Anti-Aging and Anti-Tumor Effects of Lithocholic Bile Acid

Anthony Arlia-Ciommo †, Amanda Piano †, Veronika Svistkova †, Sadaf Mohtashami † and Vladimir I. Titorenko *

Department of Biology, Concordia University, 7141 Sherbrooke Street, West, SP Building, Room 501-13, Montreal, QC H4B 1R6, Canada; E-Mails: anthony.arlia@outlook.com (A.A.-C.); amandapiano91@hotmail.com (A.P.); klubnika_veronika@hotmail.com (V.S.); sadaf.mohtashami@gmail.com (S.M.)

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: vladimir.titorenko@concordia.ca; Tel.: +1-514-848-2424 (ext. 3424); Fax: +1-514-848-2881.

Received: 24 July 2014; in revised form: 21 August 2014 / Accepted: 11 September 2014 / Published: 18 September 2014

Abstract: Bile acids are cholesterol-derived bioactive lipids that play essential roles in the maintenance of a healthy lifespan. These amphipathic molecules with detergent-like properties display numerous beneficial effects on various longevity- and healthspan-promoting processes in evolutionarily distant organisms. Recent studies revealed that lithocholic bile acid not only causes a considerable lifespan extension in yeast, but also exhibits a substantial cytotoxic effect in cultured cancer cells derived from different tissues and organisms. The molecular and cellular mechanisms underlying the robust anti-aging and anti-tumor effects of lithocholic acid have emerged. This review summarizes the current knowledge of these mechanisms, outlines the most important unanswered questions and suggests directions for future research.

Keywords: bioactive lipids; lipid metabolism; lipidomics; bile acids; aging and age-related diseases; cancer; anti-aging and anti-tumor therapeutic agents; endoplasmic reticulum; mitochondria; cell death
1. Introduction

Aging of unicellular and multicellular eukaryotic organisms is an intricate biological phenomenon [1–5]. It is believed to be caused by an age-dependent, progressive dysregulation of many processes within a eukaryotic cell [6–19]. The rates, efficiencies and spatiotemporal organization of all of these cellular processes throughout the organismal lifespan are modulated by only a few nutrient- and energy-sensing signaling pathways that converge into a network; this evolutionarily conserved network integrates the insulin/insulin-like growth factor 1, AMP-activated protein kinase/target of rapamycin and cAMP/protein kinase A (cAMP/PKA) pathways [6–13,20–35]. The flow of information along the signaling network of cellular aging can be modulated by certain dietary and pharmacological interventions that can extend lifespan and/or delay the onset of various age-related physiological changes in yeast, nematodes, fruit flies, mice and primates. These interventions are known to prolong both longevity and healthspan in organisms across phyla by beneficially influencing pathologies and diseases of old age [3–8,27–32,34–40]. These interventions include: (1) caloric restriction (CR), a dietary regimen that limits the intake of calories without reducing the supply of amino acids, vitamins and other nutrients [3,6–9,37–52]; (2) dietary restriction (DR), a group of nutrient intake interventions that limit the supply of certain amino acids or vitamins and/or alter the balance of dietary components, but do not reduce overall food or calorie intake [3,6,38–41,53–71]; and (3) certain natural chemical compounds and some pharmaceutical drugs [3,6–9,31,34–40,72–106].

The molecular and cellular mechanisms underlying the robust longevity-extending and health-improving effects of CR, certain DR regimens and some pharmacological interventions have begun to emerge. These mechanisms involve several distinct, evolutionarily conserved ways of modulating the flow of information along the signaling network, which orchestrates a pro- or anti-aging cellular pattern by controlling numerous longevity-defining cellular processes [3–13,24,35–40,44–51,91,97–99]. Among these cellular processes are certain pathways of lipid metabolism and interorganellar transport [10,11,16–18,124,131–140] (Figure 1); the proper functioning of this network is essential for maintaining lipid homeostasis in all of these cellular organelles and membranes [10,11,16–18,95,124,133,136–140] (Figure 1); and (3) the concentration of long-chain fatty acids in plasma is a probable biomarker of longevity in various species of mammals [130].

It needs to be emphasized that: (1) lipid metabolism and transport within a cell are governed by an intricate network of interorganellar communications integrating the endoplasmic reticulum (ER), lipid droplets (LD), peroxisomes, mitochondria and the plasma membrane (PM) [10,11,16–18,124,131–140] (Figure 1); (2) the proper functioning of this network is essential for maintaining lipid homeostasis in all of these cellular organelles and membranes [10,11,16–18,95,124,133,136–140] (Figure 1); and (3) the efficacy of maintaining lipid homeostasis in some or all of these cellular organelles and membranes defines the lifespan of chronologically aging yeast [10,11,16–18,95]. A current view of how the network integrating lipid metabolism and transport in different cellular locations maintains
lipid homeostasis in various cellular organelles and membranes is summarized in a model; this model is depicted schematically in Figure 1. The model posits that: (1) after being synthesized in the ER, the phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylcholine (PC) and phosphatidylinositol (PI) classes of phospholipids are transported to mitochondria via mitochondria-ER junctions and to the PM via PM-ER junctions; (2) following the synthesis of the phosphatidylethanolamine (PE) class of phospholipids in the inner mitochondrial membrane (IMM) from PS formed in the ER, PE is transported from mitochondria to the ER via mitochondria-ER junctions and subsequently from the ER to the PM via PM-ER junctions; (3) cardiolipin (CL), a signature phospholipid class of the mitochondrion, is synthesized in the IMM from PA, which is formed in the ER and then delivered to mitochondria via mitochondria-ER junctions; (4) after being synthesized in the ER, the neutral lipids triacylglycerols (TAG) and ergosteryl esters (EE) are deposited within LD; (5) the physical contact existing between peroxisomes and LD stimulates the lipolytic conversion of TAG and EE to free fatty acids, which then get imported and oxidized by peroxisomes; (6) the anaplerotic conversion of acetyl-CoA to citrate and acetyl-carnitine in peroxisomes enables the replenishment of tricarboxylic acid (TCA) cycle intermediates destined for mitochondria, thereby allowing one to maintain the efficient synthesis of PE and CL in the IMM; and (7) a pool of peroxisomally produced acetyl-CoA is also used in the cytosol for the synthesis of fatty acids, which then get imported by the ER, where they enter the biosynthetic pathways for phospholipids and neutral lipids [10,11,16–18,124,131–140] (Figure 1).

In a high-throughput screen for chemical compounds that can slow down aging in the yeast, *Saccharomyces cerevisiae*, by specifically targeting lipid metabolism and interorganellar transport, we identified lithocholic acid (LCA), a bile acid, as one of them [95]. Our screen revealed that several other bile acids are also longevity-extending molecules. These other bile acids were deoxycholic acid, chenodeoxycholic acid, dehydrocholic acid and hyodeoxycholic acid. All of them were shown to increase yeast chronological lifespan to a significantly lesser degree than LCA, which is the most hydrophobic bile acid species [95]. Although we found that LCA considerably delays the aging of chronologically aging yeast, this unicellular eukaryote does not synthesize LCA or any other bile acid produced and released into the environment by mammals [95,135]. In mammals, bile acids play essential roles in many processes known to be required for the maintenance of a healthy lifespan [141–151]; these roles are outlined in more detail below. In this review, we discuss recent progress in understanding the molecular and cellular mechanisms by which LCA, a cholesterol-derived bioactive lipid, delays chronological aging in the yeast, *S. cerevisiae*, and exhibits potent and specific anti-tumor effects in cultured cancer cells derived from different tissues and organisms.
**Figure 1.** Outline of a network governing lipid metabolism and transport within the endoplasmic reticulum (ER), lipid droplets (LD), peroxisomes, mitochondria and the plasma membrane (PM). The proper functioning of this intricate network is necessary for maintaining lipid homeostasis in all of these cellular organelles and membranes. The PA, PS, PC and PI classes of phospholipids are synthesized exclusively in the ER; these are then transported to mitochondria via mitochondria-ER junctions and to the PM via PM-ER junctions. The PE and CL classes of phospholipids are formed only in the inner mitochondrial membrane (IMM); PE is then transported from mitochondria to the ER via mitochondria-ER junctions and from the ER to the PM via PM-ER junctions. The neutral lipids TAG and EE are synthesized in the ER and then deposited within LD. The lipolytic hydrolysis of TAG and EE in LD generates FFA; these then get imported and oxidized by peroxisomes. Peroxisomally produced acetyl-CoA is converted to citrate and acetyl-carnitine, whose subsequent delivery to mitochondria enables one to maintain the efficient synthesis of PE and CL in the IMM. The use of peroxisomally produced acetyl-CoA for the synthesis of FFA in the cytosol allows FFA to enter the biosynthetic pathways for phospholipids and neutral lipids in the ER. See the text for additional details.

