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mTOR, AMPK, and Sirt1: Key Players in Metabolic Stress Management

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ABSTRACT: Cells adapt their metabolism and activities in response to signals from their surroundings, and this ability is essential for their survival in the face of environmental changes. In mammalian tissues a deficit of these mechanisms is commonly associated with cellular aging and degenerative diseases related to aging, such as cardiovascular disease, cancer, immune system decline, and neurological pathologies. Several proteins have been identified as able to respond directly to energy, nutrient, and growth factor levels and stress stimuli in order to mediate adaptations in the cell. Many of these proteins are enzymes that positively or negatively modulate the autophagic process. This review focuses on biochemical mechanisms involving enzymes—specifically, mTOR, AMPK, and Sirt1—that are currently considered important for these adaptive responses, providing an overview of the interactions of the main players in this process.

KEY WORDS: mTOR, AMPK, Sirt1, autophagy.

ABBREVIATIONS: ACC, acetyl CoA carboxylase; Akt, protein kinase B; AMPK, adenosine monophosphate–activated protein kinase; ATM, ataxia-telangiectasia mutated; Atg, autophagy-related protein; Bnip3, Bcl-2/E1B 19 kDa interacting protein; CaMKK2, calcium/calmodulin-dependent protein kinase kinase 2; CBS, cystathionine β-synthase motif; CCD, central catalytic domain; CRTC2, CREB-regulated transcription coactivator 2; deptor, DEP domain–containing mTOR-interacting protein; ER, endoplasmic reticulum; ERK1/2, extracellular signal–regulated kinase 1 and 2; FIP200, FAK-family interacting protein of 200 kDa; FoxO, forkhead box transcription factor O; FXR, farnesoid X receptor; GβL, G protein β-subunit-like protein; Hsp70, heat shock protein of 70 kDa; HIF, hypoxia-inducible factor; HM, homothallic mating; GLUT, glucose transporter; GSK3-β, gly-cogen synthase kinase 3β; IKKβ, IκB kinase β; HMGR, HMG-CoA reductase; LAMP, lysosome-associated membrane protein; LKB1, liver kinase B1; mSin1, mammalian stress-activated map kinase–interacting protein 1; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; Nam, nicotinamide; Nampt, nicotinamide phosphoribosyltransferase; ODC, ornithine decarboxylase; PDK1, 3-phosphoinositide–dependent protein ki-nase 1; PER2, period circadian protein homolog 2; PPAR-γ, peroxisome proliferator–activated receptor γ; PFK2, 6-phosphofructokinase 2; PGC-1α, PPAR-γ coactivator 1α; PKCα, protein kinase C α; pras40, proline-rich Akt substrate 40 kDa; protor1/2, protein observed with rictor 1 and 2; p70S6K1, 70-kDa ribosomal protein S6 kinase 1; raptor, regulatory-associated protein of mTOR; REDD1, DNA damage response 1; Rheb, Ras homolog enriched in brain; rictor, rapamycin-insensitive companion of mTOR; ROS, reactive oxygen species; RSK1, ribosomal S6 kinase; Sir2, silent information regulator 2; Sirt, sirtuin; SGK, serum/glucocorticoid–regulated kinase; SREBP, sterol regulatory element–binding protein; TFEB, transcription factor EB; TSC1/2, tuberous sclerosis proteins 1 and 2; ULK1, unc-51-like kinase 1; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1.

I. INTRODUCTION

Cellular aging is considered an outcome of the impairment of fundamental mechanisms, including changes in the efficiency of energy production, loss of the ability to protect the intracellular environment against oxidative stress[1] and loss of the ability to maintain a proper intracellular structural setting.[2] Emerging evidence indicates that the process of self-digestion defines autophagy as pivotal in the management of these mechanisms.[3] Three forms of autophagy, extensively reviewed in Yorimitsu et al.,[4]

have been described in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy. All promote, in different ways, the proteolytic degradation of intracellular components by the lysosomal system.

In macroautophagy (the predominant form, often termed simply autophagy), the load to degrade is carried to the lysosomes through the formation of a double-membrane vesicle, defined as an autophagosome, that becomes an autophagolysosome after fusion with the lysosome. In microautophagy, material is incorporated directly through invagination of the lysosomal membrane. Both macro- and microautophagy digest cellular structures of large dimensions in both a selective and a nonselective fashion.[5] An extremely selective mechanism characterizes chaperone-mediated autophagy, [6] in which proteins are targeted for degradation by the presence of a particular amino acid sequence[7] that recruits molecular chaperones, such as the heat shock protein of 70 kDa (Hsp70). By recognizing the lysosome-associated membrane protein (LAMP) type 2A, chaperones allow protein translocation through the lysosomal membrane without vesicular traffic.

Autophagy is involved in various human physiological and pathological conditions, such as neurodegeneration, immunity, cancer, development, myopathy, heart disease, liver disease, osteoarthritis, and aging. The biological significance of this process of self-cannibalization is complex and not completely understood. Indeed, even if in certain cases autophagy represents a mode of cell suicide,[8,9] many studies have not distinguished between cell death *accompanied* by autophagy and cell death *caused* by it.[10,11] For example, in cardiac cells palmitate-induced lipotoxicity occurs together with autophagy, and it is unclear whether this phenomenon represents an attempt by the cell to protect itself or represents a cell death mechanism parallel to apoptosis.[12] In any case, it generally performs cytoprotective functions by restoring homeostasis and promoting survival.

In fact, under basal conditions, autophagy is essential for the removal of misfolded proteins and damaged organelles, such as aged mitochondria, a main source of oxidative stress, and thus it promotes proper organelle turnover and reduces the accumulation of toxic protein aggregates. In addition, increasing evidence suggests that autophagy may have a role in hormesis by improving the responsiveness of the cell to noxious stimuli after a previous, low-grade, exposure, for instance in ischemic preconditioning.[13,14] In starvation conditions, catabolic reactions in the autophagic process may be crucial as a source of the energy essential to providing the minimum levels of ATP for cell survival. Furthermore, the products of catabolism, in particular amino acids, are available to the cell for new biosynthetic reactions and allow necessary metabolic change and remodeling of the cellular proteome.

Many stimuli (e.g., the abundance of amino acids and ATP, ammonia produced by amino acid catabolism, hypoxia, stress, growth factors, and hormones) influence the rate of cellular autophagy by affecting the activity of selected proteins—in particular, the mammalian target of rapamycin (mTOR), adenosine monophosphate–activated protein kinase (AMPK), hypoxia-inducible factors (HIFs), members of the class O of forkhead box transcription factors (FoxOs), and sirtuin 1 (Sirt1).

II. mTOR

A. mTOR Complexes: Structure and Signaling

A central hub for cellular metabolism changes is represented by mTOR, a serine/threonine protein kinase that was identified in mammalian cells in 1994 as a target of the antiproliferative molecule rapamycin.[15] This kinase participates in the formation of two large protein complexes called mTOR complex 1 and mTOR complex 2 (mTORC1 and mTORC2), which are known respectively to be sensitive and insensitive to rapamycin and are characterized by the presence of the protein raptor ("regulatory-associated protein of mTOR") or the protein rictor ("rapamycin-insensitive companion of mTOR"), as shown in Fig. 1.

