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Stem cell rejuvenation and the role of autophagy in age retardation by caloric restriction: An update

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ABSTRACT

Stem cells being pluripotent in nature can differentiate into a wide array of specific cells and asymmetrically divide to produce new ones but may undergo aging by themselves. Aging has both quantitative and qualitative effects on stem cells, and could eventually restrain them from replenishing into progenitor cells. Reactive oxygen species (ROS) accumulated in the aging cells could not only block the cell cycle but also affect autophagy by damaging the mitochondria. Autophagy could eliminate redundant production of ROS in aging stem cells and helps to maintain the proliferation capacity by restraining the expression of $p16^{INK4a}$. Current studies showed that improving autophagy could restore the regenerative ability of aging stem cells. Therefore, it is important for an organism to maintain the appropriate autophagy. Caloric restriction (CR) was shown to retard the stem cell aging by a certain basic level of autophagy, suggesting that CR was an effective way to extend longevity in mammals. However, little is known about the underlying mechanisms. In this review, we tried to explore the molecular mechanisms on how CR induces appropriate autophagy to restore aging stem cell regenerative ability.

1. Introduction

Stem cells are undifferentiated biological cells having the ability to undergo self-renewal and differentiation into specific cells. However, stem cells inevitably undergoes aging(Moore and Lemischka, 2006; Rossi et al., 2005). Aging is accompanied with the accumulation of genetic damage through lifespan, and genetic mutations resulting from misbalance between DNA damage and repair (Moskalev et al., 2013). There are nine hallmarks that were contributing to the aging process and phenotype, and these hallmarks of aging were grouped into three categories (primary hallmarks, antagonistic hallmarks and integrative hallmarks) (Lopez-Otin et al., 2013). Ultimately, stem cell exhaustion and altered intercellular communication, as the integrative hallmarks, were responsible for the decline in organ function associated with aging.

Recently, the molecular mechanisms of aging have been well elaborated for a better understanding of its biological hallmarks (Aunan et al., 2016). Besides DNA replication errors and spontaneous hydrolytic reactions, reactive oxygen species (ROS) continuously challenged the integrity and stability of DNA (Hoeijmakers, 2009). DNA damage had badly impacted the functional competence of stem cells, and compromised their role in tissue renewal (Jones and Rando, 2011). Meanwhile, oxidative stress also damaged the DNA stability of stem cells (Ahlqvist et al., 2012). Stem cell exhaustion has been included as the integrative hallmark of aging (Lopez-Otin et al., 2013), and subsequently, the differentiation and migration capacities of stem cells inevitably declined (Keyes and Fuchs, 2018; Yang et al., 2015). Organism's aging is closely associated with the regenerative ability of stem cells, the decline of which may result in some age-related degenerative diseases. For example, the expression of a stem cell gene, Sox4 was restricted to regulate progenitor functions in mammals to embryonic structures and also in some adult tissues. Moreover, deleting Sox4 was showed to accelerate aging process and decreased the lifespan in mice (Foronda et al., 2014). Regeneration of skeletal muscle re-occurred when geriatric satellite cells restored quiescence in adult life (Sousa-Victor et al., 2014a), suggesting that maintaining the regenerative ability of stem cells would be beneficial to extend the lifespan. Recent

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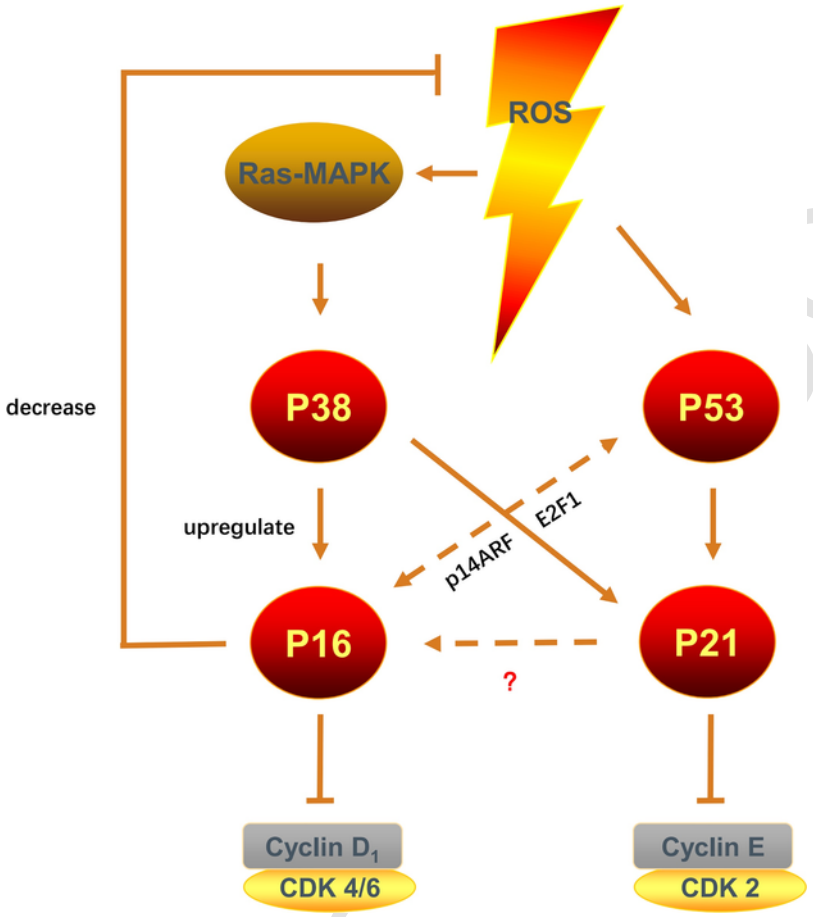

