Caloric restriction and cellular senescence

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Cellular senescence

Cellular senescence was originally described as the limited proliferative capacity of cultured human fibroblasts\(^1\). This phenomenon, now termed replicative senescence, is caused by telomere erosion. Currently, it is generally accepted that senescence can be prematurely induced by many different insults, including oxidative stress, genotoxic stress, epigenetic changes, metabolic dysfunction, over activation of oncogenes or loss of some tumor suppressor genes, and mitochondrial dysfunction\(^2\).

The main defining characteristic of senescence is permanent growth arrest, and p53-p21 and p16\(^{INK4a}\) – pRB are the two essential pathways responsible for this replicative arrest\(^3\). Interestingly, both p53 and p16\(^{INK4a}\) are the most commonly mutated genes in cancer, suggesting that one of the evolutionary advantages of the senescence response is to suppress the development of cancer\(^4\).

In addition to the growth arrest, which is a necessary but insufficient marker of senescent cells, many features and molecular markers are used for identifying senescence. For instance, a commonly used marker is the senescence-associated beta-galactosidase staining (SA-\(\beta\)gal)\(^5\). The increase in SA-\(\beta\)gal activity is thought to be a consequence of increased lysosomal mass\(^6,7\). Due the fact that there is no universal marker, the combination of many senescence-associated hallmarks is currently used for the unequivocal characterization and identification of senescent cells\(^8\).

Although p16\(^{INK4a}\) is expressed by many but not all senescent cells, it is now generally accepted to be one of the most specific senescence markers \textit{in vivo}\(^9,10\). This led to the design of two distinct mouse models where p16\(^{INK4a}\)-positive cells can be selectively eliminated\(^11,12\). These models contributed much to our understanding about the causal roles of senescent cells in many different processes that include aging, age-related diseases, and wound healing\(^11-18\).

Numerous senescence inducers can cause genomic damage that can remain unresolved. As a result, the DNA damage response (DDR) is constantly active because of the chronic persistence of DNA damage foci. Those foci are generally termed DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence)\(^19\). The persistent DDR signaling function as one of the main drivers of the expression of the senescence associated secretory phenotype (SASP)\(^20\).

The SASP is a complex mixture of secreted factors that can be divided in 3 main categories: 1) factors binding to receptors (soluble signaling molecules, such as cytokines, chemokines, and growth factors), 2) factors acting directly (matrix metalloproteases, serine proteases and small non-protein components, such as reactive oxygen (ROS) and nitrogen species), and 3) regulatory factors (tissue inhibitors of metalloproteases, the plasminogen activator inhibitor, and insulin-like growth factor binding proteins)\(^21\).
In addition to secreted factors, senescent cells can also influence their surrounding through juxtacrine NOTCH/JAG1 signaling or via intercellular transfer of different molecules through cytoplasmic bridges or exosomes release. Importantly, some SASP components can reinforce senescence and induce senescence in neighboring cells (paracrine senescence).

It is clear now that the composition of SASP factors is dependent on the type of cells and senescence inducers. Moreover, the SASP can be regulated at different levels and it seems that most of the pathways that are important for the establishment of this phenotype converge into activation of the NF-κB and the C/EBPβ pathway. Strikingly, the mechanistic target of rapamycin (mTOR) pathway, an essential player in the aging process, is an important node in SASP regulation.

Many age-related diseases, if not all, share a chronic low-grade inflammatory state referred to as inflammaging. Elimination of senescent cells reduces many proinflammatory factors such as IL-6, IL-1α and TNF-α, suggesting that the SASP is, at least in part, causing this increased inflammatory state in old tissues. Given the fact that senescent cells are present in a small percentage in old tissues, it is highly plausible that the positive effect seen after senescent cells elimination is a consequence of SASP suppression.

Growing body of evidence has demonstrated the implication of senescence in aging and age-related pathologies. Hence, targeting senescent cells either by eliminating them using “senolytics” or inhibiting the deleterious effect of SASP, might be a promising approach for enhancing healthy longevity. Several literature reviews have covered the pharmacological elimination of senescent cells elsewhere.