Abbreviations: Ac-CoA, acetyl-CoA; ADHAP, acyl dihydroxyacetone phosphate; CDP-DAG, cytidine diphosphate-diacylglycerol; CL, cardiolipin; EE, ergosteryl esters; FA-CoA, fatty acid-CoA; FFA, non-esterified (free) fatty acids; LPA, lysophosphatidic acid; MLCL, monolysocardiolipin; OMM, outer mitochondrial membrane; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TAG, triacylglycerols; WT, wild-type.
2. Bile Acids Extend Healthy Lifespan in Multicellular Eukaryotic Organisms across Species

Primary bile acids in mammals are formed from the cholesterol backbone exclusively in hepatocytes of the liver, whereas secondary bile acids (including LCA) are the products of the enzymatic modification of primary bile acids by intestinal microbial flora [141,142,144]. Bile acids are cholesterol-derived amphipathic molecules with detergent-like properties that facilitate the emulsification and absorption of dietary lipids and fat-soluble vitamins in the small intestine, influence the composition and proliferation of the intestinal microbial flora, stimulate cholesterol solubilization in bile, promote bile secretion from hepatocytes into the bile canaliculi and enable the maintenance of organismal sterol homeostasis by being first formed from cholesterol and then released into the feces [141–145]. Moreover, bile acids are potent signaling molecules. In mammals, they specifically bind to and activate the nuclear farnesoid X receptor, the nuclear pregnane X receptor, the nuclear vitamin D receptor and the plasma membrane-bound G protein-coupled TGR5 (a protein that in humans is encoded by the \textit{GPBAR1} gene) receptor, thus stimulating many longevity- and healthspan-promoting processes in various tissues [141,144,146–151]. These processes include mitochondrial oxidative metabolism, energy expenditure regulation, glucose metabolism and insulin sensitivity, metabolism of cholesterol and neutral lipids, maintenance of bile acid homeostasis, detoxification of xenobiotic and endobiotic toxins, growth of intestinal microbial organisms, hepatoprotection and liver regeneration and anti-inflammatory processes [141–144,148–151]. Because of the numerous beneficial effects of bile acids on longevity- and healthspan-promoting processes, they are used (or have the great potential to be used) as therapeutic agents for several age-related metabolic and neurodegenerative disorders caused by dysregulation of these processes [141,142,148,149]. For example, ursodeoxycholic acid has been used for solubilizing cholesterol gallstones, improving liver function in patients with primary biliary cirrhosis and preventing the occurrence of veno-occlusive disease in recipients of bone marrow transplants [141,142,149]. Furthermore, cholic and chenodeoxycholic bile acids have been successfully used for increasing lipid absorption and improving liver function, thereby preventing progressive liver disease in patients with inborn errors of bile acid synthesis [141,142,149]. Moreover, several natural and synthetic bile-acid classes of agonists of the nuclear farnesoid X receptor or TGR5 receptor are currently undergoing clinical trials for their potential use in the treatment of type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome or primary biliary cirrhosis [141,142,148,149]. It needs to be emphasized that none of the above bile acids has been reported to exhibit potent anti-aging or anti-tumor effects similar to the ones we found to be characteristic of LCA.

Importantly, recent findings suggest that bile acids may extend lifespan in mice by stimulating age-related hormetic responses and, thus, by acting as endobiotic regulators that slow down the aging process [152–155]. In fact, the levels of several bile acids have been found to be increased in the long-lived \textit{Ghrhr^{litt}} mouse, which exhibits attenuated signaling through the pro-aging insulin/insulin-like growth factor 1 pathway, due to considerably reduced circulating levels of insulin-like growth factor 1 [152,153]. Moreover, the administration of cholic bile acid to the food of wild-type mice has been shown to stimulate the transcription of many xenobiotic detoxification genes [152,153]. Furthermore, bile acid-like dafachronic acids in the nematode, \textit{C. elegans}, are known to act as cell non-autonomous molecular signals that extend organismal longevity by activating a
transcriptional program orchestrated by the DAF-12/DAF-16 signaling cascade [115,156–163]. In sum, these findings strongly suggest that bile acids in animals may function as signaling molecules that stimulate a distinct set of vital longevity- and healthspan-promoting processes. Because we found that LCA considerably (and some other bile acids to a lesser degree) increases the chronological lifespan of yeast [95], it is conceivable that the mechanisms by which bile acids extend healthy lifespan have been conserved during the course of evolution.

3. A Mechanism Underlying the Longevity-Extending Effect of LCA (Lithocholic Acid) in Chronologically Aging Yeast

Our recent studies uncovered a mechanism through which LCA prolongs the longevity of chronologically aging yeast [95,135,164,165]. With the help of subcellular fractionation by differential centrifugation, organelle separation by equilibrium density gradient centrifugation and subsequent mass spectrometric measurement of LCA in purified cellular organelles, we demonstrated that exogenously added LCA enters yeast cells and accumulates in mitochondria, but not in any other organelle [135,165] (Figure 2). Using subfractionation of purified mitochondria followed by mass spectrometric quantitation of LCA in different mitochondrial subcompartments, we revealed that confined to the mitochondria, LCA resides mainly in the IMM; a smaller portion of this bile acid also associates with the outer mitochondrial membrane (OMM) [135,165] (Figure 2). Our mass spectrometric analyses of mitochondrial membrane lipidomes provided evidence that the pools of LCA confined to the IMM and OMM alter the phospholipid composition of mitochondrial membranes. Specifically, LCA elicits a rise in the relative level of PS and a decline in the relative level of PE within mitochondrial membranes; one could assume that LCA may cause these changes by decelerating the Psd1-dependent reaction, leading to the conversion of PS to PE [135,165] (Figure 2). Furthermore, LCA causes a rise in the relative level of PG and a decline in the relative level of CL within mitochondrial membranes; it is conceivable that LCA may trigger these changes by slowing down the Crd1-dependent reaction resulting in the synthesis of CL from PG [135,165] (Figure 2). Moreover, LCA increases the relative level of PC, as well as reduces the relative levels of both CL and monolysocardiolipin (MLCL) within mitochondrial membranes; one could envisage that LCA may elicit these changes by reducing the availability of newly synthesized CL for the Cld1- and Taz1-driven reactions that enable a PC-dependent remodeling of the acyl chains of CL [135,165] (Figure 2). In addition, LCA was found to increase the relative level of PA within mitochondrial membranes [135,165] (Figure 2). One could assume that the observed LCA-driven reduction in the relative level of CL within the IMM may cause such an effect by mitigating a CL-dependent inhibition of PA translocation from the OMM to the IMM; this translocation is known to be promoted by the Ups1 protein, which shuttles PA between the two mitochondrial membranes [134,136,140] (Figure 2). The resulting acceleration of PA transport from the OMM to the IMM, in synergy with the LCA-stimulated movement of PA from the ER to the OMM via mitochondria-ER junctions, may elicit the observed rise in the relative level of PA within mitochondrial membranes [135,165] (Figure 2).
Figure 2. A mechanism through which lithocholic acid (LCA) prolongs the longevity of chronologically aging yeast. Exogenously added LCA enters a yeast cell, where it is sorted to mitochondria, but not to any other organelle. Mitochondria-associated LCA is located predominantly in the inner mitochondrial membrane (IMM) and also resides in the outer mitochondrial membrane (OMM). LCA drives a remodeling of the mitochondrial membrane lipidome, thereby enlarging mitochondria, reducing their number and causing a build-up within their matrix of cristae disconnected from the IMM. These major changes in mitochondrial abundance and morphology elevate mitochondrial respiration, membrane potential, ATP synthesis and reactive oxygen species (ROS) levels in chronologically “old” cells, thereby enhancing their long-term stress resistance and viability. Moreover, the LCA-elicited remodeling of the mitochondrial membrane lipidome mitigates mitochondrial fragmentation, thus slowing down an age-related form of apoptotic programmed cell death. All of these distinctive alterations in vital mitochondrial processes and features seen in yeast cells permanently exposed to exogenous LCA extend their chronological lifespan. See the text for additional details. Abbreviations: CDP-DAG, cytidine diphosphate-diacylglycerol; CL, cardiolipin; ER, endoplasmic reticulum; ETC, electron transport chain; MLCL, monolysocardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; $\Delta\Psi$, electrochemical membrane potential.
The observed remodeling of the mitochondrial membrane lipidome in yeast cells permanently exposed to LCA progresses with their chronological age and triggers major age-related changes in mitochondrial abundance and morphology, including: (1) an expansion of both mitochondrial membranes, which leads to a considerable enlargement of mitochondria; (2) a shift in the balance between the opposing processes of mitochondrial fission and fusion towards fusion, which causes a substantial decline in mitochondrial number; (3) a significant decrease in the fraction of mitochondria with cristae that extend from the inner boundary membrane; and (4) a massive accumulation within the mitochondrial matrix of cristae disconnected from the inner boundary membrane [135,165] (Figure 2).