Fig. 1. Interaction between ROS and p16. ROS activated p38 through Ras-MAPK pathway to upregulate p16. P38 played a critical role in the expression of p16. When p38 was down-regulated, induction of p16 attenuated accordingly. P16 in turn may decrease ROS level. ROS also activated the p53-p21 pathway. p53 and p21 were subside when the induction of p16 started rising. P16 could be induced when p53 was deficient, but induction of p53 may not eliminate p16 expression. p53 and p16 was connected with a feedback loop through p14ARF and E2F1. Both p16 and p21 inhibited cyclin-dependent kinase (CDK)4/6 and CDK2, respectively.

promising studies suggested that stem cell rejuvenation may reverse the aging phenotype at the organismal level (Rando and Chang, 2012), raising a pivotal question, "how to rejuvenate the aging of stem cells?"

Several literatures have demonstrated that caloric restriction (CR) has retarded the onset of age-related disease and maintained the physiological and behavioral functions later during life (Balasubramanian et al., 2017; Bishop and Guarente, 2007; Fontana et al., 2010; Ingram and Roth, 2015; Mattison et al., 2017; Rusli et al., 2017). Dietary restriction (DR) including timing of food intake and specific nutrients could modulate multiple systemic, neural, and cellular mechanisms that improve health and longevity (Fontana and Partridge, 2015; Ruetenik and Barrientos, 2015). Sustained research attention on the CR anti-aging mechanisms led to a new strategy that disclosed a series of CR mimetics. Metformin and spermidine have emerged as a strong candidate of CR mimetic (Ingram and Roth, 2015). Metformin was found to reduce age-related accumulation of DNA damage by down-regulating the Akt activity in intestinal stem cells (ISCs) derived from Drosophila midgut (Na et al., 2013), while knock-down of autophagy-related factor Atg6 in ISCs may induce age-related phenotypes (Na et al., 2018). Spermidine prevented stem cell senescence mainly through autophagy, and also induced keratin production of epithelial stem cells and enhanced muscle and hair follicle regeneration (Ramot et al., 2011). The cellular and molecular role of spermidine-mediated health protection had been discussed in detail (Madeo and Eisenberg, 2018). However, the impact of caloric restriction mimetics on the retardation of stem cell aging and enhancement of pluripotency still need further research. Moreover, CR is also the most physiological stimulator of autophagy, and the anti-aging effect of CR can be restrained by inhibition of autophagy (Rubinsztein et al., 2011). Technically, neither intermittent fasting nor glucose deprivation of cultured cells fit the definition of calorie restriction. The contradictory potential role of autophagy in senescence has been widely discussed in a recent review (Korolchuk et al., 2017). However, appropriate autophagy could maintain the stemness of stem cells by preventing senescence (Garcia-Prat et al., 2016a; Rubinsztein et al., 2011). In the current review, we update and discuss the underlying mechanisms of how CR induces the rejuvenation of aging stem cells through physiological autophagy within this exciting research field.

2. Accumulating ROS activates $p16^{INK4a}$

ROS, a natural byproduct of the normal metabolism of oxygen, is an endogenous threat to DNA stability. These DNA alterations may damage stem cell functions and jeopardize tissue renewal (Jones and Rando, 2011). Mitochondrial DNA (mtDNA) exhibits an extremely high rate of mutation due to the oxidative microenvironment and limited efficiency of repair mechanism (Linnane et al., 1989). Integrity of mtDNA is essential for genomic stability, however, mtDNA is continuously challenged by ROS. $P16^{INK4a}$, known as the cyclin-dependent kinase (CDK) inhibitor, showed an obvious increase in aging tissues (Krishnamurthy et al., 2004). Progenitor self-renewal potential in the sub-ventricular zone was found to be declined with age in the mouse forebrain, partly caused by an increase of $p16^{INK4a}$ (Molofsky et al., 2006). Under low-glucose culture condition, $p16^{INK4a}$ expression level were markedly reduced in mesenchymal stem cells (MSCs), leading to