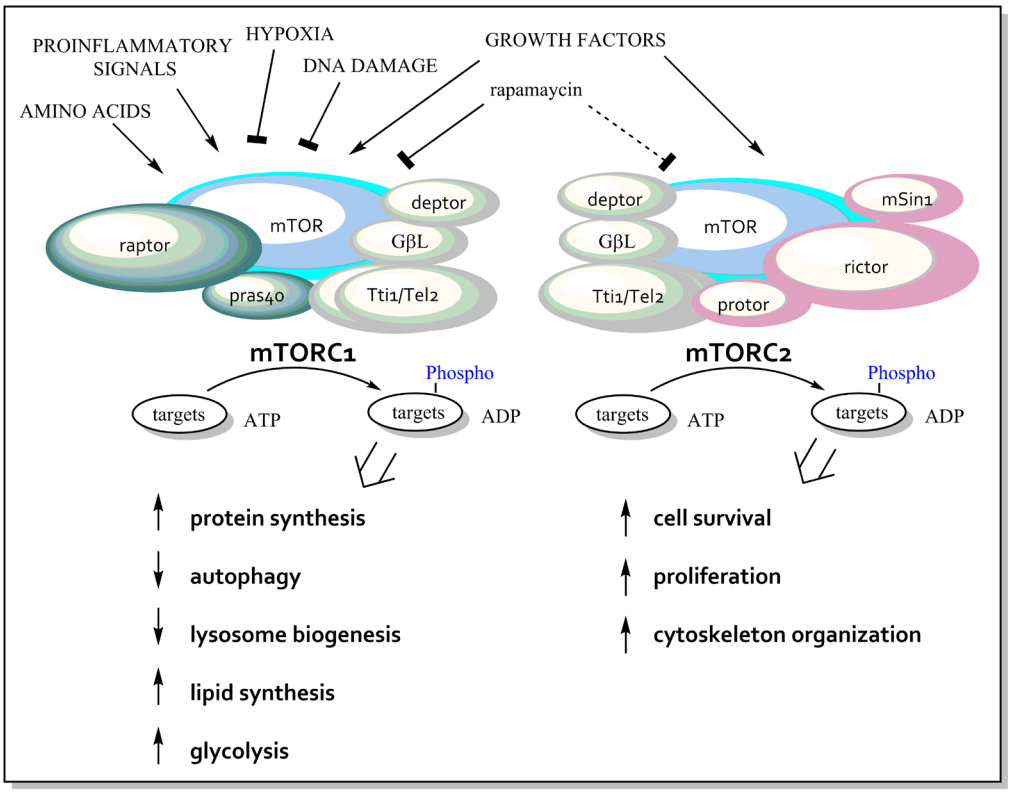

FIG. 1: Signals, mTOR complexes and their downstream processes. Shown are the main proteins associated with mTOR informing the complexes mTORC1 and mTORC2. Several signals converge and impact mTORC1 and/or mTORC2 to stimulate or inhibit the protein kinase activity of mTOR, resulting in the regulation of metabolic pathways and cell processes

Raptor is a scaffold protein that regulates the kinase activity of mTOR by interacting differently under opposite nutrient conditions. Under nutrient-rich conditions it positively modulates the mTORC1 pathway, but in starvation it associates with the complex in a different way, exerting an inhibitory effect on the kinase activity.[16] This sensitivity to rapamycin results from the interaction of this molecule with the FK506-binding protein, which recognizes a specific domain of mTOR in complex 1. In this complex, mTOR can also interact with the proline-rich Akt substrate 40 kDa (pras40), with an inhibitory effect on the kinase activity.[17] Other proteins may be found in both complexes: the G protein β-subunit-like protein (GβL), the mTOR inhibitor known as the DEP domain-containing mTOR-interacting protein (deptor), and the Tti1/Tel2 complex.

In addition to rapamycin, mTORC1 directly or indirectly responds to several cellular signals, including amino acids,[18] energy levels,[19] oxygen,[20] stress,[21] and growth factors,[22] as depicted in Fig. 1. Amino acids are considered the most important mTOR pathway activators and, among them, leucine and arginine in particular are able to stimulate mTORC1 strongly. The first evidence of this phenomenon dates back to 1995 in a study of the amino acid effect on autophagy in isolated rat hepatocytes[23]; even now, however, the precise mechanism of this process is not known in detail. Recent evidence indicates that amino acids are able to promote mTORC1 recruitment by the protein

complex named Regulator on the lysosomal surface and its binding to Rag GTPase heterodimers. There it can be activated by the protein Ras homolog enriched in brain (Rheb).[24] The main upstream key point in mTORC1 regulation is the protein complex containing tuberous sclerosis proteins 1 and 2 (TSC1/2), which convert Rheb in its inactive status bound to GDP. Rheb only in the GTP-bound form positively interacts with mTORC1; thus TSC1/2 inhibits mTOR kinase activity through Rheb inactivation.[25] TSC1/2 integrates many stimuli, as reviewed in detail by Laplante and Sabatini[26]: insulin and growth factors activate mTORC1 by TSC1/2 inhibition that is mediated by protein kinase B (Akt), extracellular signal–regulated kinase 1 and 2 (ERK1/2), and ribosomal S6 kinase (RSK1). TSC1/2 inhibition also results from cytokine-mediated IκB kinase β (IKKβ) activation. The glycogen synthase kinase 3β (GSK3-β) stimulates TSC2 by phosphorylation and therefore is a negative regulator of mTORC1; upstream this enzyme is inhibited by the canonical Wnt pathway. Hypoxia and DNA damage also modulate mTORC1 with an inhibitory effect mediated by DNA damage response 1 (REDD1) and TSC1/2 stimulation.

B. mTOR Targets and Biological Effects

mTORC1 is upstream of many biological processes and is involved overall in cell growth and proliferation. The best characterized effect of mTORC1 activation is increased protein synthesis, mainly mediated by the phosphorylation of 70-kDa ribosomal protein S6 kinase 1 (p70S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). These events positively regulate translation.[27] Moreover, protein and organelle degradation mediated by autophagy is inhibited by mTORC1 action on the protein complex containing the autophagy-initiating kinase ULK1 (unc-51-like kinase 1) and two additional protein factors, FAK-family interacting protein of 200 kDa (FIP200) and autophagy-related protein 13 (Atg13).[28]At the same time, the autophagic process is impaired by the negative modulation exerted by mTORC1 on transcription factor EB (TFEB), which is a master regulator in lysosome biogenesis.[29] The stimulating effect on cell growth and proliferation is also an outcome of the promotion of lipid synthesis by the cleavage of the sterol regulatory element–binding proteins (SREBPs) in their transcriptionally active form[30] and by the increased expression of peroxisome proliferator–activated receptor γ (PPAR-γ).[31]

mTORC1 activation also induces changes in energy metabolism, improving glycolysis-derived energy production by stimulating glucose uptake and glycolysis. Indeed, mTORC1 mediates an increase in hypoxia-inducible factor 1α (HIF1α) translation, which triggers the expression of glucose transporters and glycolytic enzymes, thereby promoting a switch from mitochondrial oxidative metabolism to glycolysis.[32]

Compared to complex 1, fewer data are available regarding the regulation and physiological functions of mTOR's complex 2. In addition to rictor, mammalian stress-activated map kinase-interacting protein 1 (mSin1) and protein observed with rictor 1 and 2 (protor1/2) are specifically present in mTORC2. Although this complex has been defined as insensitive to rapamycin, prolonged treatment actually influences its activity, at least in some cellular models.[33] mTORC2 is localized at the mitochondria-associated endoplasmic reticulum (ER) membrane, and ER stress affects the kinase activity.[34]

The main functions of complex 2 seem to be cytoskeletal organization and promotion of cell survival. In mammalian cells its activity is regulated by growth factor signaling and is upstream of several protein kinase A/G/C (AGC) members, including Akt, serum/glucocorticoid-regulated kinase (SGK), and protein kinase C α (PKCα). mTORC2 phosphorylates newly synthesized Akt and PKCα at the level of a highly conserved region typical of AGC kinases, thus facilitating its carboxyl-terminal folding and stabilizing the protein. In the presence of growth factors, Akt is further phosphorylated at other sites by mTORC2 and 3-phosphoinositide–dependent protein kinase-1 (PDK1), leading to full Akt activation.[35] These events may trigger the subsequent phosphorylation of many Akt substrates and thus affect cell growth, proliferation, apoptosis, and metabolism.