In synergy, the major changes triggered by LCA in the mitochondrial membrane lipidome and the ensuing vast changes in mitochondrial morphology elicit a distinct set of alterations in the age-related chronology of several mitochondrial processes; these vital mitochondrial processes include respiration, the preservation of electrochemical membrane potential, the synthesis of ATP and the maintenance of reactive oxygen species (ROS) homeostasis [135,165] (Figure 2). Because a permanent exposure of yeast to LCA stimulates all of these mitochondrial processes in chronologically “old” cells, they exhibit higher long-term stress resistance and viability than yeast cells cultured without LCA [135,165] (Figure 2). Moreover, a shift is elicited by LCA in the balance between the opposing processes of mitochondrial fission and fusion towards fusion attenuates mitochondrial fragmentation, thus slowing down the release of pro-apoptotic proteins from mitochondria and decelerating an age-related form of apoptotic programmed cell death [135,164,165] (Figure 2). By promoting the long-term stress resistance and viability of chronologically aging yeast cells and by slowing down their age-related apoptotic death, the permanent exposure of these cells to LCA extends their longevity [135,164,165] (Figure 2).

4. A Hypothesis: The Mitochondria-Centered Mechanism by Which LCA Prolongs Longevity Could Be Integrated into a Network of Interorganellar Communications Underlying Cellular Aging

As discussed in the Introduction, the homeostasis of the cellular lipidome in yeast is maintained via an intricate network of interorganellar communications; this network orchestrates lipid metabolism and transport within the ER, LD, peroxisomes, mitochondria and the PM [10,11,16–18,124,131–140] (Figure 1). We hypothesize that the mechanism centered on the mitochondria through which LCA extends yeast chronological lifespan [95,135,164,165] (Figure 2) could converge into the network of interorganellar communications orchestrating lipid dynamics within the ER, LD, peroxisomes, mitochondria and the PM. Our hypothesis posits that the observed LCA-elicited changes in mitochondrial membrane lipidome [135,165] (Figure 2) trigger age-related alterations in the lipidomes of all other cellular organelles and membranes integrated into this network of interorganellar communication. Such age-related alterations in the lipidomes of the ER, LD, peroxisomes, mitochondria and the PM are known to define yeast chronological lifespan by modulating the flow of interorganellar information, which is essential for establishing a pro- or anti-aging cellular pattern [10,11,14–18,95,135,138]. It is conceivable therefore that the mitochondria-centered mechanism by which LCA prolongs yeast longevity (Figure 2) is dynamically integrated into a network of interorganellar communications underlying cellular aging in yeast; the term “an endomembrane system that governs cellular aging” has been coined to reflect the essential role of crosstalk between different

Int. J. Mol. Sci. 2014, 15

16529
intracellular compartments in regulating cellular aging [10,11,18]. One could envision the existence of the following two ways for such an integration: (1) the observed remodeling of the mitochondrial membrane lipidome in yeast cells permanently exposed to exogenous LCA may cause an age-related remodeling of lipid metabolism and transport in the ER, LD and peroxisomes, thereby postponing a recently discovered age-related mode of programmed cell death called “liponecrosis” [165]; and (2) such LCA-elicited remodeling of the mitochondrial membrane lipidome may also trigger an age-related remodeling of the central metabolism in the cytosol, thus altering the coordinated metabolite flow within glycolytic and non-glycolytic pathways of carbohydrate metabolism known to define yeast longevity by modulating a distinct set of vital cellular processes [11].

5. A Mechanism Underlying an Anti-Tumor Effect of LCA in Cultured Human Cancer Cells

Incidence rates of many types of cancer are known to increase with age; therefore, cancer is considered as one of the diseases associated with aging [3,38,166,167]. Significant progress has been made in our understanding of the intricate relationship that exists between the convergent and divergent mechanisms underlying aging and cancer [5,167–179]. Because our studies revealed that LCA slows down cellular aging in yeast by modulating several cellular processes known for their essential roles in such mechanisms [95,135,164,165], we recently investigated how this bile acid affects cultured cancer cells derived from different tissues and organisms [180,181].

We found that, at concentrations that are not toxic to cultured non-cancerous cells, LCA kills cultured human neuroblastoma, breast cancer and prostate cancer cells, as well as cultured rat glioma cells [180,181]. Thus, LCA exhibits a potent and selective anti-tumor effect in cultured cancer cells that originate from diverse tissues and organisms. These studies uncovered a mechanism underlying such an anti-tumor effect of LCA in cultured human neuroblastoma cells [180] (Figure 3); a similar mechanism is responsible for the anti-tumor action of LCA in cultured human prostate cancer cells [181]. In this mechanism, LCA does not enter human neuroblastoma cells. It interacts with the plasma membrane-bound protein TGR5 on the cell surface, thereby stimulating this G protein-coupled receptor of LCA [180] (Figure 3). Such stimulation of TGR5 by LCA, its most potent natural agonist [141,149,182,183], triggers three different pathways that compromise viability and/or proliferation of human neuroblastoma cells. First, LCA-stimulated TGR5 activates the cAMP/PKA signaling pathway, thereby altering redox processes in mitochondria and mitochondrial morphology [141,143,149,184,185] (Figure 3). The ensuing activation of mitochondrial outer membrane permeabilization (MOMP) initiates the intrinsic (mitochondrial) pathway of apoptotic death by eliciting a cascade of sequential events that include the fragmentation of mitochondria, the release of cytochrome c from the mitochondrial intermembrane space into the cytosol, the formation of the apoptosome, the activation of the initiator caspase-9, caspase-9-dependent proteolytic activation of the executioner caspase-3, caspase-3-driven proteolytic processing and activation of the executioner caspase-6 and, ultimately, cell demolition by the executioner caspases through proteolytic cleavage of their numerous protein substrates [180] (Figure 3). Second, LCA-stimulated TGR5 also initiates the extrinsic (death receptor) pathway of apoptosis, which leads to activation of the initiator caspase-8 [180] (Figure 3); a mechanism underlying such activation remains to be characterized. The active form of caspase-8 not only proteolytically stimulates the executioner caspase-3, but can also cleave and activate the
BH3-only protein, BID (BH3 interacting-domain death agonist), thus causing MOMP and triggering the intrinsic pathway of apoptosis [186,187]. Third, LCA-stimulated TGR5 reduces the activity of the inflammatory caspase-1 via a currently unknown mechanism [180] (Figure 3). This caspase is involved in the processing and secretion of interleukin-1β and interleukin-18 [188–191], two cytokines known for their essential roles in stimulating cell growth and proliferation [192–194]. It is conceivable therefore that the observed inhibition of the inflammatory caspase-1 in human neuroblastoma cells treated with LCA may attenuate the growth and proliferation of neighboring cancer cells, thereby contributing to the anti-tumor effect of LCA in these cells [180] (Figure 3).

**Figure 3.** A mechanism underlying an anti-tumor effect of lithocholic acid (LCA) in cultured human neuroblastoma cells. LCA is the most potent natural agonist of TGR5, a plasma membrane-bound G protein-coupled receptor. LCA binding to TGR5 on the cell surface compromises viability and/or proliferation of human neuroblastoma cells by triggering three different pathways. The first pathway is initiated when LCA-stimulated TGR5 activates the cAMP/PKA signaling cascade. The ensuing specific changes in mitochondrial redox processes and morphology cause an activation of mitochondrial outer membrane permeabilization (MOMP), thus triggering the intrinsic (mitochondrial) pathway of apoptotic death. The second pathway leads to activation of the initiator caspase-8. Activated caspase-8 cleaves and stimulates both the executioner caspase-3 and BID (BH3-interacting domain death agonist), thus eliciting both the extrinsic (death receptor) and intrinsic (mitochondrial) pathways of apoptotic death, respectively. The third pathway operates via an inhibition of the inflammatory caspase-1, thus slowing down the processing and secretion of the cytokines interleukin-1β and interleukin-18 and, ultimately, attenuating the growth and proliferation of neighboring neuroblastoma cells. See the text for additional details. Abbreviations: Csp-1, -3, -6, -8 and -9, caspases-1, -3, -6, -8 and -9.

6. Conclusions and Future Perspectives

Recent studies revealed that LCA, a bile acid, not only extends the longevity of chronologically aging yeast, but also compromises the viability and proliferation of cultured cancer cells derived from
different tissues and organisms. The molecular and cellular mechanisms underlying the robust anti-aging and anti-tumor effects of this cholesterol-derived bioactive lipid have emerged. Despite significant progress in the understanding of these mechanisms, we are still lacking answers to the following important questions.