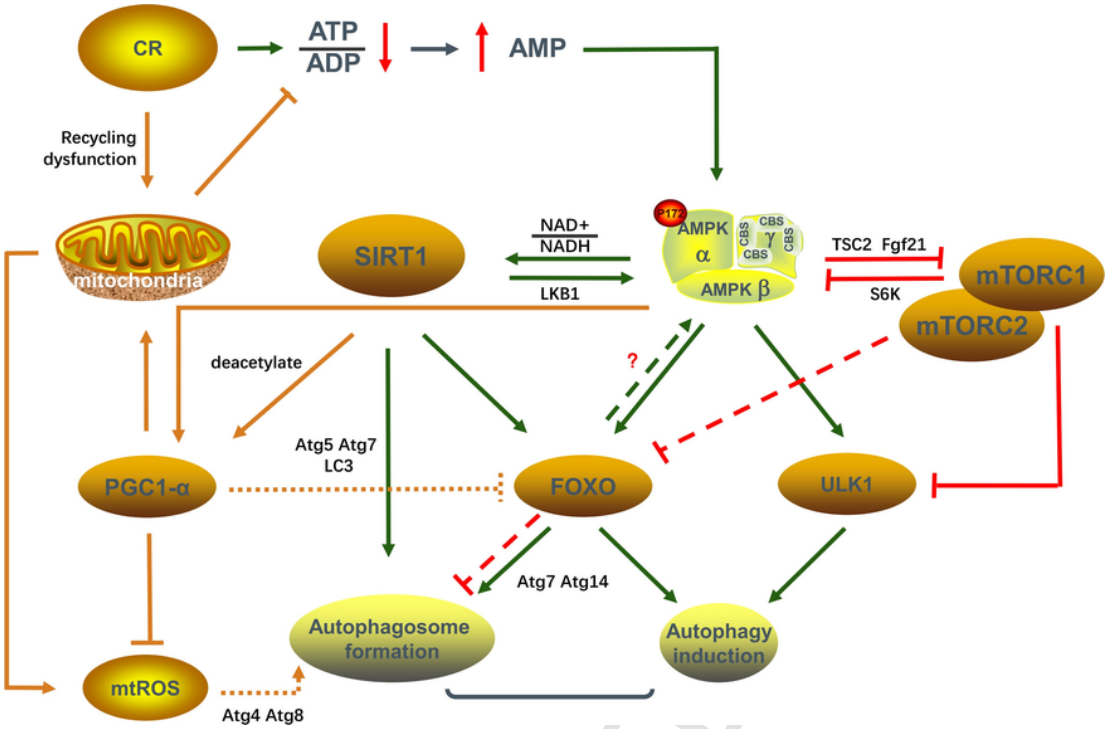

Fig. 2. The network-regulating mechanisms of CR-inducing autophagy. CR decreased the ATP/ADP ratio and activated AMPK. AMPK could directly upregulate SIRT1, FOXO and ULK1 to induce autophagy. a) LKB1 targeted SIRT1 to promote AMPK activity. SIR1 transcribed central Atgs to deacetylate FOXO to induce autophagy and regulate autophagosome formation. SIR1 also directly participated in autophagosome formation through Atg5 and Atg7. b) AMPK regulated ULK1 by inhibiting mTORC1 through phosphorylating TSC2, mTOR in turn inhibited AMPK by effector S6K. Sufficient nutrients activated mTOR which blocked the interaction between AMPK and ULK1, whereas AMPK could phosphorylate ULK1 by inhibiting mTOR under nutrients deprivation. (Red line indicates "suppression" and green line points to "activation". Dotted line means the relationship needs further exploration.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).Under oxidative stress, AMPK activated PGC-1α expression and reduced ROS production in skeleton muscle cell. Stimulation of SIR1 may deacetylate PGC-1α to reorganize mitochondrial metabolism and change the ATP/ADP ratio as a feedback. Elevated PGC-1α level may suppress FoxO1 and FoxO3 activation to prevent autophagy induction in muscle atrophy models. Increased mitochondrial ROS levels may stimulate autophagosome formation by Atg4 and Atg8. (Orange line indicates mitochondrial metabolism and dotted line means experiments in specific cell or yeast.)

significantly increasing cycling cells and decreasing cellular senescence (Lo et al., 2011). In mammals, SIRT1 (a NAD-dependent histone deacetylase) acts as a nutrient sensor either directly or indirectly involved in the regulation of various gene expressions, which has positive correlation with CR-induced longevity (Leibiger and Berggren, 2006). During glucose restriction, elevated SIRT1 activated Akt/p70S6K1 signaling and suppressed p16 by its deacetylation effects in human lung fibroblasts (Li and Tollefsbol, 2011). Similar results were found in human bone marrow-derived MSCs, and overexpression of SIRT1 enhanced cell growth and delayed the accumulation of p16 (Yuan et al., 2012). Another study showed that expression of p16 in transgenic mice leads to inhibition of intestinal stem cell proliferation and premature aging features, proposing that inhibition of p16 may reverse aging progress (Boquoi et al., 2015). The lifelong assessment of p16 in knock-in mice showed an exponential increase in their levels, and $p16^{INK4a}$ activation was noted in the emerging neoplasm and surrounding stromal cells, suggesting that $p16^{INK4a}$ activation characterized the emergence of cancers (Burd et al., 2013).

However, the impact of p16 in longevity and aging was considered both during pro-aging and anti-aging. Mice exhibited an extended longevity with a mild increase of $p16^{INK4a}$, but accumulation of $p16^{INK4a}$ during adulthood was negative in relation to lifespan and led to age-dependent changes in several organs (Baker et al., 2016). The expression of $p16^{INK4a}$ could intervene with the proliferation of stem cells and impair their regenerative capacity, yet defects in $p16^{INK4a}$ could regain the stem cell proliferation in aging (Janzen et al., 2006; Krishnamurthy et al., 2006; Stepanova and Sorrentino, 2005). Surprisingly, activation of p19 (Arf) display an opposite effect against $p16^{INK4a}$. However, during aging, these two genes appeared in concert with each other. If the balance tipped more towards p19 (Arf), aging might be attenuated (Baker et al., 2008).

