Research Progress in Signaling Pathways That Regulate Life Span

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Abstract. Aging is a complex multifactorial biological process shared by all living organisms. It shows a gradual decline in normal physiological function in a time-dependent manner. Various model organisms are used to study the mechanisms of aging, and important discoveries in this area have contributed to the development of new therapies for age-related diseases. In this review, we discuss the important signaling pathways that have been shown to affect longevity, including the insulin/insulin-like growth factor (IGF-1) signaling pathway, the target of rapamycin (TOR) signaling pathway, and the AMP-activated protein kinase (AMPK) signaling pathway, providing a basic understanding of the mechanisms of the aging process.

1. Introduction
Longevity is the ability of the body to sustain life. The life expectancy under suitable living conditions is determined by the rate of aging. Aging is a complex multifactorial biological process shared by all organisms. It shows normal physiological function gradually decreased with time. Biological aging has important implications for human health because of the increases of the susceptibility to many diseases, including cancer, metabolic disorders such as neurodegenerative diseases, cardiovascular disease and diabetes [2-5]. Both genetic factors and non-genetic factors affect the development of age-related diseases and the lifespan of human. Among the life-style and environmental factors, dietary restriction (DR) is the most widely studied longevity promoting intervention. Studies in various model organisms, such as mice, nematodes, fruit flies, yeasts, rats and primates, have shown that effective restriction of food intake can significantly improve the physiological function of an organism and extend the lifespan [6]. In human, restricting dietary intake lowers serum insulin levels [7], decreases the risk of cardiovascular diseases [8], and increases the lifespan [7]. Although the exact cause of aging and the longevity-promoting effect of DR are still unclear, a large number of longevity-modulating genes and signal pathways have been discovered in the past a few decades through research efforts in understanding the molecular mechanisms of DR. In this paper, we discuss three important signaling pathways that regulate aging and longevity, including insulin/insulin-like growth factor (IGF-1) signaling pathway, the target of rapamycin (TOR) signaling pathway and the AMP-activated protein kinase (AMPK) signaling pathway.
2. Insulin/IGF-1 signaling pathway
The insulin/IGF-1 signaling pathway was the first genetic pathway discovered which regulates aging and longevity. Its regulation of aging and longevity has been confirmed in research using model organisms and in human studies [9]. Biologists have discovered that *daf-2* mutants of nematodes live nearly twice as long as the wild-type nematodes [10]. A homolog of the mammalian IGF-1 receptor, DAF-2 is one of the key players that mediate insulin/IGF-1 signaling pathway [11]. In *Caenorhabditis elegans* (*C. elegans*), DAF-2 receptor binds to its ligands, such as INS-7 and DAF-28 [12], and activates downstream phosphatidylinositol 3-kinase (PI3K) signaling. Mutations in *daf-2* gene or in the PI3K homolog *age-1* gene in *C. elegans* cause the nematodes to arrest in dauer state, shifting metabolism to fat storage, and living longer [10, 11, 13]. DAF-16, an important insulin/IGF-1 signaling target homologous to the mammalian Forkhead box O (FoxO) family, controls dauer arrest and life span by acting in opposition to DAF-2 and *AGE-1* [14]. FoxO transcription factors are related to a plethora of cellular processes and are major determinants of animal lifespan [15]. The nuclear translocation of DAF-16 and its transcriptional activity can be regulated by insulin/IGF-1 signaling. The activated PI3K transforms phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3), recruiting 3-phosphoinositide-dependent kinase-1 (PDK-1) to the inner cytoplasmic membrane and causing the activation of the downstream protein kinase B (AKT/PKB) signaling pathway [16, 17]. AKT-1, AKT-2 and the serine- and glucocorticoid-inducible kinase-1 (SGK-1), which are activated by PDK-1, phosphorylate the cytoplasmic DAF-16 and enhance the binding of DAF-16 to cytoskeleton, thereby inhibiting the distribution of DAF-16 to the nucleus, which in turn inhibits the transcriptional activity of DAF-16, directly shortening the lifespan of nematodes [15]. *Tub 1* (mammalian homolog of *tubby*) is a gene related to longevity and fat storage located in the upstream of *daf-16*. Deletion of *tub 1* gene in nematode promotes the transfer of *daf-16* into the nucleus, thus extending the lifespan of nematodes [18].

It has been shown that DAF-16 participates the anti-aging effects associated with DR. In *C. elegans*, the gene expression of both *daf-2* and its ligand insulin like peptide *ins-7* are down-regulated by DR. The inhibition of the insulin/IGF-1 signaling pathway triggers the nuclear translocation of DAF-16, activating the expression of genes related to longevity and extending the lifespan of nematodes [19, 20]. Enhancing the stress resistance appears to play a role in the lifespan extension effect of DAF-16 activation [21]. The oxygen free radicals have been viewed as a likely major contributor to aging [22]. The superoxide dismutase (SOD), which removes superoxide free radical, contributes to longevity assurance [22]. It has been shown that the *sod-3* gene is a downstream effector of *daf-16* [23]. When nematodes are subjected to oxidative stress, the DAF-16 protein rapidly transfers to the nucleus and induces the expression of *sod-3* gene resisting the external stress and extending the lifespan of nematodes [22, 24].

The importance of insulin/IGF-1 signaling pathway in longevity has also been found in model organisms such as flies and mice. In *Drosophila*, inhibition of insulin/IGF-1 signaling pathway-associated molecules such as DAF-2 effectively activates the transcriptional activity of forhead domain transcription factor dFOXO and promotes longevity [25]. In mice, it has been found that the level of IGF-1 in circulatory system is negatively correlated with the lifespan, so the loss of IGF-1 signal may effectively prolong the lifespan of mice [26].