Do the LCA-driven alterations in the mitochondrial membrane lipidome seen in yeast cells [135,165] (Figure 2) elicit any changes in the membrane lipidomes of other cellular organelles and membranes known to be integrated into a network of interorganellar communication that underlies cellular aging [10,11,16–18,95,124,131–140] (Figure 1)? If so, how do these changes in the lipidomes of the ER, LD, peroxisomes, mitochondria and/or the PM in yeast cells permanently exposed to LCA contribute to the “liponecrotic” mode [165] of their age-related programmed cell death?

Does the remodeling of the mitochondrial membrane lipidome driven by LCA in chronologically aging yeast [135,165] (Figure 2) alter the coordinated metabolite flow within certain pathways of the central metabolism in the cytosol? If so, how do these alterations in the metabolome of yeast cells treated with LCA impact several longevity-defined cellular processes [11] that are known to be modulated by the metabolite flow within glycolytic and non-glycolytic pathways of carbohydrate metabolism?

How does LCA binding to TGR5 on the surface of human neuroblastoma cells activate the initiator caspase-8 [180] (Figure 3)? What is the mechanism underlying the ability of LCA-stimulated TGR5 to inhibit the inflammatory caspase-1 in these cancer cells [180] (Figure 3)? How does such LCA-driven inhibition of caspase-1 impact the growth and proliferation of human neuroblastoma cells?

We shall have to answer these important questions if we want to understand the inherent complexity of mechanisms through which LCA and other bioactive lipids influence cellular aging, define organismal longevity and impact diseases of old age.

Acknowledgments

We are grateful to current and former members of the Titorenko laboratory for discussions. This research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada and Concordia University Chair Fund to Vladimir I. Titorenko. Amanda Piano was supported by a Frederick Banting and Charles Best Canada Master’s Scholarship Award from the Canadian Institutes of Health Research. Veronika Svistkova was supported by an Undergraduate Summer Award from the NSERC of Canada. Vladimir I. Titorenko is a Concordia University Research Chair in Genomics, Cell Biology and Aging.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Guarente, L.P.; Partridge, L.; Wallace, D.C. Molecular Biology of Aging; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2008; p. 610.
2. Masoro, E.J.; Austad, S.N. Handbook of the Biology of Aging, 7th ed.; Elsevier Inc.: Oxford, UK, 2011; p. 572.
3. Niccoli, T.; Partridge, L. Ageing as a risk factor for disease. *Curr. Biol.* 2012, 22, R741–R752.

4. Gems, D.; Partridge, L. Genetics of longevity in model organisms: Debates and paradigm shifts. *Annu. Rev. Physiol.* 2013, 75, 621–644.

5. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* 2013, 153, 1194–1217.

6. Fontana, L.; Partridge, L.; Longo, V.D. Extending healthy life span—From yeast to humans. *Science* 2010, 328, 321–326.

7. Kaeberlein, M. Lessons on longevity from budding yeast. *Nature* 2010, 464, 513–519.

8. Kenyon, C.J. The genetics of ageing. *Nature* 2010, 464, 504–512.

9. Longo, V.D.; Shadel, G.S.; Kaeberlein, M.; Kennedy, B. Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metab.* 2012, 16, 18–31.

10. Leonov, A.; Titorenko, V.I. A network of interorganellar communications underlies cellular aging. *IUBMB Life* 2013, 65, 665–674.

11. Arlia-Ciommo, A.; Leonov, A.; Piano, A.; Svistkova, V.; Titorenko, V.I. Cell-autonomous mechanisms of chronological aging in the yeast *Saccharomyces cerevisiae*. *Microbial Cell* 2014, 1, 163–178.

12. Greer, E.L.; Brunet, A. Signaling networks in aging. *J. Cell Sci.* 2008, 121, 407–412.

13. Finley, L.W.; Haigis, M.C. The coordination of nuclear and mitochondrial communication during aging and calorie restriction. *Ageing Res. Rev.* 2009, 8, 173–188.

14. Goldberg, A.A.; Bourque, S.D.; Kyryakov, P.; Boukh-Viner, T.; Gregg, C.; Beach, A.; Burstein, M.T.; Machkalyan, G.; Richard, V.; Rampersad, S.; et al. A novel function of lipid droplets in regulating longevity. *Biochem. Soc. Trans.* 2009, 37, 1050–1055.

15. Goldberg, A.A.; Bourque, S.D.; Kyryakov, P.; Gregg, C.; Boukh-Viner, T.; Beach, A.; Burstein, M.T.; Machkalyan, G.; Richard, V.; Rampersad, S.; et al. Effect of calorie restriction on the metabolic history of chronologically aging yeast. *Exp. Gerontol.* 2009, 44, 555–571.

16. Beach, A.; Titorenko, V.I. In search of housekeeping pathways that regulate longevity. *Cell Cycle* 2011, 10, 3042–3044.

17. Titorenko, V.I.; Terlecky, S.R. Peroxisome metabolism and cellular aging. *Traffic* 2011, 12, 252–259.

18. Beach, A.; Burstein, M.T.; Richard, V.R.; Leonov, A.; Levy, S.; Titorenko, V.I. Integration of peroxisomes into an endomembrane system that governs cellular aging. *Front. Physiol.* 2012, 3, doi:10.3389/fphys.2012.00283.

19. Kyryakov, P.; Beach, A.; Richard, V.R.; Burstein, M.T.; Leonov, A.; Levy, S.; Titorenko, V.I. Caloric restriction extends yeast chronological lifespan by altering a pattern of age-related changes in trehalose concentration. *Front. Physiol.* 2012, 3, doi:10.3389/fphys.2012.00256.

20. Yan, L.; Vatner, D.E.; O’Connor, J.P.; Ivessa, A.; Ge, H.; Chen, W.; Hirotani, S.; Ishikawa, Y.;Sadoshima, J.; Vatner, S.F. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 2007, 130, 247–258.

21. Enns, L.C.; Ladiges, W. Protein kinase A signaling as an anti-aging target. *Ageing Res. Rev.* 2010, 9, 269–272.
22. Enns, L.C.; Morton, J.F.; Mangalindan, R.S.; McKnight, G.S.; Schwartz, M.W.; Kaeberlein, M.R.; Kennedy, B.K.; Rabinovitch, P.S.; Ladiges, W.C. Attenuation of age-related metabolic dysfunction in mice with a targeted disruption of the Cβ subunit of protein kinase A. *J. Gerontol. A Biol. Sci. Med. Sci.* 2009, 64, 1221–1231.

23. Enns, L.C.; Morton, J.F.; Treuting, P.R.; Emond, M.J.; Wolf, N.S.; Dai, D.F.; McKnight, G.S.; Rabinovitch, P.S.; Ladiges, W.C. Disruption of protein kinase A in mice enhances healthy aging. *PLoS One* 2009, 4, e5963.

24. Narasimhan, S.D.; Yen, K.; Tissenbaum, H.A. Converging pathways in lifespan regulation. *Curr. Biol.* 2009, 19, R657–R664.

25. Zoncu, R.; Efeyan, A.; Sabatini, D.M. mTOR: From growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell Biol.* 2011, 12, 21–35.

26. Hardie, D.G.; Ross, F.A.; Hawley, S.A. AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* 2012, 13, 251–262.

27. Inoki, K.; Kim, J.; Guan, K.L. AMPK and mTOR in cellular energy homeostasis and drug targets. *Annu. Rev. Pharmacol. Toxicol.* 2012, 52, 381–400.

28. Lapierre, L.R.; Hansen, M. Lessons from *C. elegans*: Signaling pathways for longevity. *Trends Endocrinol. Metab.* 2012, 23, 637–644.

29. Laplante, M.; Sabatini, D.M. mTOR signaling in growth control and disease. *Cell* 2012, 149, 274–293.

30. Yan, L.; Park, J.Y.; Dillinger, J.G.; de Lorenzo, M.S.; Yuan, C.; Lai, L.; Wang, C.; Ho, D.; Tian, B.; Stanley, W.C.; et al. Common mechanisms for calorie restriction and adenylyl cyclase type 5 knockout models of longevity. *Aging Cell* 2012, 11, 1110–1120.

31. Cornu, M.; Albert, V.; Hall, M.N. mTOR in aging, metabolism, and cancer. *Curr. Opin. Genet. Dev.* 2013, 23, 53–62.

32. Jewell, J.L.; Guan, K.L. Nutrient signaling to mTOR and cell growth. *Trends Biochem. Sci.* 2013, 38, 233–242.

33. Vatner, S.F.; Park, M.; Yan, L.; Lee, G.J.; Lai, L.; Iwatsubo, K.; Ishikawa, Y.; Pessin, J.; Vatner, D.E. Adenylyl cyclase type 5 in cardiac disease, metabolism, and aging. *Am. J. Physiol. Heart Circ. Physiol.* 2013, 305, H1–H8.