It should be noted that, as mentioned earlier, Akt is upstream of TSC1/2, which means that mTORC2 supports mTORC1 activity.[36] FoxOs are targets and mediators of Akt inhibitory action that inhibit mTORC1 and increase Akt and rictor expression. An intriguing two-part role has been proposed by Chen et al[34]. for FoxOs that seem to be able to maintain mTORC1, mTORC2, and Akt in a homeostatic balance in different conditions: (1) when growth factor and nutrient numbers are excessive, Akt inactivates the FoxOs and activates mTORC1, which in turn stimulates a negative feedback loop, thereby inhibiting Akt and subsequently activating the FoxOs and rictor and finally inhibiting mTORC1 and activate Akt; (2) oxidative or nutrient stress activates the FoxOs, resulting in mTORC1 suppression to reduce energy consumption but maintaining basal Akt activity to sustain energy production.[37] mTORC2-mediated phosphorylation of PKCα may modulate cellular processes such as proliferation, apoptosis, differentiation, motility, and inflammation.[38] mTORC2 is also required for the SGK activities of phosphorylating regulatory proteins that control cellular processes such as ion transport and growth. This complex is upstream of Rho GTPases to regulate the actin cytoskeleton and to control actin polymerization[39]—an effect that seems to be particularly important in neurons.[40]

Taken together, this information, representing only some of the data available in the literature, describes a highly structured framework in which mTOR controls cell functions by integrating a variety of stimuli in very complex signaling.

III. AMPK

A. AMPK: Structure and Signaling

The main cellular sensor of energy status is AMPK, which regulates the supply and use of energy substrates in metabolic stress conditions. Indeed, this enzyme activates pathways for ATP production and simultaneously blocks energy-consuming processes. In addition to energy balance control in a single cell, emerging evidence ascribes to AMPK a broader role as a regulator of whole-body metabolism.[41]

The typical mammalian AMPK structure is a heterotrimer consisting of a catalytic subunit, α, and two regulatory subunits, β and γ.[42] AMPK subunits can be found in different isoforms: α exists as an α1 or α2 isoform[43]; β, as either a β1 or a β2 isoform (the latter is highly expressed in muscle).[44] Finally, there are three possible isoforms γ (γ1, γ2, and γ3).[45] Although the expression pattern of the isoforms varies dramatically between tissues and it is possible to hypothesize a tissue-specific role for the different isoforms, the enzyme formed by the α1, β1, and γ1 subunits is the prevailing one in most cells.

In the catalytic α-subunit, the Ser/Thr kinase domain is located at the amino terminus, followed by an autoinhibitory domain and, at the C-terminal end, by a region necessary for the formation of the trimeric complex. The β-subunit includes two highly conserved domains: a glycogen-binding domain in the central region and a C-terminal region that interacts with the other subunits. The γ-subunit has a highly variable N-terminal region followed by four identical cystathionine β-synthase motifs (CBS1–CBS4), forming two domains termed Bateman.[46] Each CBS sequence has a potential adenosine derivative–binding site, but in mammals CBS2 always appears empty and CBS4 is tied to AMP; thus only CBS1 and CBS3 can modify their state by binding AMP, ADP, or ATP.

In normal conditions, ATP is abundant and occupies the CBS1 and CBS3 sites. If the cell's energy status changes, particularly if the cell is subjected to physiological stress, ATP in site 3 may be replaced by ADP or AMP, stimulating the catalytically competent α-subunit. Moreover, nucleotide substitution promotes the phosphorylation of a conserved Thr in an activation loop sequence present in this subunit, which strongly improves kinase activity. The Thr residue can be phosphorylated by liver kinase B1 (LKB1)[47]or calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2).[48]In a severe stress situation, AMP can cause a further allosteric activation of the enzyme by interacting with site 1 in the γ-subunit. When the cell's energy content normalizes, ATP replaces ADP or AMP and in con-

sequence the Thr in the activation loop is dephosphorylated.[42]

Most known stimuli that activate AMPK (summarized in Fig. 2) operate by causing changes in the cell's energy state—namely, increasing the intracellular concentration of ADP, AMP, or calcium. This is referred as the "canonical" mechanism, and glucose starvation, physical exercise, and hypoxia are typical of stimuli that act in this way. Also, metformin, a very widely used drug for type 2 diabetes, activates AMPK by increasing intracellular levels of AMP, although other mechanisms have been supposed to justify its therapeutic effects.[49]

Many molecules derived from plants, such as resveratrol[50] and curcumin,[51] have been shown to have a stimulatory effect on AMPK. Usually their mode of action involves mitochondrial ATP synthesis inhibition with consequent ATP depletion. Thus the activation of AMPK results from the operation of these molecules as mimetics of caloric restriction. In addition to this classical activation mechanism, a more direct mechanism has been identified that is performed by reactive oxygen species (ROS) such as hydrogen peroxide: AMPK is activated when two conserved Cys residues in the autoinhibitory domain of the enzyme are oxidized.[52] Some reports indicate that AMPK can be activated by ataxia-telangiectasia mutated (ATM), a cellular damage sensor responsible for coordinating damage-response checkpoints and DNA repair during the cell cycle.[53] Oxidative stress, genotoxic stimuli, and treatments causing DNA damage seem to involve ATM for AMPK activation. Several studies indicate that hypoxia can positively modulate AMPK activity and its downstream pathway as well,[54,55]and the activation seems to be related to the increase of ROS and intracellular calcium.[56]

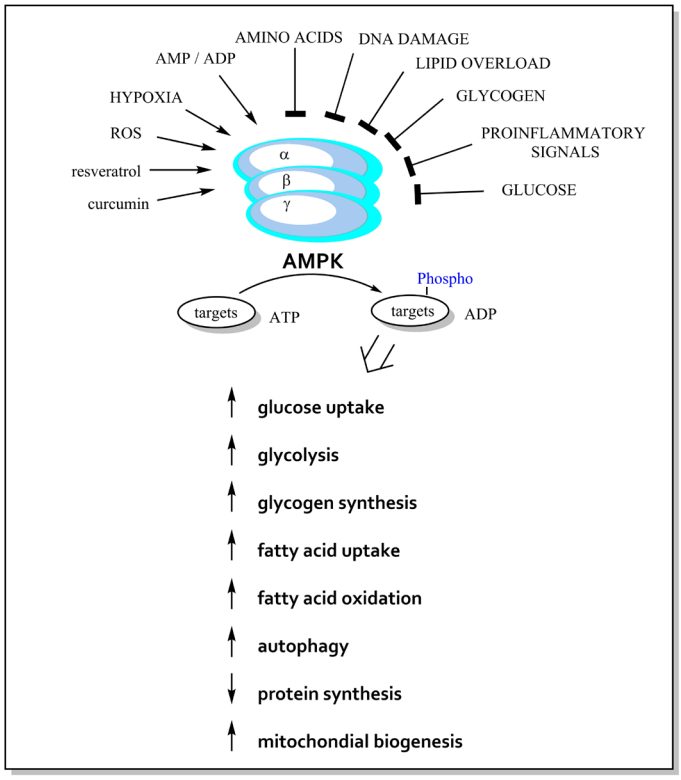

FIG. 2: Signals, AMPK, and its downstream processes. Shown are the heterotrimeric composition of AMPK, consisting of a catalytic α-subunit and two regulatory subunits, β and γ. Several signals converge and impact AMPK to stimulate or inhibit its catalytic activity, resulting in the regulation of metabolic pathways and cellular processes

Mechanisms of AMPK inhibition also actively participate in the control of energy homeostasis both in the single cell and in the whole organism.[57] Increasing evidence supports a correlation between conditions related to a high-fat diet (such as insulin resistance and obesity) and low AMPK activity. Indeed, a lipid overload (palmitate in particular) may inhibit this enzyme.[58] High glucose and glycogen accumulation negatively modulate AMPK,[58] and some studies have demonstrated that this kinase may be inhibited by amino acids,[59,60] assigning it a role in amino acid sensing opposite to that of mTOR. Proinflammatory signals (tumor necrosis factor α, for example) inhibit AMPK activity,[61] and interestingly increasing evidence indicates that AMPK is a negative regulator of NF-κB–mediated proinflammatory pathways.[62,63]