Stem cells are particularly sensitive to the accumulation of mtDNA mutations (Ahlqvist et al., 2012). An abnormal increase of ROS production could inhibit Hematopoietic stem cells (HSCs) self-renewal and induce HSCs senescence, resulting in premature exhaustion of HSCs and hematopoietic dysfunction (Shao et al., 2011). An increase of ROS also play a key role in aging and apoptosis of bone marrow-derived MSCs, and antagonizing oxidant led to promote MSC survival and therapeutic effect (Feng et al., 2018; Matsuda et al., 2018). Mechanism by which ROS activates $p16^{INK4a}$ remains to be elucidated, probably via the MAPK-p38-p16 pathway. Inhibition of p38 MAPK pathway may restore asymmetric division in dysfunctional satellite cell caused by aging (Sousa-Victor et al., 2014b). ROS activated p38 MAPK, which played a critical role in the induction of senescence by up regulation of p16 and p21 (Kim et al., 2017; Li et al., 2017; Shao et al., 2011). It was reported that knockdown of p16 increased intracellular ROS and DNA damage, while the re-expression of p16 in turn may decrease the ROS level (Jenkins et al., 2011). p16 is one of the downstream target of p53, and activation of p53 could induce p16 activation (Shao et al., 2011). On the other way, the activation of ROS-p53-p21 pathway may affect the expression of p16. It was reported that, up regulation of p53 were subsided upon rising the levels of p16, connecting with a feedback mechanism through p14ARF and E2F1 (Shao et al., 2011). Under physiological circumstances, there is a dynamic balance and mutual restriction between ROS and p16 (Fig. 1).

Satellite cells undergo an irreversible transition from quiescence to senescence during aging. Silencing of $p16^{INK4a}$ restored geriatric stem cell regeneration and maintained the quiescence state to reestablish