34. Burkewitz, K.; Zhang, Y.; Mair, W.B. AMPK at the nexus of energetics and aging. *Cell Metab.* 2014, 20, 10–25.

35. Hu, F.; Liu, F. Targeting tissue-specific metabolic signaling pathways in aging: The promise and limitations. *Protein Cell* 2014, 5, 21–35.

36. Evans, D.S.; Kapahi, P.; Hsueh, W.C.; Kockel, L. TOR signaling never gets old: Aging, longevity and TORC1 activity. *Ageing Res. Rev.* 2011, 10, 225–237.

37. Mercken, E.M.; Carbonneau, B.A.; Krzysik-Walker, S.M.; de Cabo, R. Of mice and men: The benefits of caloric restriction, exercise, and mimetics. *Ageing Res. Rev.* 2012, 11, 390–398.

38. Kaeberlein, M. Longevity and aging. *F1000Prime Rep.* 2013, 5, doi:10.12703/P5-5.

39. Kaeberlein, M. mTOR inhibition: From aging to autism and beyond. *Scientifica* 2013, 2013, doi:10.1155/2013/849186.

40. de Cabo, R.; Carmona-Gutierrez, D.; Bernier, M.; Hall, M.N.; Madeo, F. The search for antiaging interventions: From elixirs to fasting regimens. *Cell* 2014, 157, 1515–1526.
41. Weindruch, R.; Walford, R.L. *The Retardation of Aging and Disease by Dietary Restriction*; Charles C. Thomas: Springfield, IL, USA, 1988.

42. Masoro, E.J. *Caloric Restriction: A Key to Understanding and Modulating Aging*, 1st ed.; Elsevier Inc.: Oxford, UK, 2002; p. 192.

43. Min, K.J.; Flatt, T.; Kulaots, I.; Tatar, M. Counting calories in *Drosophila* diet restriction. *Exp. Gerontol.* **2007**, *42*, 247–251.

44. Mair, W.; Dillin, A. Aging and survival: The genetics of life span extension by dietary restriction. *Annu. Rev. Biochem.* **2008**, *77*, 727–754.

45. Colman, R.J.; Anderson, R.M.; Johnson, S.C.; Kastman, E.K.; Kosmatka, K.J.; Beasley, T.M.; Allison, D.B.; Cruzen, C.; Simmons, H.A.; Kemnitz, J.W.; *et al*. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **2009**, *325*, 201–204.

46. Anderson, R.M.; Weindruch, R. Metabolic reprogramming, caloric restriction and aging. *Trends Endocrinol. Metab.* **2010**, *21*, 134–141.

47. Speakman, J.R.; Mitchell, S.E. Caloric restriction. *Mol. Aspects Med.* **2011**, *32*, 159–221.

48. Mattison, J.A.; Roth, G.S.; Beasley, T.M.; Tilmont, E.M.; Handy, A.M.; Herbert, R.L.; Longo, D.L.; Allison, D.B.; Young, J.E.; Bryant, M.; *et al*. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **2012**, *489*, 318–321.

49. Chung, K.W.; Kim, D.H.; Park, M.H.; Choi, Y.J.; Kim, N.D.; Lee, J.; Yu, B.P.; Chung, H.Y. Recent advances in caloric restriction research on aging. *Exp. Gerontol.* **2013**, *48*, 1049–1053.

50. Guarente, L. Calorie restriction and sirtuins revisited. *Genes Dev.* **2013**, *27*, 2072–2085.

51. Libert, S.; Guarente, L. Metabolic and neuropsychiatric effects of caloric restriction and sirtuins. *Annu. Rev. Physiol.* **2013**, *75*, 669–684.

52. Willcox, B.J.; Willcox, D.C. Caloric restriction, caloric restriction mimetics, and healthy aging in Okinawa: Controversies and clinical implications. *Curr. Opin. Clin. Nutr. Metab. Care* **2014**, *17*, 51–58.

53. Miller, R.A.; Buehner, G.; Chang, Y.; Harper, J.M.; Sigler, R.; Smith-Wheelock, M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell* **2005**, *4*, 119–125.

54. Troen, A.M.; French, E.E.; Roberts, J.F.; Selhub, J.; Ordovas, J.M.; Parnell, L.D.; Lai, C.Q. Lifespan modification by glucose and methionine in *Drosophila melanogaster* fed a chemically defined diet. *Age* **2007**, *29*, 29–39.

55. Lee, K.P.; Simpson, S.J.; Clissold, F.J.; Brooks, R.; Ballard, J.W.; Taylor, P.W.; Soran, N.; Raubenheimer, D. Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2498–2503.

56. Alvers, A.L.; Fishwick, L.K.; Wood, M.S.; Hu, D.; Chung, H.S.; Dunn, W.A., Jr.; Aris, J.P. Autophagy and amino acid homeostasis are required for chronological longevity in *Saccharomyces cerevisiae*. *Aging Cell* **2009**, *8*, 353–369.

57. Fanson, B.G.; Weldon, C.W.; Pérez-Staples, D.; Simpson, S.J.; Taylor, P.W. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* **2009**, *8*, 514–523.

58. Flatt, T. Ageing: Diet and longevity in the balance. *Nature* **2009**, *462*, 989–990.
59. Grandison, R.C.; Piper, M.D.; Partridge, L. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* 2009, 462, 1061–1064.

60. Greer, E.L.; Brunet, A. Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. *Aging Cell* 2009, 8, 113–127.

61. Piper, M.D.; Partridge, L.; Raubenheimer, D.; Simpson, S.J. Dietary restriction and aging: A unifying perspective. *Cell Metab.* 2011, 14, 154–160.

62. Tatar, M. The plate half-full: Status of research on the mechanisms of dietary restriction in *Drosophila melanogaster*. *Exp. Gerontol.* 2011, 46, 363–368.

63. Swindell, W.R. Dietary restriction in rats and mice: A meta-analysis and review of the evidence for genotype-dependent effects on lifespan. *Aging Res. Rev.* 2012, 11, 254–270.

64. Aris, J.P.; Alvers, A.L.; Ferraiuolo, R.A.; Fishwick, L.K.; Hanvivatpong, A.; Hu, D.; Kirlew, C.; Leonard, M.T.; Losin, K.J.; Marraffini, M.; et al. Autophagy and leucine promote chronological longevity and respiration proficiency during calorie restriction in yeast. *Exp. Gerontol.* 2013, 48, 1107–1119.

65. Cabreiro, F.; Au, C.; Leung, K.Y.; Vergara-Irigaray, N.; Cochemé, H.M.; Noori, T.; Weinkove, D.; Schuster, E.; Greene, N.D.; Gems, D. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* 2013, 153, 228–239.

66. Liao, C.Y.; Johnson, T.E.; Nelson, J.F. Genetic variation in responses to dietary restriction—an unbiased tool for hypothesis testing. *Exp. Gerontol.* 2013, 48, 1025–1029.

67. Sanchez-Roman, I.; Barja, G. Regulation of longevity and oxidative stress by nutritional interventions: Role of methionine restriction. *Exp. Gerontol.* 2013, 48, 1030–1042.

68. Watson, E.; MacNeil, L.T.; Arda, H.E.; Zhu, L.J.; Walhout, A.J. Integration of metabolic and gene regulatory networks modulates the *C. elegans* dietary response. *Cell* 2013, 153, 253–266.

69. Wu, Z.; Song, L.; Liu, S.Q.; Huang, D. Independent and additive effects of glutamic acid and methionine on yeast longevity. *PLoS One* 2013, 8, e79319.

70. Ruckenstuhl, C.; Netzberger, C.; Entfellner, I.; Carmona-Gutierrez, D.; Kickenweiz, T.; Stekovic, S.; Gleixner, C.; Schmid, C.; Klug, L.; Sorgo, A.G.; et al. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLoS Genet.* 2014, 10, e1004347.

71. Watson, E.; MacNeil, L.T.; Ritter, A.D.; Yilmaz, L.S.; Rosebrock, A.P.; Caudy, A.A.; Walhout, A.J. Interspecies systems biology uncovers metabolites affecting *C. elegans* gene expression and life history traits. *Cell* 2014, 156, 759–770.

72. Howitz, K.T.; Bitterman, K.J.; Cohen, H.Y.; Lamming, D.W.; Lavu, S.; Wood, J.G.; Zipkin, R.E.; Chung, P.; Kisielewski, A.; Zhang, L.L.; et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003, 425, 191–196.

73. Bauer, J.H.; Goupil, S.; Garber, G.B.; Helfand, S.L. An accelerated assay for the identification of lifespan-extending interventions in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 2004, 101, 12980–12985.