B. AMPK Targets and Biological Effects

Many effects have been described downstream of AMPK on cellular glucose, lipid and protein metabolism and, because of its actions on autophagy and mitochondria biogenesis, on the production of energy in general. In glucose metabolism, AMPK promotes glucose uptake by glucose transporter types 4 and 1 (GLUT4 and GLUT1)[64,65] and promotes glycolysis via phosphorylation of the isoforms of 6-phosphofructokinase-2 (PFK2), which are present in cardiomyocytes,[66] monocytes, and macrophages.[54] Moreover, the anabolic pathways in glucose metabolism are down-regulated by glycogen synthase inhibition[67]and by the transcription arrest of the gluconeogenic enzymes.[68,69] At the same time, fatty acid uptake in cardiac cells, mediated by the transporter CD36, and fatty acid oxidation are positively influenced by AMPK,[70,71] which phosphorylates and inhibits acetyl CoA carboxylase 2 (ACC2). AMPK also inhibits the ACC1 isoform, which is involved in fatty acid synthesis in other tissues[72,73] and affects the transcription of lipogenic enzymes in liver by SREBP phosphorylation.[74] It also inhibits cholesterol synthesis by phosphorylating the rate-limiting enzyme HMG-CoA reductase (HMGR),[75] and triglyceride synthesis.[76] AMPK's energy control function is also exerted by activating mitochondrial biogenesis. This effect is realized by direct phosphorylation of PPAR-γ coactivator 1α (PGC-1α), the master regulator of this process.[77] Moreover, if on the one hand AMPK promotes the synthesis of new mitochondria, on the other hand it influences their turnover by activating their degradation through the autophagic process (in this case called mitophagy). This provides effective quality control of energy output efficiency and minimizes ROS production by aged mitochondria. The modulation of autophagy is performed by direct ULK1 phosphorylation, which triggers the process,[78] as well as by a negative modulation on the mTOR axis,[79] thus adding an inhibitory effect on protein synthesis to the previously described catabolic and antianabolic actions of this kinase.

IV. SIRT1

A. Sirt1: Structure and Signaling

The mammalian sirtuin family has been the object of several studies, spurring a huge interest in its fundamental role as a longevity factor and metabolic sensor. It was discovered at least 30 years ago when Sir2 (silent information regulator 2) was identified in yeast and found to be a silencer of specific genome regions, such as telomeric ends and homothallic mating (HM) loci. In this way it influences the replicative aging process of yeast.[80] When the exact activity of Sir2 was discovered and characterized as NAD^+-dependent histone deacetylase,[81] a mechanistic explanation became available for the actual role of this protein as a metabolic link between the energetic state and life span.

Sir2 has seven orthologs (Sirt1–Sirt7) in mammals that are ubiquitously expressed in tissues and have different subcellular localizations and activities. While Sirt1, Sirt6, and Sirt7 lie mainly in the nucleus, Sirt3, Sirt4, and Sirt5 are mitochondrial proteins and Sirt2 is found to act in the cytoplasm. The best studied and described member of this family is Sirt1. Despite being principally a nuclear protein, it is provided with two nuclear localization signals and two nuclear exportation signals,

via which it shuttles between nucleus and cytosol.[82] Human Sirt1 shows a central catalytic domain (CCD) that is conserved and is flanked by N- and C-terminal ends. The functions of these extensions are unclear, particularly that of the C-terminal region, but it is likely that they serve as regulators. Sirt1 is rich in editable residues that allow it to respond to homeostatic changes caused by events such as phosphorylation, sumoylation, methylation, and nitrosylation.[82]

As shown in Fig. 3, only in the presence of the cosubstrate NAD^+ does the reaction lead to removal of acetyl groups from an acetylated substrate, releasing nicotinamide, 2'-O-acetyl-ADP-ribose and the deacetylated target (e.g., histonic lysines). Thus, the NAD^+ biosynthetic rate is crucial in Sirt1 regulation. It has been shown that NAD^+ increases in mammals during exercise, fasting, and caloric restriction,[83-85] and the same conditions are associated with intensive Sirt1 activity. Furthermore, Sirt1 seems to be implicated in regulation of the circadian rhythm. Two studies have reported an interaction between Sirt1 and CLOCK-BMAL1, joint master regulators of the circadian clock, [86,87] which implies that Sirt1 is the molecular link among several cellular processes, playing a regulatory role in the circadian clock, aging, cellular metabolism, and stress response. These different processes are characterized by changes in NAD^+ levels and in turn influence Sirt1 activity in a fine and detailed circuit. Because this scenario identifies Sirt1 as a pivotal modulator of adaptive responses to the metabolic environment, its functional deficiency may imply cellular deterioration and aging.

B. Sirt1 Targets and Biological Effects

The important role of Sirt1 mirrors the fundamental role of its targets as effectors of the processes just described. One of the best studied Sirt1 sub-

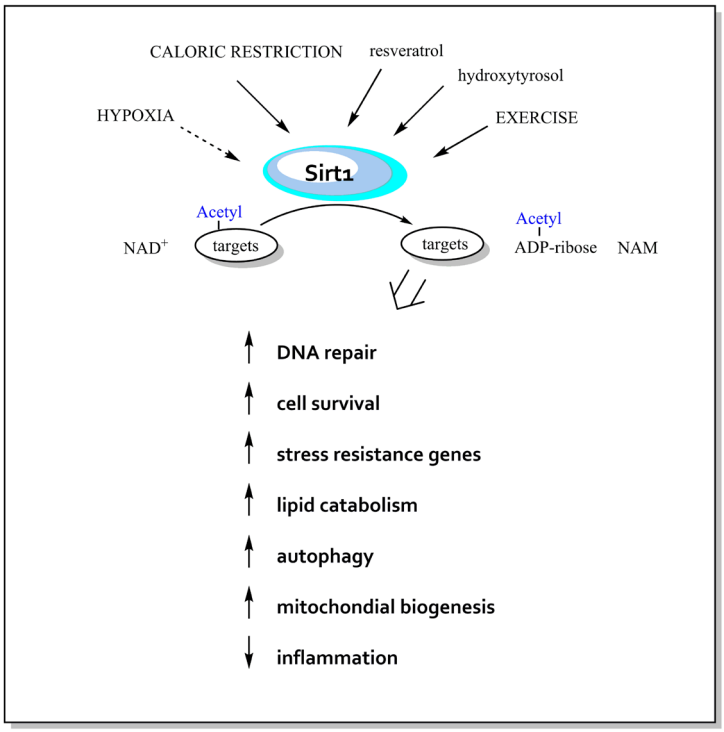

FIG. 3: Signals, Sirt1, and its downstream processes. Several signals converge on Sirt1 to stimulate its protein deacetylase activity, which uses NAD^+ as a cosubstrate and releases acetyl-ADP-ribose and nicotinamide (NAM). Deacetylation of protein substrates results in the regulation of metabolic pathways and cellular processes, whereas NAM can be regenerated to NAD^+ by a salvage pathway

strates is p53,[88,89] the identification of which as a target has shown that Sirt1 does not act merely as a histone deacetylase. It seems as well to deacetylate p53 on Lys^{382} in humans and inhibit p53-dependent apoptosis.[88,89] This prosurvival function is in line with Sirt1's effect in tumoral promotion. However, besides Lys^{382}, six deacetylable residues occur on p53 and it is possible that its specific activity varies according to different acetylated sites and to other subsequent post-translational modifications, and whose pattern is recognized like a "code."