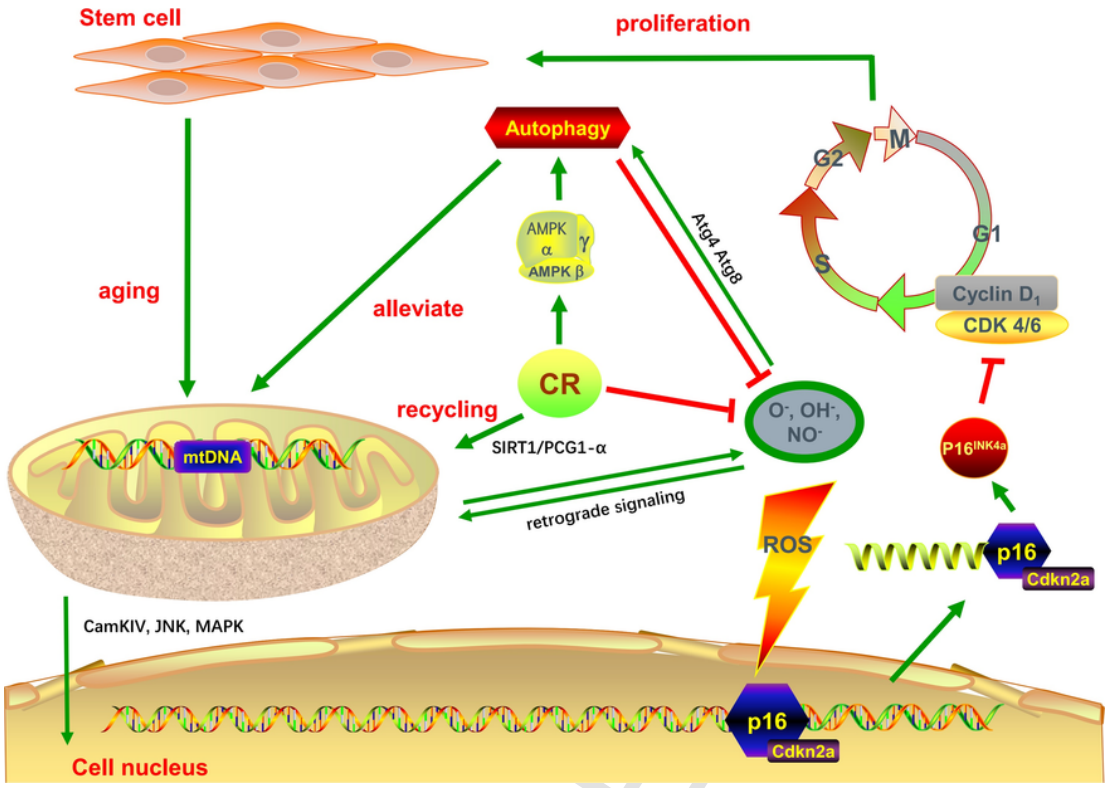

Fig. 3. CR induced autophagy to maintain aging stem cell regenerative ability. Accumulation of ROS easily damaged mitochondria DNA in aging stem cells, which activated $p16^{INK4a}$ to prevent the interaction between CDK4/6 and cyclin D. Activated $p16^{INK4a}$ led to the pre-senescence state of stem cells and hurt their potential of proliferation. CR could recycle dysfunction mitochondria and reorganize mitochondrial metabolism by SIRT1/PGC-1α. Overwhelmed mtROS caused cytotoxicity and accelerated aging process. mtROS was also essential for cell biology and survival when acting as retrograde signaling cooperated with nuclear genome to promote mitochondria recycling. Increased mtROS levels induced autophagy through Atg4 and Atg8 to increase autophagosome formation in yeast. Dysfunctional mitochondria activated transcription factors probably through CamKIV, JNK, and MAPK, etc. CR improved oxidative phosphorylation and antioxidation levels to decrease ROS level. Autophagy induced by CR through AMPK pathway to eliminate accumulating mutated mtDNA and redundant ROS. Finally, CR reduced excess generation of ROS and inhibited expression of $p16^{INK4a}$.

stem cell pool. Dysregulation of $p16^{INK4a}$ may cause defective regeneration ability by preventing stem cell activation (Sousa-Victor et al., 2014a,b). As previously described, CR reduced the activation of $p16^{INK4a}$ and maintained the stem cells regenerative ability.

3. Autophagy alleviates stem cell aging

Autophagy is a highly conserved process in eukaryotic organisms, involving the digestion of damaged cellular structure, aging organelles, and some macromolecules such as proteins to provide nutrients for essential metabolic processes. Under the condition of cell nutrient depletion, autophagy was stimulated by transducers to restore intracellular nutrient stores (Galluzzi et al., 2014). The knock-in of ATG proteins in infant mice was lethal, because autophagy is indispensable for transporting intracellular energy reserves (Rubinsztein et al., 2011).

ROS produced by mitochondria is important to metabolic adaptations such as hypoxia. Physiological level of mitochondrial ROS is considered to be important for metabolic adaptation and is essential for cell biology and survival. Interestingly, acting as a signal, ROS triggered retrograde signaling (mitochondria-to-nucleus) to regulate cell response to mitochondrial dysfunction in *Saccharomyces cerevisiae* and in mammals (Arnould et al., 2015; Torelli et al., 2015). Dysfunctional mitochondria increases intracellular calcium and subsequently activates transcription factors by a number of signaling pathways, probably through calcineurin, CamKIV, JNK, and MAPK in mammalian cells (Arnould et al., 2002; Biswas et al., 1999; Butow and Avadhani, 2004; Luo et al., 1997). Recent study found that G-Protein Pathway Suppressor 2 (GPS2), which mediate mitochondrial retrograde signaling is a transcriptional activator of mitochondrial genes and are involved in regulating the translocation from mitochondria to nucleus in mice cells (Cardamone et al., 2018). However, abnormal increase of ROS would inevitably damage cell functions, and an excessive formation of ROS in the generation of ATP by oxidative phosphorylation would eventually lead to cytotoxicity and accelerate aging process. Collectively, these faulty mitochondria gradually depleted the cell from ATP and increased ROS production (Kim et al., 2007).

The molecular interaction between ROS and autophagy may involve signal pathways such as ROS-FOXO3-LC3/BNIP3-autophagy, ROS-NRF2-P62-autophagy, ROS-HIF1-BNIP3/NIX-autophagy and ROS-TIGAR-autophagy (Li et al., 2015). Increasing mitochondrial ROS (mtROS) levels triggered by starvation of cells could induce autophagy mainly through ATG4, a redox-sensitive autophagy gene. It was reported that, once ATG4 is activated, it might regulate ATG8 to increase autophagosome formation in yeast (Perez-Perez et al., 2014). Extreme intracellular stress may lead to autophagic cell death, but how the autophagic cells undergo survival pathway or apoptosis pathway responding to stimuli remain inconclusive (Liu and Levine, 2015). In general, autophagic cell death is accompanied by high levels of ROS, which speculate that ROS levels are both essential in stress-induced autophagy and autophagic cell death (Finkel, 2012). In aged satellite cells, autophagy plays an important role in reducing ROS accumulation (Garcia-Prat et al., 2016b). As the body gets older, the physiological activity of autophagy declines, and accumulating evidences also highlighted the anti-aging functions of autophagy in animal cells. However, the role of autophagy in anti-aging is still controversial. For example, autophagy was found to promote oncogene-induced-senescence due to the over-expression of ULK3 in human fibroblasts (Young et al., 2009). The autophagy process is so complex that it may degrade either active

or negative regulators of senescence (Korolchuk et al., 2017). Selective autophagy of mitochondria was required for maintenance of muscle stem cells in their healthy quiescent state, which could sustain the regeneration of skeletal muscle. Senescence-associated mitochondrial dysfunction is considered to be a significant cause of aging. Moreover, the damaged mitochondria was attributed to defective mitophagy and caused increased levels of ROS, leading to the loss of regenerative capacity of stem cells in muscles an at old age (Garcia-Prat et al., 2016a; Korolchuk et al., 2017).