74. Wood, J.G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S.L.; Tatar, M.; Sinclair, D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 2004, 430, 686–689.
75. Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-Lluch, G.; Lewis, K.; et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337–342.

76. Powers, R.W., 3rd.; Kaeberlein, M.; Caldwell, S.D.; Kennedy, B.K.; Fields, S. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* **2006**, *20*, 174–184.

77. Valenzano, D.R.; Terzibasi, E.; Genade, T.; Cattaneo, A.; Domenici, L.; Cellerino, A. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* **2006**, *16*, 296–300.

78. Bonawitz, N.D.; Chatenay-Lapointe, M.; Pan, Y.; Shadel, G.S. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metab.* **2007**, *5*, 265–277.

79. Medvedik, O.; Lamming, D.W.; Kim, K.D.; Sinclair, D.A. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PLoS Biol.* **2007**, *5*, e261.

80. Petrascheck, M.; Ye, X.; Buck, L.B. An antidepressant that extends lifespan in adult *Caenorhabditis elegans*. *Nature* **2007**, *450*, 553–556.

81. Anisimov, V.N.; Berstein, L.M.; Egormin, P.A.; Piskunova, T.S.; Popovich, I.G.; Zabezhinski, M.A.; Tyndyk, M.L.; Yurova, M.V.; Kovalenko, I.G.; Poroshina, T.E.; et al. Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle* **2008**, *7*, 2769–2773.

82. Benedetti, M.G.; Foster, A.L.; Vantipalli, M.C.; White, M.P.; Sampaio, J.N.; Gill, M.S.; Olsen, A.; Lithgow, G.J. Compounds that confer thermal stress resistance and extended lifespan. *Exp. Gerontol.* **2008**, *43*, 882–891.

83. McColl, G.; Killilea, D.W.; Hubbard, A.E.; Vantipalli, M.C.; Melov, S.; Lithgow, G.J. Pharmacogenetic analysis of lithium-induced delayed aging in *Caenorhabditis elegans*. *J. Biol. Chem.* **2008**, *283*, 350–357.

84. Pearson, K.J.; Baur, J.A.; Lewis, K.N.; Peshkin, L.; Price, N.L.; Labinskyy, N.; Swindell, W.R.; Kamara, D.; Minor, R.K.; Perez, E.; et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* **2008**, *8*, 157–168.

85. Engel, N.; Mahlknecht, U. Aging and anti-aging: Unexpected side effects of everyday medication through sirtuin1 modulation. *Int. J. Mol. Med.* **2008**, *21*, 223–232.

86. Evason, K.; Collins, J.J.; Huang, C.; Hughes, S.; Kornfeld, K. Valproic acid extends *Caenorhabditis elegans* lifespan. *Aging Cell* **2008**, *7*, 305–317.

87. Wanke, V.; Cameroni, E.; Uotila, A.; Piccolis, M.; Urban, J.; Loewith, R.; de Virgilio, C. Caffeine extends yeast lifespan by targeting TORC1. *Mol. Microbiol.* **2008**, *69*, 277–285.

88. Demidenko, Z.N.; Blagosklonny, M.V. At concentrations that inhibit mTOR, resveratrol suppresses cellular senescence. *Cell Cycle* **2009**, *8*, 1901–1904.

89. Demidenko, Z.N.; Shuttman, M.; Blagosklonny, M.V. Pharmacologic inhibition of MEK and PI-3K converges on the mTOR/S6 pathway to decelerate cellular senescence. *Cell Cycle* **2009**, *8*, 1896–1900.
90. Demidenko, Z.N.; Zubova, S.G.; Bukreeva, E.I.; Pospelov, V.A.; Pospelova, T.V.; Blagosklonny, M.V. Rapamycin decelerates cellular senescence. *Cell Cycle* **2009**, *8*, 1888–1895.
91. Eisenberg, T.; Knauer, H.; Schauer, A.; Büttner, S.; Ruckenstuhl, C.; Carmona-Gutierrez, D.; Ring, J.; Schroeder, S.; Magnes, C.; Antonacci, L.; *et al.* Induction of autophagy by spermidine promotes longevity. *Nat. Cell Biol.* **2009**, *11*, 1305–1314.
92. Harrison, D.E.; Strong, R.; Sharp, Z.D.; Nelson, J.F.; Astle, C.M.; Flurkey, K.; Nadon, N.L.; Wilkinson, J.E.; Frenkel, K.; Carter, C.S.; *et al.* Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* **2009**, *460*, 392–395.
93. Skulachev, V.P.; Anisimov, V.N.; Antonenko, Y.N.; Bakeeva, L.E.; Chernyak, B.V.; Erichev, V.P.; Filenko, O.F.; Kalinina, N.I.; Kapelko, V.I.; Kolosova, N.G.; *et al.* An attempt to prevent senescence: A mitochondrial approach. *Biochim. Biophys. Acta* **2009**, *1787*, 437–461.
94. Bjedov, I.; Toivonen, J.M.; Kerr, F.; Slack, C.; Jacobson, J.; Foley, A.; Partridge, L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* **2010**, *11*, 35–46.
95. Goldberg, A.A.; Richard, V.R.; Kyryakov, P.; Bourque, S.D.; Beach, A.; Burstein, M.T.; Glebov, A.; Koupaki, O.; Boukh-Viner, T.; Gregg, C.; *et al.* Chemical genetic screen identifies lithocholic acid as an anti-aging compound that extends yeast chronological life span in a TOR-independent manner, by modulating housekeeping longevity assurance processes. *Aging* **2010**, *2*, 393–414.
96. Onken, B.; Driscoll, M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* healthspan via AMPK, LKB1, and SKN-1. *PLoS One* **2010**, *5*, e8758.
97. Baur, J.A.; Ungvari, Z.; Minor, R.K.; le Couteur, D.G.; de Cabo, R. Are sirtuins viable targets for improving healthspan and lifespan? *Nat. Rev. Drug Discov.* **2012**, *11*, 443–461.
98. Chung, J.H.; Manganiello, V.; Dyck, J.R. Resveratrol as a calorie restriction mimetic: Therapeutic implications. *Trends Cell Biol.* **2012**, *22*, 546–554.
99. Pan, M.H.; Lai, C.S.; Tsai, M.L.; Wu, J.C.; Ho, C.T. Molecular mechanisms for anti-aging by natural dietary compounds. *Mol. Nutr. Food Res.* **2012**, *56*, 88–115.
100. Park, S.J.; Ahmad, F.; Philp, A.; Baar, K.; Williams, T.; Luo, H.; Ke, H.; Rehmann, H.; Taussig, R.; Brown, A.L.; *et al.* Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. *Cell* **2012**, *148*, 421–433.
101. Pan, Y.; Nishida, Y.; Wang, M.; Verdin, E. Metabolic regulation, mitochondria and the life-prolonging effect of rapamycin: A mini-review. *Gerontology* **2012**, *58*, 524–530.
102. Liu, X.; Wu, W.Y.; Jiang, B.H.; Yang, M.; Guo, D.A. Pharmacological tools for the development of traditional Chinese medicine. *Trends Pharmacol. Sci.* **2013**, *34*, 620–628.
103. Rallis, C.; Codlin, S.; Bähler, J. TORC1 signaling inhibition by rapamycin and caffeine affect lifespan, global gene expression, and cell proliferation of fission yeast. *Aging Cell* **2013**, *12*, 563–573.
104. Stephan, J., Franke, J., Ehrenhofer-Murray, A.E. Chemical genetic screen in fission yeast reveals roles for vacuolar acidification, mitochondrial fission, and cellular GMP levels in lifespan extension. *Aging Cell* **2013**, *12*, 574–583.
105. Rallis, C.; López-Maury, L.; Georgescu, T.; Pancaldi, V.; Bähler, J. Systematic screen for mutants resistant to TORC1 inhibition in fission yeast reveals genes involved in cellular ageing and growth. *Biol. Open* **2014**, *3*, 161–171.

106. Wu, Z.; Song, L.; Liu, S.Q.; Huang, D. Tanshinones extend chronological lifespan in budding yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **2014**, doi:10.1007/s00253-014-5890-5.

107. Blüher, M.; Kahn, B.B.; Kahn, C.R. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **2003**, *299*, 572–574.

108. Chiu, C.H.; Lin, W.D.; Huang, S.Y.; Lee, Y.H. Effect of a C/EBP gene replacement on mitochondrial biogenesis in fat cells. *Genes Dev.* **2004**, *18*, 1970–1975.

109. Picard, F.; Kurtev, M.; Chung, N.; Topark-Ngarm, A.; Senawong, T.; Machado De Oliveira, R.; Leid, M.; McBurney, M.W.; Guarente, L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. *Nature* **2004**, *429*, 771–776.

110. Bordone, L.; Guarente, L. Caloric restriction, SIRT1 and metabolism: Understanding longevity. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 298–305.