3. TOR signaling pathway
The TOR signaling pathway is a nutritional sensor for individual organisms, which can sense the levels of growth factors and amino acids and regulate a series of downstream signaling pathways that control cell survival, proliferation, movement and protein synthesis [27, 28]. TOR is composed of two highly conserved protein complexes, TORC1 and TORC2. TORC1 mainly regulates cell growth, protein synthesis and autophagy, while TORC2 regulates cytoskeleton recombination. The regulation of metabolic state of an organism is accomplished by the combined action of the two [29]. Inhibiting TOR signal can effectively promote cells to maintain the basic physiological functions [28]. Studies in yeast, nematode, *Drosophila* and mice have shown that decrease of the activity of the TOR complex...
by genetic deletion of its subunits or pharmacological inhibition extends the lifespan of the organisms [30]. Mutation of let-363 gene, the homologous gene of TOR in C. elegans, shifts metabolism to accumulate fat, and extend the life span of the nematodes [31, 32].

The mTOR signaling pathways play important roles in regulating ribosomal biosynthesis including the transcription process of ribosomal protein, ribosomal RNA and the transporter RNA [33]. Ribosomal S6 protein kinase 1 (S6K1) is a key regulator of mRNA translation and a component of the nutrient-responsive mTOR signaling pathway [34]. Hansen et al. [35] found that reducing S6K mRNA levels by RNAi extended the mean lifespan of C. elegans. Pan et al. [36] showed that reducing the expression of ifg-1, the worm homologue of eukaryotic translation initiation factor 4G (eIF4G), which is a scaffold protein in the cap-binding complex, decreased translation and increased longevity. Although the molecular mechanisms by which TOR affects longevity remain elusive, inhibition of TOR activity may increases the lifespan and resistance to age-related pathologies by inhibiting the protein synthesis of an organism [34]. In addition, TOR signaling may participate in regulating aging and longevity through affecting the process of autophagy. Autophagy is a major degradation pathway that is essential for cells to remove damaged organelles and macromolecules [37]. As a rejuvenating process, autophagy seems to play an important role in longevity. FoxA factors are regulators of embryonic development and postembryonic life. In C. elegans, pha-4 gene encodes a FoxA transcription factor that regulates the expression of autophagy genes, and is required to establish the foregut in embryos [38-40]. Reducing the activity of mTOR can activate the transcriptional activity of pha-4 [38], enhancing the degradation of damaged organelles and reducing their harmful influence on normal cell function by promoting autophagy [41].

4. AMPK signaling pathway

AMPK is another important metabolic energy sensor that links nutrients to longevity. In normal physiological conditions, in order to maintain biological homeostasis and basal metabolism, high concentration of ATP and low concentration of AMP are maintained in cells. When the ratio of AMP to ATP is low, AMPK protein is in an inactive state [42]. When cells are hypoxic, have low glucose levels or the synthesis of ATP is impeded, the ratio of AMP/ATP is increased and AMPK is activated [42]. The AMPK encoding gene in C. elegans is aak-2. Worms show a shortened lifespan if aak-2 is mutated, extended lifespan if aak-2 is overexpressed [43]. In the mammalian and nematodes system, AAK-2 (AMPK α2) mediated longevity requires the downregulation of the insulin/IGF-1 signaling pathway and the subsequent upregulation and translocation of DAF-16/FOXO [43]. In worms, the activation of AMPK and its downstream targets often relies on the distinct methods of DR, evidence suggests that DR regimens that reduce carbohydrates more prominently than amino acids might render it more dependent on AMPK pathway [44, 45].

The longevity effects of AMPK are mediating by a network of its downstream effectors. cAMP response element-binding protein (CREB)-regulated transcription coactivator-1 (CRTC-1) is a direct AMPK target. It interacts with the CREB homologue-1 (CRH-1) transcription factor in vivo. Reducing crh-1 gene expression has been shown to increase longevity [46]. In C. elegans, overexpression of constitutionally activated AMPK causes phosphorylation and deactivation of CRTC-1 and significantly extends the lifespan of nematodes [46]. The longevity effects of CRTC-1 inactivation are mediated by CRH-1 and the downregulation of crtc-1 gene expression extends lifespan in a crh-1-dependent manner, suggesting a possible role for CREB in AMPK-mediated anti-ageing process [46]. In mouse models, studies have shown that AMPK regulates mitochondrial biosynthesis and cell senescence by directly phosphorylating peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) [47]. Liang et al. found transgenic mice overexpressing PGC-1α had an increased life expectancy compared to the control group [48]. In C. elegans, it has been found that the reduction of glucose uptake can activate AMPK, which in turn accelerates the oxidative phosphorylation in mitochondria and leads to an instantaneous increase in ROS levels. This increase of ROS level elevates the activity of intracellular catalase thus enhancing the resistance of cells to long-term oxidative pressure, and ultimately promoting the longevity of nematodes [49].
In summary, as a sensor of energy, AMPK plays a crucial part in the metabolic regulation of biological macromolecules. The dysfunction of the AMPK signaling pathway greatly impairs the normal physiological activities of an organism and may lead to shortened life span of the organism.

5. Conclusion
The existing evidence shows that a variety of signaling pathways play important roles in the realization of physiological functions and normal aging process of organisms. The challenge for the future will be to determine how these different pathways map onto and interact with each other, and to decipher their molecular mechanisms. For some, basic questions such as when and where these signals are needed and whether these signals are cell-autonomous or non-cell-autonomous need to be addressed. Understanding the essential causes of aging and how they can be offset may promote human health and longevity.

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