In contrast, autophagy flux of hematopoietic stem cells is enhanced with aging. Autophagy suppresses hematopoietic stem cell metabolism by clearing active mitochondria to maintain quiescent state, which are essential to preserve the regenerative capacity of old hematopoietic stem cells during advanced aging process (Ho et al., 2017). Although the decline of autophagy is connected with the alteration of multiple age-related pathways, the exact mechanism is still not clearly understood (Garcia-Prat et al., 2016b). The process of aging seems to be accelerated by long-lasting inhibition of autophagy, and a prolonged stimulation of autophagy might retard senescence *in vivo* (Cavallini et al., 2008; Donati, 2006). Autophagy inhibits the accumulation of ROS in the mitochondria, while its dysfunction is accompanied by stem cell aging. To some extent, the life span of stem cells depends on the strength of the autophagy (Bergamini, 2006; Garcia-Prat et al., 2016a).

4. CR promotes stem cell regeneration through autophagy

CR is considered as a powerful non-genetic interventional factor in delaying senescence in mammals, even though the underlying mechanisms of CR have not been clearly defined. Recently, a study mentioned that CR changes the oxidative stress level to extend the longevity (Lee and Min, 2013). Gradually, more and more evidences tend to support that CR effect was largely based on specific genotypes. For example, CR was found to have little effect on the lifespan of wild mice compared with laboratory adapted mice (Harper et al., 2006), proposing that CR lowers the magnitude of energy imbalance (lower body temperature, lower rate of metabolism and lower ROS production) in specific genotypes (Sohal and Forster, 2014). Furthermore, a recent study pointed out that CR was able to delay age-related methylation drift in mammals, which was considered to be an important determinant of lifespan in mammals (Maegawa et al., 2017).

In general, CR was defined as the long-term reduction in calorie intake rather than reduction of any specific nutritional requirement. This indicates that the dietary restriction (DR) and CR are two independent factors. It was reported that, decreasing caloric intake may shorten the lifespan in many insects, while deducing the protein : carbohydrate ratio of the diet may cause positive effect on survival rate in *Drosophila* (Cooper et al., 2004; Lee et al., 2008b).

An elegant study of CR on rhesus monkeys reported that, CR improved survival in adult rhesus monkeys by reducing the incidence of age-associated diseases such as diabetes, cancer and cardiovascular diseases (Colman et al., 2009). Conversely, another study reported that CR improved the overall health and body functions of rhesus monkeys, but failed to improve the mean survival rate compared to their control group (Mattison et al., 2012). The contradictory result in these two studies might have appeared due to the different study design followed in two experiments. The experimental factors such as food intake, source of monkeys, diet composition and feeding practices were not equivalent between these two studies. Furthermore, the beneficial onset of CR in nonhuman primates was not the same as that in rodents, as CR improved survival when implemented in adulthood rather than during childhood (Mattison et al., 2017). As reported, few beneficial effects of adult-onset CR has been observed in transcriptional level in rhesus monkey studies, despite the early onset of CR was found to suppress more than 80% of age-related gene expression changes in mice skeletal muscles (Lopez-Lluch and Navas, 2016). Recent study showed that CR was associated with processing of RNA genes and altered hepatic metabolism reprogramming, indicating that RNA processing is the central mechanism of CR response (Rhoads et al., 2018). CR was also found to reduce mitochondrial H_2O_2 release in rat liver (Hagopian et al., 2005; Lambert and Merry, 2004), rat skeletal muscles and mouse liver (Bevilacqua et al., 2005; Faulks et al., 2006).

Autophagy can be induced by CR, anoxia, endoplasmic reticulum stress and other environmental stresses. CR is the most common physiological stimulator of autophagy, and the anti-aging effect of CR could be restrained by the inhibition of autophagy (Rubinsztein et al., 2011; Yen and Klionsky, 2008). CR reduced protein synthesis and metabolic changes through attenuating mammalian target of rapamycin (mTOR), and subsequently reversed the age-related decline of autophagy activity (Toth et al., 2008; Wohlgemuth et al., 2007; Yen and Klionsky, 2008). Autophagic dysfunction is ascribed to age; thus, its age-dependent changes including biological signals may be suppressed by CR (Cavallini et al., 2008). CR causes modulation of mitochondria recycling and improve its efficiency. As in stem cells, SIRT1 mediates the effects of CR through redox status and influences the stem cell maintenance by controlling growth-factor responses (Mazzoccoli et al., 2014). CR stimulates both the SIRT1 deacetylase activity and SIRT1/PGC-1α interaction in order to reorganize mitochondrial metabolism (Anderson and Weindruch, 2010). It was reported that elevated PGC-1α levels reduced LC3II/LC3I ratio, and may inhibit FoxO1 and FoxO3 to prevent mitophagy-related proteins from overexpression in immobilization-remobilization-induced muscle atrophy models (Kang and Ji, 2016). It has been widely demonstrated that the specific autophagy recycling of mitochondria (mitophagy) was appearing in many conditions of dysfunctional mitochondria (Lemasters, 2005), speculating that autophagy may be a crucial part of the anti-aging mechanism of CR (Ferreira-Marques et al., 2016; Hansen et al., 2008).