111. Grönke, S.; Mildner, A.; Fellert, S.; Tennagels, N.; Petry, S.; Müller, G.; Jäckle, H.; Kühnlein, R.P. Brummer lipase is an evolutionarily conserved fat storage regulator in *Drosophila*. *Cell Metab.* **2005**, *1*, 323–330.

112. Haemmerle, G.; Lass, A.; Zimmermann, R.; Gorkiewicz, G.; Meyer, C.; Rozman, J.; Heldmaier, G.; Maier, R.; Theussl, C.; Eder, S.; et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* **2006**, *312*, 734–737.

113. Gerhart-Hines, Z.; Rodgers, J.T.; Bare, O.; Lerin, C.; Kim, S.H.; Mostoslavsky, R.; Alt, F.W.; Wu, Z.; Puigserver, P. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *EMBO J.* **2007**, *26*, 1913–1923.

114. Gregor, M.F.; Hotamisligil, G.S. Adipocyte stress: The endoplasmic reticulum and metabolic disease. *J. Lipid Res.* **2007**, *48*, 1905–1914.

115. Russell, S.J.; Kahn, C.R. Endocrine regulation of ageing. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 681–691.

116. Olefsky, J.M. Fat talks, liver and muscle listen. *Cell* **2008**, *134*, 914–916.

117. Rodgers, J.T.; Lerin, C.; Gerhart-Hines, Z.; Puigserver, P. Metabolic adaptations through the PGC-1α and SIRT1 pathways. *FEBS Lett.* **2008**, *582*, 46–53.

118. Wang, M.C.; O’Rourke, E.J.; Ruvkun, G. Fat metabolism links germ line stem cells and longevity in *C. elegans*. *Science* **2008**, *322*, 957–960.

119. Narbonne, P.; Roy, R. *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature* **2009**, *457*, 210–214.

120. Lapierre, L.R.; Gelino, S.; Meléndez, A.; Hansen, M. Autophagy and lipid metabolism coordinately modulate life span in germ line-less *C. elegans*. *Curr. Biol.* **2011**, *21*, 1507–1514.

121. Ackerman, D.; Gems, D. The mystery of *C. elegans* aging: An emerging role for fat. *Bioessays* **2012**, *34*, 466–471.

122. Hou, N.S.; Taubert, S. Function and regulation of lipid biology in *Caenorhabditis elegans* aging. *Front. Physiol.* **2012**, *3*, 143.
123. Lapierre, L.R.; Meléndez, A.; Hansen, M. Autophagy links lipid metabolism to longevity in *C. elegans*. *Autophagy* 2012, 8, 144–146.

124. Beach, A.; Titorenko, V.I. Essential roles of peroxisomally produced and metabolized biomolecules in regulating yeast longevity. *Subcell. Biochem.* 2013, 69, 153–167.

125. Huang, X.; Liu, J.; Dickson, R.C. Down-regulating sphingolipid synthesis increases yeast lifespan. *PLoS Genet.* 2012, 8, e1002493.

126. Huang, X.; Liu, J.; Withers, B.R.; Samide, A.J.; Leggas, M.; Dickson, R.C. Reducing signs of aging and increasing lifespan by drug synergy. *Aging Cell* 2013, 12, 652–660.

127. Liu, J.; Huang, X.; Withers, B.R.; Blalock, E.; Liu, K.; Dickson, R.C. Reducing sphingolipid synthesis orchestrates global changes to extend yeast lifespan. *Aging Cell* 2013, 12, 833–841.

128. Huang, X.; Withers, B.R.; Dickson, R.C. Sphingolipids and lifespan regulation. *Biochim. Biophys. Acta* 2014, 1841, 657–664.

129. O’Rourke, E.J.; Kuballa, P.; Xavier, R.; Ruvkun, G. ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes Dev.* 2013, 27, 429–440.

130. Jové, M.; Naudí, A.; Aledo, J.C.; Cabré, R.; Ayala, V.; Portero-Otin, M.; Barja, G.; Pamplona, R. Plasma long-chain free fatty acids predict mammalian longevity. *Sci. Rep.* 2013, 3, doi:10.1038/srep03346.

131. Carrasco, S.; Meyer, T. STIM proteins and the endoplasmic reticulum-plasma membrane junctions. *Annu. Rev. Biochem.* 2011, 80, 973–1000.

132. Friedman, J.R.; Voeltz, G.K. The ER in 3D: A multifunctional dynamic membrane network. *Trends Cell Biol.* 2011, 21, 709–717.

133. Henry, S.A.; Kohlwein, S.D.; Carman, G.M. Metabolism and regulation of glycerolipids in the yeast *Saccharomyces cerevisiae*. *Genetics* 2012, 190, 317–349.

134. Rowland, A.A.; Voeltz, G.K. Endoplasmic reticulum-mitochondria contacts: Function of the junction. *Nat. Rev. Mol. Cell Biol.* 2012, 13, 607–625.

135. Beach, A.; Richard, V.R.; Leonov, A.; Burstein, M.T.; Bourque, S.D.; Koupaki, O.; Juneau, M.; Feldman, R.; Iouk, T.; Titorenko, V.I. Mitochondrial membrane lipidome defines yeast longevity. *Aging* 2013, 5, 551–574.

136. Horvath, S.E.; Daum, G. Lipids of mitochondria. *Prog. Lipid Res.* 2013, 52, 590–614.

137. Kohlwein, S.D.; Veenhuis, M.; van der Klei, I.J. Lipid droplets and peroxisomes: Key players in cellular lipid homeostasis or a matter of fat—store ’em up or burn ’em down. *Genetics* 2013, 193, 1–50.

138. Richard, V.R.; Leonov, A.; Beach, A.; Burstein, M.T.; Koupaki, O.; Gomez-Perez, A.; Levy, S.; Pluska, L.; Mattie, S.; Rafesh, R.; *et al.* Macromitophagy is a longevity assurance process that in chronologically aging yeast limited in calorie supply sustains functional mitochondria and maintains cellular lipid homeostasis. *Aging* 2013, 5, 234–269.

139. Tavassoli, S.; Chao, J.T.; Young, B.P.; Cox, R.C.; Prinz, W.A.; de Kroon, A.I.; Loewen, C.J. Plasma membrane-endoplasmic reticulum contact sites regulate phosphatidylcholine synthesis. *EMBO Rep.* 2013, 14, 434–440.