PGC-1α has been reported as Sirt1 target, and its deacetylation appears to be necessary for its activation.[84] As for FoxOs, an energy stress stimulus is required for Sirt1-mediated activation of PGC-1α. This enigma has been resolved with the entry of another actor in this play, AMPK, described earlier. During an imbalance in the AMP/ATP ratio, AMPK phosphorylates PGC-1α, priming it as a substrate for deacetylation/activation by Sirt1. Furthermore, the relationship between Sirt1 and AMPK is not limited to targeting the same factor, but seems to be closer than that. For instance, it has been described how AMPK inhibition or siRNA-mediated knockdown can affect Sirt1 expression.[90]

Important targets of Sirt1 deacetylase activity are HIF1α and HIF2α. Dioum et al. were the first to show that HIF2α is stimulated by Sirt1 during hypoxia and in this way may regulate protective factors downstream.[91] However, it was later reported by Lim and colleagues that HIF1α, in its deacetylated form, fails to mediate metabolic adaptation to hypoxia. Indeed, in this condition lowered NAD^+ levels result in Sirt1 inhibition and thus in acetylation and activation of HIF1α. Lim et al. also found that hypoxia down-regulates Sirt1 mRNA.[92]

The list of Sirt1 substrates has been growing and now includes liver X receptor (LXR); SREBPs and farnesoid X receptor (FXR), which are involved in lipid metabolism; NF-κB in inflammatory response; period circadian protein homolog 2 (PER2) in the circadian clock; CREB-regulated transcription coactivator 2 (CRTC2) in gluconeogenesis; E2F1 in cell survival; and KU70 in DNA repair.[93,94]

Not surprisingly, scientific interest is growing in strategies to stimulate Sirt1 exogenously. The most talked about compound is resveratrol, a polyphenol commonly identified in red wine. Howitz et al. in 2003 described resveratrol-mediated enhancement of Sirt1 and life span.[95] The direct activation of Sirt1 by resveratrol was questioned by Bora et al. in 2005,[96] who showed that resveratrol cannot stimulate Sirt1 activity without a functional AMPK and proposed that Sirt1 is a downstream AMPK effector.[97] It would be interesting to characterize the mechanisms underlying Sirt1 stimulation as induced by other phenolic compounds. In a recent study we showed that hydroxytyrosol, a molecule mainly derived from olives, increases Sirt1 expression.[98] Further investigations will be necessary to better understand the molecular mechanisms underlying this event.

Brunet et al. showed that Sirt1 interacts with and deacetylates FoxO transcription factors. They reported how, on the one hand, Sirt1 switches off the expression of apoptosis-related genes and, on the other hand, directs FoxOs toward the expression of resistance stress–related genes.[99] In accordance with this finding, a 2005 study on hepatocytes demonstrated that deacetylation targets FoxO for nuclear retention, which is necessary for its transcriptional activity.[100]

However, until 2008 the actual potential of Sirt1 as modulator of cellular processes was unclear. The missing piece in this complicated puzzle was the discovery that Sirt1 may be involved in autophagy by deacetylating Atg5, Atg7, and Atg8.[101] Actually, this finding did not come as a surprise, because both autophagy and Sirt1 are stimulated during starvation and fasting. It has been shown how Sirt1 deacetylates these autophagy-related proteins in a NAD^+-dependent fashion.[101] Moreover, it has been found that FoxO3 deacetylation may control the transcription of autophagy-related genes such as LC3 and Bcl-2/E1B 19 kDa interacting protein (Bnip3) in skeletal muscle,[102] and that Sirt1 promotes starvation-induced autophagy by deacetylating FoxO in cardiac myocytes.[103]

A better understanding of the biochemical relationship between Sirt1 and autophagy may pro-

vide new insight into the actual functions of Sirt1 in energy stress response, energy sensing, and energy recovery.

V. CONCLUSIONS

According to data currently available in the literature, AMPK and Sirt1, as opposed to mTOR, are involved in the regulation of many cellular and metabolic processes, including autophagy. The cross-talk among these three enzymes occurs at several levels: not only is it that they influence biological processes independently of each other but the action of one may be mediated or modulated by the actions of the other(s) in some cases. For example, AMPK's autophagy-promoting activity results from direct phosphorylation of ULK1[78] independent of mTOR's control of autophagy , as well as from inhibition of an mTOR pathway[79] through AMPK-mediated phosphorylation of raptor[104] and TSC2.[105] Likely via mTOR inhibition, AMPK negatively modulates the expression of ornithine decarboxylase (ODC),[106] the first and rate-limiting enzyme in the biosynthesis of polyamines, which are essential for cell growth and proliferation. Interestingly, the presence of certain amino acids, primarily Asn and Gln, is required—via mTOR modulation—for ODC induction by growth factors and insulin.[107] However, polyamine depletion following treatment with an ODC inhibitor can down-regulate AMPK expression and activation[108] or accelerate the decline of its activation.[109] This suggests a role for polyamines in sustaining AMPK activity, which means that these organic polycations may represent homeostatic factors operating in the interplay between mTOR and AMPK, at least in some contexts. Eisenberg et al. presented evidence that the polyamine spermidine acts as a longevity factor in model organisms by inducing autophagy.[110] Subsequently, it was found that resveratrol and spermidine in human cultured cells promote autophagy in a Sirt1-dependent and Sirt1-independent way, respectively, even if these compounds stimulate convergent pathways that culminate in concordant modifications of the acetylproteome.[111] It should be noted that polyamines not only are synthesized in cells from the amino acids Arg and Met, but can derive from diet, and it has been reported that polyamine-rich food decreases age-associated pathology and mortality in aged mice.[112]

A further difficulty in this picture is that, despite many convergent biological effects of AMPK and Sirt1, the complex cross-talk between them does not allow a clear determination of which one is upstream and which one is downstream. It has been demonstrated that AMPK does not directly phosphorylate Sirt1, but the effect of this kinase on lipid oxidation is to modify the NAD^+/NADH ratio, thus modulating Sirt1 deacetylase activity.[83] Moreover, some evidence indicates that the expression of Nampt (Nicotinamide phosphoribosyltransferase), which is involved in the synthesis of NAD^+ by the NAD^+ salvage pathway, is induced by AMPK.[85,113] Even so, LKB1, upstream of AMPK activation, is a target of Sirt1, which may regulate its acetylation status, intracellular localization, and activity.[114] Finally, an interesting study by Guo et al. showed that Sirt1 overexpression in neurons is associated with a reduction in mTOR protein levels and activity, thus providing a new Sirt1 negative modulation of mTOR signaling.[115]

A large and increasing number of reports, only some of which were cited in this review, delineate an intricate and complex molecular system that senses nutrient and energy status, growth factor levels, and stress stimuli. This system has been only partly unraveled, but it is known that its core consists of three master enzymes—mTOR, AMPK, and Sirt1—which cross-talk and integrate signals, ensuring tight management of autophagy and cell homeostasis, as shown in Fig. 4. Switching on autophagy by careful nutritional, pharmacological, and/or molecular regulation and manipulation of these key players may promote longevity and protection against degenerative and aging-associated diseases.

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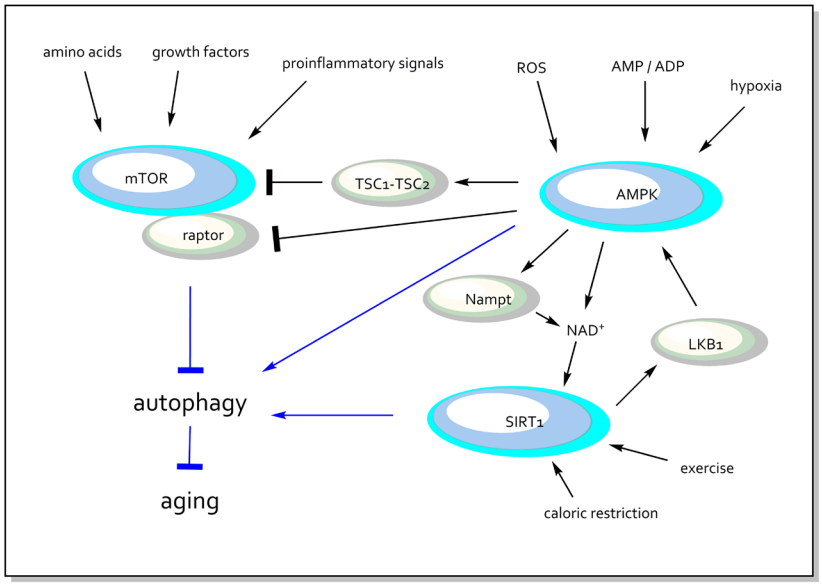

FIG. 4: Interactions between mTOR, AMPK, and Sirt1. Sensing and integration of nutrients, growth, energy, and stress signals require intricate cross-talk, only partly defined, among mTOR, AMPK, and Sirt1, resulting in the regulation of autophagy. Promotion of autophagy has antiaging effects at the cellular and organ level and can increase the life span of the organism. Nampt is an enzyme of the NAD^+ salvage pathway

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DISCLAIMER

The authors declare that they have no conflicts of interest.