5. CR mediates autophagy through AMPK

Four pathways have been primarily implied in regulating the effect of CR: insulin-like growth factor 1 signaling pathway, silent mating type information regulation 2 homolog (SIRTUIN) pathway, adenosine monophosphate–activated protein kinase (AMPK) pathway, and mammalian target of rapamycin (mTOR) pathway (Speakman and Mitchell, 2011). The sirtuin family is the potent factor in anti-aging, and in mammals, SIRT1, SIRT3 and SIRT6 interact to modulate genomic stability and proteostasis (Lopez-Otin et al., 2013). AMPK, an energy sensor, is a central node in a network of nutrient-sensing pathways regulating longevity, and is involved in maintaining homeostasis and integrating cellular functions (Burkewitz et al., 2016). AMPK was activated when ATP synthesis is compromised (under hypoxia, ischemia, low nutrient) or when ATP consumption is accelerated (under exercise or fasting) (Canto and Auwerx, 2011). CR-induced energy deficiency could lead to elevated intracellular ADP content (Chen et al., 2013). AMPK tends to detect the ratio of ATP/AMP to evaluate the energy status of the cells. As long as the ratio of ATP:AMP decreases under the threshold, AMPK is about to be activated (Hawley et al., 2010). CR was found to lower AMP:ATP ratios in worms and flies (Canto and Auwerx, 2011). In AMPKα2 knock-out mice hearts, CR down-regulated the expression of ATP5g2 (subunit of mitochondrial ATP synthase) and decreased the ATP level (Chen et al., 2013). Several lines of evidence implicated that AMPK sensed nutrient scarcity in CR (Chen et al., 2013; Lopez-Lluch and Navas, 2016). However, long-term or short-term regimens of CR may have different effects on AMPK (Cerletti et al., 2012; Edwards et al., 2010; Noyan et al., 2015), and several nutrient-sensing signaling pathways are also included in autophagy. The AMPK/mTOR signaling pathway is indispensable to such an adaptive response (Yen and Klionsky, 2008). Short-term CR alleviated age-related epithelial-

mesenchymal transition through AMPK-mTOR pathway, which could reduce renal fibrosis during aging (Dong et al., 2017). Long-term CR up regulated Fgf21 to phosphorylates neuronal AMPK and inhibited mTOR signaling, which then decreased the neurofibrillary tangles to treat neurodegeneration in ApoE-deficient mice (Ruhlmann et al., 2016). A recent study highlighted the role of splicing homeostasis in aging, pointing out that pre-mRNA splicing in the course of aging were reduced by dietary restriction through TORC1 modulator-splicing factor 1 (the *C. elegans* branch point binding protein) (Heintz et al., 2017). Rapamycin may increase the lifespan by delaying multiple aspect of senescence, the mechanism of which was strictly dependent on the induction of autophagy in yeast (Rubinsztein et al., 2011). It was found that the activation of AMPK diminished with aging (Reznick et al., 2007). Intriguingly, CR could modify the aging-associated reductions of AMPK, subsequently stimulating the autophagic activity (Moroz et al., 2014; Ye et al., 2013).

5.1. AMPK–SIR1–FOXO pathway

CR could induce the expression of autophagy-related genes (Atgs), mediated by the forkhead box protein O (FOXO) family of forkhead transcription factors in AMPK–SIRT1 signaling pathway (Brunet et al., 2004; Salminen and Kaarniranta, 2012). CR decreases ATP production and activates AMPK, and regulate autophagy by SIRT1 or directly by phosphorylating the FOXO family of transcription factors (Canto and Auwerx, 2011; Greer et al., 2007; Nakashima and Yakabe, 2007; Pallauf and Rimbach, 2013). In return, FOXO may activate atypical γ isoform AMP-binding sites in *C. elegans*, but a similar response in mammalian cell is unknown (Burkewitz et al., 2016). Sirtuin family depends on deacetylation of NAD^+ for their function, and AMPK promotes activation of SIRT1 by NAD^+/NADH ratio in cells. The AMPK kinase, LKB1 promotes AMPK activity by directly targeting SIRT1 deacetylation (Burkewitz et al., 2016). Later on, either SIRT1 induces autophagy by deacetylation of FOXO transcription factors, or SIRT1 directly acts on Atg5, Atg7, and Atg8 to participate in autophagosome formation (Lee et al., 2008a). The FOXO family was also confirmed to transcribe Atg7 and Atg14 to regulate autophagosome formation (Hong-Brown et al., 2017; Pallauf and Rimbach, 2013). Therefore, the organism depends on the interaction among AMPK, FOXO, and SIRT1 to adapt to the CR environment and extend its life span (Hariharan et al., 2010).