140. Tatsuta, T.; Scharwey, M.; Langer, T. Mitochondrial lipid trafficking. *Trends Cell Biol.* 2014, 24, 44–52.
141. Thomas, C.; Pellicciari, R.; Pruzanski, M.; Auwerx, J.; Schoonjans, K. Targeting bile-acid signalling for metabolic diseases. *Nat. Rev. Drug Discov.* 2008, 7, 678–693.
142. De Aguiar Vallim, T.Q.; Tarling, E.J.; Edwards, P.A. Pleiotropic roles of bile acids in metabolism. *Cell Metab.* 2013, 17, 657–669.
143. Hylemon, P.B.; Zhou, H.; Pandak, W.M.; Ren, S.; Gil, G.; Dent, P. Bile acids as regulatory molecules. *J. Lipid Res.* 2009, 50, 1509–1520.
144. Lefebvre, P.; Cariou, B.; Lien, F.; Staels, B. Role of bile acids and bile acid receptors in metabolic regulation. *Physiol. Rev.* 2009, 89, 147–191.
145. Vallim, T.Q.; Edwards, P.A. Bile acids have the gall to function as hormones. *Cell Metab.* 2009, 10, 162–164.
146. Chiang, J.Y. Bile acids: Regulation of synthesis. *J. Lipid Res.* 2009, 50, 1955–1966.
147. Monte, M.J.; Marin, J.J.; Antelo, A.; Vazquez-Tato, J. Bile acids: Chemistry, physiology, and pathophysiology. *World J. Gastroenterol.* 2009, 15, 804–816.
148. Tiwari, A.; Maiti, P. TGR5: An emerging bile acid G-protein-coupled receptor target for the potential treatment of metabolic disorders. *Drug Discov. Today* 2009, 14, 523–530.
149. Pols, T.W.; Noriega, L.G.; Nomura, M.; Auwerx, J.; Schoonjans, K. The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. *J. Hepatol.* 2011, 54, 1263–1272.
150. Chiang, J.Y. Bile acid metabolism and signaling. *Compr. Physiol.* 2013, 3, 1191–1212.
151. Li, T.; Chiang, J.Y. Nuclear receptors in bile acid metabolism. *Drug Metab. Rev.* 2013, 45, 145–155.
152. Amador-Noguez, D.; Yagi, K.; Venable, S.; Darlington, G. Gene expression profile of long-lived Ames dwarf mice and Little mice. *Aging Cell* 2004, 3, 423–441.
153. Amador-Noguez, D.; Dean, A.; Huang, W.; Setchell, K.; Moore, D.; Darlington, G. Alterations in xenobiotic metabolism in the long-lived Little mice. *Aging Cell* 2007, 6, 453–470.
154. Gems, D. Long-lived dwarf mice: Are bile acids a longevity signal? *Aging Cell* 2007, 6, 421–423.
155. Gems, D.; Partridge, L. Stress-response hormesis and aging: “That which does not kill us makes us stronger”. *Cell Metab.* 2008, 7, 200–203.
156. Motola, D.L.; Cummins, C.L.; Rottiers, V.; Sharma, K.K.; Li, T.; Li, Y.; Suino-Powell, K.; Xu, H.E.; Auchus, R.J.; Antebi, A.; et al. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell* 2006, 124, 1209–1223.
157. Wollam, J.; Magomedova, L.; Magner, D.B.; Shen, Y.; Rottiers, V.; Motola, D.L.; Mangelsdorf, D.J.; Cummins, C.L.; Antebi, A. The Rieske oxygenase DAF-36 functions as a cholesterol 7-desaturase in steroidogenic pathways governing longevity. *Aging Cell* 2011, 10, 879–884.
158. Lee, S.S.; Schroeder, F.C. Steroids as central regulators of organismal development and lifespan. *PLoS Biol.* 2012, 10, e1001307.
159. Wollam, J.; Magner, D.B.; Magomedova, L.; Rass, E.; Shen, Y.; Rottiers, V.; Habermann, B.; Cummins, C.L.; Antebi, A. A novel 3-hydroxysteroid dehydrogenase that regulates reproductive development and longevity. *PLoS Biol.* 2012, 10, e1001305.
160. Groen, A.K.; Kuipers, F. Bile acid look-alike controls life span in *C. elegans*. *Cell Metab.* 2013, 18, 151–152.
161. Magner, D.B.; Wollam, J.; Shen, Y.; Hoppe, C.; Li, D.; Latza, C.; Rottiers, V.; Hutter, H.; Antebi, A. The NHR-8 nuclear receptor regulates cholesterol and bile acid homeostasis in *C. elegans*. *Cell Metab.* 2013, 18, 212–224.
162. Mahanti, P.; Bose, N.; Bethke, A.; Judkins, J.C.; Wollam, J.; Dumas, K.J.; Zimmerman, A.M.; Campbell, S.L.; Hu, P.J.; Antebi, A.; et al. Comparative metabolomics reveals endogenous ligands of DAF-12, a nuclear hormone receptor, regulating C. elegans development and lifespan. *Cell Metab.* 2014, 19, 73–83.

163. Burstein, M.T.; Kyryakov, P.; Beach, A.; Richard, V.R.; Koupaki, O.; Gomez-Perez, A.; Leonov, A.; Levy, S.; Noohi, F.; Titorenko, V.I. Lithocholic acid extends longevity of chronologically aging yeast only if added at certain critical periods of their lifespan. *Cell Cycle* 2012, 11, 3443–3462.

164. Burstein, M.T.; Titorenko, V.I. A mitochondrially targeted compound delays aging in yeast through a mechanism linking mitochondrial membrane lipid metabolism to mitochondrial redox biology. *Redox Biol.* 2014, 2, 305–307.

165. Sheibani, S.; Richard, V.R.; Beach, A.; Leonov, A.; Feldman, R.; Mattie, S.; Khelghatybana, L.; Piano, A.; Greenwood, M.; Vali, H.; et al. Macromitophagy, neutral lipids synthesis, and peroxisomal fatty acid oxidation protect yeast from “liponecrosis”, a previously unknown form of programmed cell death. *Cell Cycle* 2014, 13, 138–147.

166. Weinberg, R.A. *The Biology of Cancer*, 2nd ed.; Garland Science, Taylor & Francis Group, LLC: New York, NY, USA, 2007; p. 876.

167. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* 2011 144, 646–674.

168. Campisi, J. Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell* 2005, 120, 513–522.

169. Blagosklonny, M.V. Aging and immortality: Quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* 2006, 5, 2087–2102.

170. Campisi, J.; d’Adda di Fagagna, F. Cellular senescence: When bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* 2007, 8, 729–740.

171. Collado, M.; Blasco, M.A.; Serrano, M. Cellular senescence in cancer and aging. *Cell* 2007, 130, 223–233.

172. Finkel, T.; Serrano, M.; Blasco, M.A. The common biology of cancer and ageing. *Nature* 2007, 448, 767–774.

173. Serrano, M.; Blasco, M.A. Cancer and ageing: Convergent and divergent mechanisms. *Nat. Rev. Mol. Cell Biol.* 2007, 8, 715–722.

174. Adams, P.D. Healing and hurting: Molecular mechanisms, functions, and pathologies of cellular senescence. *Mol. Cell* 2009, 36, 2–14.

175. Blagosklonny, M.V.; Hall, M.N. Growth and aging: A common molecular mechanism. *Aging* 2009, 1, 357–362.

176. Ohtani, N.; Mann, D.J.; Hara, E. Cellular senescence: Its role in tumor suppression and aging. *Cancer Sci.* 2009, 100, 792–797.

177. Rodier, F.; Campisi, J. Four faces of cellular senescence. *J. Cell Biol.* 2011, 192, 547–556.

178. Campisi, J. Aging, cellular senescence, and cancer. *Annu. Rev. Physiol.* 2013, 75, 685–705.

179. Piano, A.; Titorenko, V.I. The intricate interplay between mechanisms underlying aging and cancer. *Aging Dis.* 2014, 5, in press.
180. Goldberg, A.A.; Beach, A.; Davies, G.F.; Harkness, T.A.A.; LeBlanc, A.; Titorenko, V.I. Lithocholic bile acid selectively kills neuroblastoma cells, while sparing normal neuronal cells. *Oncotarget* 2011, 2, 761–782.

181. Goldberg, A.A.; Beach, A.; Titorenko, V.I.; Sanderson, J.T. Bile acids induce apoptosis selectively in androgen-dependent and -independent prostate cancer cells. *Peer J.* 2013, 1, doi:10.7717/peerj.122.

182. Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H.; Sugiyama, E.; Nakamura, T.; Itadani, H.; Tanaka, K. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem. Biophys. Res. Commun.* 2002, 298, 714–719.

183. Kawamata, Y.; Fujii, R.; Hosoya, M.; Harada, M.; Yoshida, H.; Miwa, M.; Fukusumi, S.; Habata, Y.; Itoh, T.; Shintani, Y.; et al. A G protein-coupled receptor responsive to bile acids. *J. Biol. Chem.* 2003, 278, 9435–9440.

184. Wachs, F.P.; Krieg, R.C.; Rodrigues, C.M.; Messmann, H.; Kullmann, F.; Knüchel-Clarke, R.; Schölmerich, J.; Rogler, G.; Schlottmann, K. Bile salt-induced apoptosis in human colon cancer cell lines involves the mitochondrial transmembrane potential but not the CD95 (Fas/Apo-1) receptor. *Int. J. Colorectal Dis.* 2005, 20, 103–113.

185. Merrill, R.A.; Dagda, R.K.; Dickey, A.S.; Cribbs, J.T.; Green, S.H.; Usachev, Y.M.; Strack, S. Mechanism of neuroprotective mitochondrial remodeling by PKA/AKAP1. *PLoS Biol.* 2011, 9, e1000612.

186. Taylor, R.C.; Cullen, S.P.; Martin, S.J. Apoptosis: Controlled demolition at the cellular level. *Nat. Rev. Mol. Cell Biol.* 2008, 9, 231–241.

187. Tait, S.W.; Green, D.R. Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Biol.* 2010, 11, 621–632.

188. Kersse, K.; Vanden Berghe, T.; Lamkanfi, M.; Vandenabeele, P. A phylogenetic and functional overview of inflammatory caspases and caspase-1-related CARD-only proteins. *Biochem. Soc. Trans.* 2007, 35, 1508–1511.

189. Dinarello, C.A. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 2009, 27, 519–550.

190. Keller, M.; Rüegg, A.; Werner, S.; Beer, H.D. Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 2009, 132, 818–831.

191. Yi, C.H.; Yuan, J. The Jekyll and Hyde functions of caspases. *Dev. Cell* 2009, 16, 21–34.

192. Li, X.; Jiang, S.; Tapping, R.I. Toll-like receptor signaling in cell proliferation and survival. *Cytokine* 2010, 49, 1–9.

193. Donath, M.Y.; Shoelson, S.E. Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 2011, 11, 98–107.

194. Grivennikov, S.I.; Karin, M. Inflammatory cytokines in cancer: Tumour necrosis factor and interleukin 6 take the stage. *Ann. Rheum. Dis.* 2011, 70, i104–i108.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).