However, another plausible approach is to avoid the formation of senescent cells by preventing cellular damage, and accumulating data in experimental animal models and humans suggest that caloric restriction (CR) without malnutrition may be a promising intervention in this context.

**Calorie restriction**

Calorie restriction (CR) with adequate nutrient intake is the most powerful non-genetic intervention for extending healthspan and lifespan in multiple animal models, including yeast, fruit flies, worms, and rodents. In most strains of rats and mice a reduction of dietary calories by 20 up to 50% results in a substantial extension of both average and maximal lifespan, even if mice with different genotypes respond differently to the same degree of restriction. Not only these CR animals, supplied with the appropriate amount of calories and nutrients, live significant longer, but many of the typical age-associated chronic diseases are either prevented or delayed. For example, the incidence of cancer, the
leading cause of death in rodents, is drastically reduced in CR animals; similar reductions or slowing down of disease progression have been observed for nephropathy, cardiomyopathy, diabetes, chronic lung diseases, autoimmune diseases and neurodegenerative disease.\textsuperscript{36,37}

Accumulating data indicate that CR extends lifespan also in non-human primates\textsuperscript{38}. In Rhesus monkeys, CR significantly improves metabolic health, prevents obesity, glucose intolerance/type 2 diabetes, and postpones the onset of sarcopenia, hearing loss and atrophy of certain key subcortical regions of the brain, including the caudate and putamen and the left insula \textsuperscript{(34)}. Moreover, CR in monkeys reduces morbidity and mortality for cancer, cardiovascular disease and frailty\textsuperscript{39,40}. Indeed, in contrast to the scientifically unsupported opinion that long-term CR promotes frailty\textsuperscript{41-43}, recent data from the Wisconsin CR Primate study clearly show that the levels of weakness, poor endurance, slowness, low physical activity and frailty were significantly lower in the CR than in the ad-libitum fed monkeys\textsuperscript{44}.

In humans, calorie restriction with adequate intake of vitamins and minerals causes many of the same physiological, metabolic and molecular adaptations observed in CR animals. For example, moderate CR leads to major improvements in all the classical cardiovascular risk factors, over and above those conferred by weight loss, even when implemented in healthy young and middle-aged non-obese men and women. Interestingly, these cardiometabolic adaptations are coupled with improvements in cardiac and arterial function, including improvements in left ventricular diastolic function and heart rate variability\textsuperscript{45}. Like in small mammals, CR in humans causes major modifications of several hormones that are implicated in the pathogenesis of cancer and in the biology of aging\textsuperscript{46}. Serum concentrations of insulin, testosterone, estradiol and several inflammatory markers were significantly lower, while IGFBP-1, SHBG, adiponectin and cortisol concentration were higher in people practicing CR group than in controls eating Western diets ad-libitum\textsuperscript{45}. Plasma triiodothyronine concentration, and as a consequence average 24- hour, day- time and night- time core body temperature, were also significantly reduced, supporting a strong CR-mediated inhibitory effect on metabolic rate and oxidative stress\textsuperscript{47}.