REFERENCES

1. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239-47. Cited in PubMed; PMID 11089981.
2. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217. Cited in PubMed; PMID 23746838; PubMed Central PMCID 3836174.
3. Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. Cell. 2011;146(5):682-95. Cited in PubMed; PMID 21884931.
4. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. Cell Death Differ. 2005;12(Suppl 2):1542-52. Cited in PubMed; PMID 16247502; PubMed Central PMCID 1828868.
5. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy. 2011;7(3):279-96. Cited in PubMed; PMID 21189453. PubMed Central PMCID 3060413.
6. Dice JF. Chaperone-mediated autophagy. Autophagy. 2007;3(4):295-9. Cited in PubMed; PMID 17404494.
7. Dice JF. Peptide sequences that target cytosolic proteins for lysosomal proteolysis. Trends Biochem Sci. 1990;15(8):305-9. Cited in PubMed; PMID 2204156.
8. Xu Y, Kim SO, Li Y, Han J. Autophagy contributes to caspase-independent macrophage cell death. J Biol Chem. 2006;281(28):19179-87. Cited in PubMed; PMID 16702227.
9. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, Lenardo M. Autophagic programmed cell death by selective catalase degradation. Proc Natl Acad Sci USA. 2006;103(13):4952-7. Cited in PubMed; PMID 16547133. PubMed Central PMCID 1458776.
10. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ. 2005;12(Suppl 2):1528-34. Cited in PubMed; PMID 16247500.
11. Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. Nat Rev Mol Cell Biol. 2008;9(12):1004-10. Cited in PubMed; PMID 18971948. PubMed Central PMCID 2727358.
12. Cetrullo S, Tantini B, Flamigni F, Pazzini C, Facchini A, Stefanelli C, Caldarera CM, Pignatti C. Antiapop-

totic and antiautophagic effects of eicosapentaenoic acid in cardiac myoblasts exposed to palmitic acid. Nutrients. 2012;4(2):78-90. Cited in PubMed; PMID WOS:000300718900002. English.

13. Gottlieb RA, Mentzer RM. Autophagy during cardiac stress: joys and frustrations of autophagy. Annu Rev Physiol. 2010;72:45-59. Cited in PubMed; PMID 20148666. PubMed Central PMCID 3682821.
14. Sheng R, Zhang LS, Han R, Liu XQ, Gao B, Qin ZH. Autophagy activation is associated with neuroprotection in a rat model of focal cerebral ischemic preconditioning. Autophagy. 2010;6(4):482-94. Cited in PubMed; PMID 20400854.
15. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell. 1994;78(1):35-43. Cited in PubMed; PMID 7518356.
16. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell. 2002;110(2):163-75. Cited in PubMed; PMID 12150925.
17. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell. 2007;25(6):903-15. Cited in PubMed; PMID 17386266.
18. Meijer AJ, Lorin S, Blommaart EF, Codogno P. Regulation of autophagy by amino acids and MTOR-dependent signal transduction. Amino Acids. 2014. Cited in PubMed; PMID 24880909.
19. Dimitroff B, Howe K, Watson A, Campion B, Lee HG, Zhao N, O'Connor MB, Neufeld TP, Selleck SB. Diet and energy-sensing inputs affect TorC1-mediated axon misrouting but not TorC2-directed synapse growth in a Drosophila model of tuberous sclerosis. PLoS One. 2012;7(2):e30722. Cited in PubMed; PMID 22319582. PubMed Central PMCID 3272037.
20. Vadysirisack DD, Ellisen LW. mTOR activity under hypoxia. Methods Mol Biol. 2012;821:45-58. Cited in PubMed; PMID 22125059. PubMed Central PMCID 3960283.
21. Aramburu J, Ortells MC, Tejedor S, Buxade M, Lopez-Rodriguez C. Transcriptional regulation of the stress response by mTOR. Sci Signal. 2014;7(332):re2. Cited in PubMed; PMID 24985347.
22. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell. 2010;40(2):310-22. Cited in PubMed; PMID 20965424. PubMed Central PMCID 2993060.
23. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ. Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J Biol Chem. 1995;270(5):2320-6. Cited in PubMed; PMID 7836465.
24. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010;141(2):290-303. Cited in PubMed; PMID 20381137. PubMed Central PMCID 3024592.
25. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol. 2003;13(15):1259-68. Cited in PubMed; PMID 12906785.
26. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149(2):274-93. Cited in PubMed; PMID 22500797. PubMed Central PMCID 3331679.
27. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol. 2009;10(5):307-18. Cited in PubMed; PMID 19339977.
28. Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem. 2009;284(18):12297-305. Cited in PubMed; PMID 19258318. PubMed Central PMCID 2673298.
29. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, Huynh T, Ferron M, Karsenty G, Vellard MC, Facchinetti V, Sabatini DM, Ballabio A. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. EMBO J. 2012;31(5):1095-108. Cited in PubMed; PMID 22343943. PubMed Central PMCID 3298007.
30. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, Vander Heiden MG, MacKeigan JP, Finan PM, Clish CB, Murphy LO, Manning BD. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell. 2010;39(2):171-83. Cited in PubMed; PMID 20670887. PubMed Central PMCID 2946786.
31. Kim JE, Chen J. regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes. 2004;53(11):2748-56. Cited in PubMed; PMID 15504954.
32. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol. 2002;22(20):7004-14. Cited in PubMed; PMID 12242281. PubMed Central PMCID 139825.
33. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM. Prolonged ra-

pamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 2006;22(2):159-68. Cited in PubMed; PMID 16603397.

34. Chen CH, Shaikenov T, Peterson TR, Aimbetov R, Bissenbaev AK, Lee SW, Wu J, Lin HK, Sarbassov dos D. ER stress inhibits mTORC2 and Akt signaling through GSK-3beta-mediated phosphorylation of rictor. Sci Signal. 2011;4(161):ra10. Cited in PubMed; PMID 21343617.
35. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ, Sessa WC, Qin J, Zhang P, Su B, Jacinto E. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J. 2008;27(14):1932-43. Cited in PubMed; PMID 18566586. PubMed Central PMCID 2486276.
36. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129(7):1261-74. Cited in PubMed; PMID 17604717. PubMed Central PMCID 2756685.
37. Chen CC, Jeon SM, Bhaskar PT, Nogueira V, Sundararajan D, Tonic I, Park Y, Hay N. FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. Dev Cell. 2010;18(4):592-604. Cited in PubMed; PMID 20412774. PubMed Central PMCID 3031984.
38. Nakashima S. Protein kinase C alpha (PKC alpha): regulation and biological function. J Biochem. 2002;132(5):669-75. Cited in PubMed; PMID 12417014.
39. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol. 2004;6(11):1122-8. Cited in PubMed; PMID 15467718.
40. Huang W, Zhu PJ, Zhang S, Zhou H, Stoica L, Galiano M, Krnjevic K, Roman G, Costa-Mattioli M. mTORC2 controls actin polymerization required for consolidation of long-term memory. Nat Neurosci. 2013;16(4):441-8. Cited in PubMed; PMID 23455608. PubMed Central PMCID 3615448.
41. Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med. 2010;16(9):1001-8. Cited in PubMed; PMID 20802499. PubMed Central PMCID 2935934.
42. Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D, Jing C, Walker PA, Eccleston JF, Haire LF, Saiu P, Howell SA, Aasland R, Martin SR, Carling D, Gamblin SJ. Structure of mammalian AMPK and its regulation by ADP. Nature. 2011;472(7342):230-3. Cited in PubMed; PMID 21399626. PubMed Central PMCID 3078618.
43. Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, House CM, Fernandez CS, Cox T, Witters LA, Kemp BE. Mammalian AMP-activated protein kinase subfamily. J Biol Chem. 1996;271(2):611-4. Cited in PubMed; PMID 8557660.
44. Thornton C, Snowden MA, Carling D. Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. J Biol Chem. 1998;273(20):12443-50. Cited in PubMed; PMID 9575201.
45. Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem J. 2000;346(Pt 3):659-69. Cited in PubMed; PMID 10698692. PubMed Central PMCID 1220898.
46. Bateman A. The structure of a domain common to archaebacteria and the homocystinuria disease protein. Trends Biochem Sci. 1997;22(1):12-3. Cited in PubMed; PMID 9020585.
47. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlattner U, Wallimann T, Carlson M, Carling D. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr Biol. 2003;13(22):2004-8. Cited in PubMed; PMID 14614828.
48. Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell Metab. 2005;2(1):9-19. Cited in PubMed; PMID 16054095.
49. Foretz M, Hebrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, Sakamoto K, Andreelli F, Viollet B. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J Clin Invest. 2010;120(7):2355-69. Cited in PubMed; PMID 20577053. PubMed Central PMCID 2898585.
50. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. Proc Natl Acad Sci USA. 2007;104(17):7217-22. Cited in PubMed; PMID 17438283. PubMed Central PMCID 1855377.
51. Um MY, Hwang KH, Ahn J, Ha TY. Curcumin attenuates diet-induced hepatic steatosis by activating AMP-activated protein kinase. Basic Clin Pharmacol Toxicol. 2013;113(3):152-7. Cited in PubMed; PMID 23574662.
52. Zmijewski JW, Banerjee S, Bae H, Friggeri A, Lazarowski ER, Abraham E. Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. J Biol Chem. 2010;285(43):33154-64. Cited in PubMed; PMID 20729205. PubMed Central PMCID 2963401.
53. Alexander A, Cai SL, Kim J, Nanez A, Sahin M, MacLean KH, Inoki K, Guan KL, Shen J, Person MD, Kuse-