5.2. AMPK–mTOR–ULK1 pathway

CR mainly depends on the up regulation of AMPK and down regulation of mTOR to activate autophagy. The two distinct cellular complexes, mTOR complex 1 and 2 (mTORC1 and mTORC2), played an important role in this process, respectively. While mTORC1 is clearly identified in autophagosome formation, there is little evidence whether mTORC2 is involved (Dunlop and Tee, 2014). Though mTORC2 may suppress autophagy through Akt/FOXO3A signaling pathway (Mammucari et al., 2007), inhibition of mTORC2 remain impaired with autophagosome precursor formation, suggesting that mTORC2 paly a dual role in autophagy (Dunlop and Tee, 2014). CR initiates AMPK and subsequently suppresses the activity of mTOR indirectly (Matsui et al., 2007; Yang and Klionsky, 2010). AMPK phosphorylates Tuberous Sclerosis Complex 2 (TSC2) to inhibit mTORC1 complex (Inoki et al., 2003), and AMPKα is then inhibited by the phosphorylation of TOR effector S6 kinase (S6K) (Dagon et al., 2012). Another important pathway of AMPK regulating autophagy is by directly phosphorylating ULK1. ULK1, the mammalian autophagy-initiating kinase, is the key downstream factor of AMPK during the whole process (Kim et al., 2011; Lee et al., 2010). When nutrient availability is not limited, a high mTORC1 activity disrupts ULK1 activation to stop the interaction between ULK1 and AMPK. However, in response to nutrient deprivation, autophagy is promoted by the AMPK-modulated phosphorylation of ULK1 (Dunlop and Tee, 2014; Egan et al., 2011; Khan and Kumar, 2012). Under oxygen stress conditions such as anoxia and CR, AMPK–mTOR signaling act as a positive regulator of autophagy, thereby enhancing the survival rates of mesenchymal stem cells (Liang et al., 2016; Zhang et al., 2012). In the usual course of events, multiple signal pathways are involved in the induction of autophagy by CR, and the activation of AMPK is one among the vital step in this process (Fig. 2).

6. Conclusions

It is well known that the decline of functional activities in organism with aging is closely associated with the regenerative ability of stem cells. Several experimental results showed that CR could increase HSC quiescence and promote HSC self-renewal by reducing IGF-1 expression (Cheng et al., 2014; Mendelsohn and Larrick, 2014; Tang et al., 2016). It was previously mentioned that CR could also enhance the regenerative capacity of the intestinal epithelium through preservation of an injury-resistant reserve intestinal stem cell (ISC) pool by down regulating mTORC1 signaling in mice (Yousefi et al., 2018). Intriguingly, short-term fasting promoted intestinal stem and progenitor cell function in young and aged mice by inducing a robust fatty acid oxidation (FAO) program (Mihaylova et al., 2018). Recently, ascorbic acid was reported to inhibit senescence of mesenchymal stem cells through inhibiting the production of ROS and activating AKT/mTOR signaling (Yang et al., 2018). Therefore, it is important to uncover the precise molecular mechanisms of stem cells anti-aging property.

Experiments with *C. elegans*, Drosophila and *Mus musculus* have shown that induction of autophagy could lead to life-span extension (Pallauf and Rimbach, 2013). Meanwhile, in Drosophila, autophagy preserved the proper stem cell function for the continuous renewal of the intestinal epithelium, contributing to stable intestinal homeostasis (Nagy et al., 2018). ROS could directly activate autophagy under some circumstances, and the transcriptional and post-transcriptional regulations are the possible mechanism indicated (Li et al., 2015). Under oxidative stress, selective autophagy of mitochondria may play a key role in retarding accumulation of aging-related-mtDNA mutations, however, this process known as mitophagy declines with aging (Kim et al., 2007; Lemasters, 2005). Generation of mtROS activated $p16^{INK4a}$ and led to the pre-senescence state of stem cells (Sousa-Victor et al., 2014a). CR tends to induce autophagy through AMPK pathway to decrease the expression of $p16^{INK4a}$-related genes by clearing redundant ROS and mutated mtDNA of stem cells. By relieving de-repression of $p16^{INK4a}$, CR may promote stem cell self-renewal and slow down their progress into pre-senescence state (Fig. 3).

Though mitophagy was considered to be the central regulator of cell senescence, there was compelling evidence showing that the mtDNA mutation was not accompanied by the ROS production in mitochondrial DNA polymerase γ deficient mutant mice. Moreover, whether decreasing this mtDNA mutations could lead to life extension was not determined (Hiona et al., 2010; Korolchuk et al., 2017). CR could induce autophagy through AMPK signaling to strengthen stem cells in an unfavorable cellular environment, however, this needs to be further explored. Furthermore, the effect of AMPK in oxidative stress modulated by CR is controversial, possibly because the experimental design and methodology followed for CR evaluation was far from standardization (Jeon, 2016). Experiments have confirmed that autophagosomes were able to control cell cycle through hypoxia-inducible factor, but studies on the direct interaction between autophagy and cell cycle are lacking (Hubbi and Semenza, 2015).

Several molecules of AMPK activators have been found to improve the lifespan. Metformin mimics CR on cell metabolism to gain health benefits by activating AMPK and autophagy, which could eventually reduce inflammation and toxicity (Garg et al., 2017). Drugs targeted on

AMPK sustained untoward side effects rather than extending life in organism, mainly because CR effect are connected with a series of nutrient sensors, which are closely interlinked with each other (Burkewitz et al., 2016). Furthermore, understanding this complex network of aging-determining pathways, instead of a singular molecular node, would be beneficial to lengthen healthy lifespan (Korolchuk et al., 2017). It is hard to separate a single target from the binding network, as they may initiate a chain reaction beyond our expectation. Adopting a network approach to the complex interweaved nutrient-sensing signaling pathways may initiate new perceptive on aging process in cells. A quantitative strategy in Alzheimer's disease research has been outlined to predict cognitive aging by using network metrics as biomarkers (Ash and Rapp, 2014). Such quantitative analysis strategies could also be applied in modification of molecular changes in aging cell to predict the aging trajectories of organism. The complex molecular systems may enable to make use of artificial intelligent neural networks to understand and explore the complex interaction between CR, autophagy and anti-aging.

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