witt D, Mills GB, Kastan MB, Walker CL. ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proc Natl Acad Sci USA. 2010;107(9):4153-8. Cited in PubMed; PMID 20160076. PubMed Central PMCID 2840158.

54. Marsin AS, Bouzin C, Bertrand L, Hue L. The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. J Biol Chem. 2002;277(34):30778-83. Cited in PubMed; PMID 12065600.
55. Mu J, Brozinick JT Jr., Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell. 2001;7(5):1085-94. Cited in PubMed; PMID 11389854.
56. Gusarova GA, Trejo HE, Dada LA, Briva A, Welch LC, Hamanaka RB, Mutlu GM, Chandel NS, Prakriya M, Sznajder JI. Hypoxia leads to Na,K-ATPase downregulation via Ca(2+) release-activated Ca(2+) channels and AMPK activation. Mol Cell Biol. 2011;31(17):3546-56. Cited in PubMed; PMID 21730292. PubMed Central PMCID 3165547.
57. Viollet B, Horman S, Leclerc J, Lantier L, Foretz M, Billaud M, Giri S, Andreelli F. AMPK inhibition in health and disease. Crit Rev Biochem Mol Biol. 2010;45(4):276-95. Cited in PubMed; PMID 20522000. PubMed Central PMCID 3132561.
58. Wu Y, Song P, Xu J, Zhang M, Zou MH. Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. J Biol Chem. 2007;282(13):9777-88. Cited in PubMed; PMID 17255104.
59. Leclerc I, Rutter GA. AMP-activated protein kinase: a new beta-cell glucose sensor?: Regulation by amino acids and calcium ions. Diabetes. 2004;53(Suppl 3):S67-74. Cited in PubMed; PMID 15561925.
60. Gleason CE, Lu D, Witters LA, Newgard CB, Birnbaum MJ. The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. J Biol Chem. 2007;282(14):10341-51. Cited in PubMed; PMID 17287212.
61. Steinberg GR, Michell BJ, van Denderen BJ, Watt MJ, Carey AL, Fam BC, Andrikopoulos S, Proietto J, Gorgun CZ, Carling D, Hotamisligil GS, Febbraio MA, Kay TW, Kemp BE. Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metab. 2006;4(6):465-74. Cited in PubMed; PMID 17141630.
62. Peairs A, Radjavi A, Davis S, Li L, Ahmed A, Giri S, Reilly CM. Activation of AMPK inhibits inflammation in MRL/lpr mouse mesangial cells. Clin Exp Immunol. 2009;156(3):542-51. Cited in PubMed; PMID 19438609. PubMed Central PMCID 2691985.
63. Kim HG, Hien TT, Han EH, Hwang YP, Choi JH, Kang KW, Kwon KI, Kim BH, Kim SK, Song GY, Jeong TC, Jeong HG. Metformin inhibits P-glycoprotein expression via the NF-kappaB pathway and CRE transcriptional activity through AMPK activation. Br J Pharmacol. 2011;162(5):1096-108. Cited in PubMed; PMID 21054339. PubMed Central PMCID 3051382.
64. Chen S, Murphy J, Toth R, Campbell DG, Morrice NA, Mackintosh C. Complementary regulation of TBC1D1 and AS160 by growth factors, insulin and AMPK activators. Biochem J. 20085;409(2):449-59. Cited in PubMed; PMID 17995453.
65. Barnes K, Ingram JC, Porras OH, Barros LF, Hudson ER, Fryer LG, Foufelle F, Carling D, Hardie DG, Baldwin SA. Activation of GLUT1 by metabolic and osmotic stress: potential involvement of AMP-activated protein kinase (AMPK). J Cell Sci. 2002;115(Pt 11):2433-42. Cited in PubMed; PMID 12006627.
66. Marsin AS, Bertrand L, Rider MH, Deprez J, Beauloye C, Vincent MF, Van den Berghe G, Carling D, Hue L. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr Biol. 2000;10(20):1247-55. Cited in PubMed; PMID 11069105.
67. Carling D, Hardie DG. The substrate and sequence specificity of the AMP-activated protein kinase: phosphorylation of glycogen synthase and phosphorylase kinase. Biochim Biophys Acta. 1989;1012(1):81-6. Cited in PubMed; PMID 2567185.
68. Leclerc I, Kahn A, Doiron B. The 5'-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. FEBS Lett. 1998;431(2):180-4. Cited in PubMed; PMID 9708898.
69. Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferre P, Foufelle F, Carling D. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol Cell Biol. 2000;20(18):6704-11. Cited in PubMed; PMID 10958668. PubMed Central PMCID 86183.
70. Long YC, Barnes BR, Mahlapuu M, Steiler TL, Martinsson S, Leng Y, Wallberg-Henriksson H, Andersson L, Zierath JR. Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. Diabetologia. 2005;48(11):2354-64. Cited in PubMed; PMID 16237515.
71. Hutber CA, Hardie DG, Winder WW. Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. Am J Physiol. 1997;272(2 Pt 1):E262-266. Cited in PubMed; PMID 9124333.
72. Sim AT, Hardie DG. The low activity of acetyl-CoA carboxylase in basal and glucagon-stimulated hepatocytes

is due to phosphorylation by the AMP-activated protein kinase and not cyclic AMP-dependent protein kinase. FEBS Lett. 1988;233(2):294-8. Cited in PubMed; PMID 2898386.

73. Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, Saha AK. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem. 2002;277(36):32571-7. Cited in PubMed; PMID 12065578.
74. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab. 2011;13(4):376-88. Cited in PubMed; PMID 21459323. PubMed Central PMCID 3086578.
75. Clarke PR, Hardie DG. Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver. EMBO J. 1990;9(8):2439-46. Cited in PubMed; PMID 2369897. PubMed Central PMCID 552270.
76. Costford SR, Kavaslar N, Ahituv N, Chaudhry SN, Schackwitz WS, Dent R, Pennacchio LA, McPherson R, Harper ME. Gain-of-function R225W mutation in human AMPKgamma(3) causing increased glycogen and decreased triglyceride in skeletal muscle. PLoS One. 2007;2(9):e903. Cited in PubMed; PMID 17878938. PubMed Central PMCID 1964808.
77. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci USA. 2007;104(29):12017-22. Cited in PubMed; PMID 17609368. PubMed Central PMCID 1924552.
78. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science. 2011;331(6016):456-61. Cited in PubMed; PMID 21205641. PubMed Central PMCID 3030664.
79. Kimura N, Tokunaga C, Dalal S, Richardson C, Yoshino K, Hara K, Kemp BE, Witters LA, Mimura O, Yonezawa K. A possible linkage between AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. Genes Cells. 2003;8(1):65-79. Cited in PubMed; PMID 12558800.
80. Shore D, Squire M, Nasmyth KA. Characterization of two genes required for the position-effect control of yeast mating-type genes. EMBO J. 1984;3(12):2817-23. PubMed PMID 6098447. PubMed Central PMCID 557771.
81. Imai S, Johnson FB, Marciniak RA, McVey M, Park PU, Guarente L. Sir2: an NAD-dependent histone deacetylase that connects chromatin silencing, metabolism, and aging. Cold Spring Harb Symp Quant Biol. 2000;65:297-302. Cited in PubMed; PMID 12760043.
82. Tanno M, Sakamoto J, Miura T, Shimamoto K, Horio Y. Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. J Biol Chem. 2007;282(9):6823-32. Cited in PubMed; PMID 17197703.
83. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature. 2009;458(7241):1056-60. Cited in PubMed; PMID 19262508. PubMed Central PMCID 3616311.
84. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature. 2005;434(7029):113-8. Cited in PubMed; PMID 15744310.
85. Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, Sartorelli V. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell. 2008;14(5):661-73. Cited in PubMed; PMID 18477450. PubMed Central PMCID 2431467.
86. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell. 2008;134(2):317-28. Cited in PubMed; PMID 18662546.
87. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell. 2008;134(2):329-40. Cited in PubMed; PMID 18662547. PubMed Central PMCID 3526943.
88. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell. 2001;107(2):137-48. Cited in PubMed; PMID 11672522.
89. Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell. 2001;107(2):149-59. Cited in PubMed; PMID 11672523.
90. Passariello CL, Zini M, Nassi PA, Pignatti C, Stefanelli C. Upregulation of SIRT1 deacetylase in phenylephrine-treated cardiomyoblasts. Biochem Biophys Res Commun. 2011;407(3):512-6. Cited in PubMed; PMID 21414296.
91. Dioum EM, Chen R, Alexander MS, Zhang QY, Hogg

RT, Gerard RD, Garcia JA. Regulation of hypoxia-inducible factor 2 alpha signaling by the stress-r deacetylase sirtuin 1. Science. 2009;324(5932):1289-93. Cited in PubMed; PMID WOS:000266635100035.

92. Lim JH, Lee YM, Chun YS, Chen J, Kim JE, Park JW. Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1 alpha. Molecular Cell. 2010;38(6):864-78. Cited in PubMed; PMID WOS:000279297500011.
93. Li XL, Kazgan N. Mammalian sirtuins and energy metabolism. Int J Biol Sci. 2011;7(5):575-87. Cited in PubMed; PMID WOS:000291307900007.
94. Brooks CL, Gu W. How does SIRT1 affect metabolism, senescence and cancer? Nat Rev Cancer. 2009;9(2):123-8. Cited in PubMed; PMID 19132007. PubMed Central PMCID 2857763.
95. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature. 2003;425(6954):191-6. Cited in PubMed; PMID 12939617.
96. Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. J Biol Chem. 2005;280(17):17187-95. Cited in PubMed; PMID 15749705.
97. Canto C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab. 2010;11(3):213-9. Cited in PubMed; PMID 20197054. PubMed Central PMCID 3616265.
98. Facchini A, Cetrullo S, D'Adamo S, Guidotti S, Minguzzi M, Facchini A, Borzi RM, Flamigni F. Hydroxytyrosol prevents increase of osteoarthritis markers in human chondrocytes treated with hydrogen peroxide or growth-related oncogene alpha. PLoS One. 2014;9(10):e109724. Cited in PubMed; PMID 25279550. PubMed Central PMCID 4184903.
99. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science. 2004;303(5666):2011-5. Cited in PubMed; PMID 14976264.
100. Frescas D, Valenti L, Accili D. Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. J Biol Chem. 2005;280(21):20589-95. Cited in PubMed; PMID 15788402.
101. Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc Natl Acad Sci USA. 2008;105(9):3374-9. Cited in PubMed; PMID 18296641. PubMed Central PMCID 2265142.
102. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, Goldberg AL, Schiaffino S, Sandri M. FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab. 2007;6(6):458-71. Cited in PubMed; PMID 18054315.
103. Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA, Sadoshima J. Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. Circ Res. 2010;107(12):1470-82. Cited in PubMed; PMID 20947830. PubMed Central PMCID 3011986.
104. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell. 2008;30(2):214-26. Cited in PubMed; PMID 18439900. PubMed Central PMCID 2674027.
105. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;115(5):577-90. Cited in PubMed; PMID 14651849.
106. Passariello CL, Gottardi D, Cetrullo S, Zini M, Campana G, Tantini B, Pignatti C, Flamigni F, Guarnieri C, Caldarera CM, Stefanelli C. Evidence that AMP-activated protein kinase can negatively modulate ornithine decarboxylase activity in cardiac myoblasts. Biochim Biophys Acta. 2012;1823(4):800-7. Cited in PubMed; PMID 22230191.
107. Ray RM, Johnson LR. Regulation of intestinal mucosal growth by amino acids. Amino Acids. 2014;46(3):565-73. Cited in PubMed; PMID 23904095. PubMed Central PMCID 3875634.
108. Zou T, Liu L, Rao JN, Marasa BS, Chen J, Xiao L, Zhou H, Gorospe M, Wang JY. Polyamines modulate the subcellular localization of RNA-binding protein HuR through AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1. Biochem J. 2008;409(2):389-98. Cited in PubMed; PMID 17919121.
109. Cetrullo S, Tantini B, Facchini A, Pignatti C, Stefanelli C, Caldarera CM, Flamigni F. A pro-survival effect of polyamine depletion on norepinephrine-mediated apoptosis in cardiac cells: role of signaling enzymes. Amino Acids. 2011;40(4):1127-37. Cited in PubMed; PMID 20835736.
110. Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstein B, Herker E, Fahrenkrog B, Frohlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, Madeo F. Induction of autophagy by spermidine promotes longevity. Nat Cell

Biol. 2009;11(11):1305-14. Cited in PubMed; PMID 19801973.

111. Morselli E, Marino G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, Cabrera S, Benit P, Rustin P, Criollo A, Kepp O, Galluzzi L, Shen S, Malik SA, Maiuri MC, Horio Y, Lopez-Otin C, Andersen JS, Tavernarakis N, Madeo F, Kroemer G. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J Cell Biol. 2011;192(4):615-29. Cited in PubMed; PMID 21339330. PubMed Central PMCID 3044119.
112. Soda K, Dobashi Y, Kano Y, Tsujinaka S, Konishi F. Polyamine-rich food decreases age-associated pathology and mortality in aged mice. Exp Gerontol. 2009;44(11):727-32. Cited in PubMed; PMID 19735716.
113. Zhang T, Berrocal JG, Frizzell KM, Gamble MJ, DuMond ME, Krishnakumar R, Yang T, Sauve AA, Kraus WL. Enzymes in the NAD+ salvage pathway regulate SIRT1 activity at target gene promoters. J Biol Chem. 2009;284(30):20408-17. Cited in PubMed; PMID 19478080. PubMed Central PMCID 2740465.
114. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1—possible role in AMP-activated protein kinase activation. J Biol Chem. 2008;283(41):27628-35. Cited in PubMed; PMID WOS:000259719200034.
115. Guo WJ, Qian L, Zhang J, Zhang W, Morrison A, Hayes P, Wilson S, Chen TS, Zhao J. Sirt1 overexpression in neurons promotes neurite outgrowth and cell survival through inhibition of the mTOR signaling. J Neurosci Res. 2011;89(11):1723-36. Cited in PubMed; PMID WOS:000295430100003.