CR-mediated molecular mechanisms promoting health and longevity

The mechanisms mediating the health benefits of CR are not fully understood. Multiple systemic metabolic, neuroendocrine and immunological adaptations coupled with cell-specific molecular mechanisms are involved. For example, calorie restriction without malnutrition exerts a powerful effect in improving insulin sensitivity and in reducing protein glycation, oxidative stress and free radical-induced cellular damage. The CR induced reduction of multiple anabolic hormones and growth factors causes a down-regulation of the nutrient-sensing insulin/IGF signaling network and an activation of FOXO,
which modifies several “longevity genes”\textsuperscript{51}, including endogenous antioxidant enzymes (e.g. SOD2, catalase), DNA repair (e.g. DDB1) and autophagy (e.g. beclin-1, autophagin-1) genes\textsuperscript{52}. Autophagy and mitophagy are important for the removal of dysfunctional organelles, amyloid and other protein aggregates that interfere with normal cell function\textsuperscript{53}. FOXO activation is also a powerful inhibitor of cyclin D, a master regulator of cell cycle progression and cell proliferation. Another major adaptation to CR is the reduction in plasma concentrations of inflammatory cytokines and a modest increase in circulating cortisol that results in a reduction in systemic inflammation together with a protection against aging-associated deterioration in immune function\textsuperscript{54,55}. Increased expression of protein chaperones such as heat shock protein-70 is also important to improve proteostasis, the removal of damaged cellular proteins and cellular stress resistance\textsuperscript{50,56}. As we will discuss later, other molecular effectors that have been shown to mediate the health effects of CR include TOR\textsuperscript{57}, AMPK\textsuperscript{58}, sirtuins\textsuperscript{59}, and NRF2\textsuperscript{60}. Energy and amino acids restriction cause an inhibition of mTORC1 activity, which in turn enhances autophagy, improves proteostasis and stem cell function\textsuperscript{61}. Overexpression of sirtuins (i.e. SIRT1, SIRT3 and SIRT6) improves metabolic homeostasis through histone deacetylation, inhibits NF-kB signaling and increases genomic stability\textsuperscript{59}. In addition, activation of AMPK and SIRT1 up-regulates PGC-1a, a key transcriptional factor regulating mitochondrial function, antioxidant defenses, and fatty acid oxidation\textsuperscript{62}.

**CR and cellular senescence**

Different studies have shown that CR reduce senescence markers in different mouse organs and human colon mucosa\textsuperscript{9,63-66}. One of the main inducers of senescence is cellular damage. Therefore, it is highly plausible that CR reduces the generation of senescent cells by preventing damage to occur. CR can protect cellular deterioration in at least two major ways: interfering with the source of damage, for example via decreasing oxidative stress and inflammation, or repairing/eliminating already present damage, for example by increasing autophagy (Figure 1)\textsuperscript{49,50,54}.

An important source of cellular damage is oxidative stress, mainly caused by an accumulation of reactive oxygen species (ROS). Historically, the beneficial effects of CR were thought to be the result of slow metabolism resulting in reduced production of ROS \textsuperscript{67}, but it is now known that CR can actively regulate many other defense mechanisms against oxidative stress\textsuperscript{52,68}. Numerous experiments have established that sirtuins are critically required to reduce oxidative stress. In accordance, CR induces the expression of SIRT3, which was shown to play an essential role in reducing oxidative damage and its related pathologies\textsuperscript{69,70}. It is interesting to note that mitochondrial dysfunction caused by SIRT3 downregulation can, in fact, induces senescence\textsuperscript{71}. Another member of the sirtuin family, SIRT1, was reported to mediate aspects of CR response\textsuperscript{72}. Due to the fact that some studies have demonstrated the important role of SIRT1 in regulating oxidative status within the cell\textsuperscript{73}, it is highly possible that SIRT1 mediates its effect
during CR by reducing levels of oxidative stress. These notions suggest that CR might prevent senescence, in part, by upregulating the antioxidant defense program partly through the increase of sirtuins function and in part through up-regulation of FOXO.

Autophagy, a major lysosomal degradation pathway that is activated by CR, might act as an anti-senescence mechanism by clearance of damaged proteins and organelles including damaged mitochondria$^{50,74}$. Intriguingly, selective elimination of dysfunctional mitochondria by mitophagy alleviate many aspects of the senescence phenotype$^{75}$.

Another mechanism by which CR can prevent senescence is through enhancement of DNA repair mechanisms. Interestingly, CR activates many pathways that can prevent and aid in resolving DNA lesions. For instance, CR reduces age-dependent decline in non-homologous end joining (NHEJ)$^{76}$. In addition, it can improve nucleotide excision repair (NER), increase the fidelity of polymerase alpha and beta, and decrease their age dependent decline$^{77,78}$. Moreover, CR can also protect DNA by inducing the base excision repair pathway (BER), both in young and aged animals$^{79}$.

It has been shown that CR can regulate longevity pathways that include the insulin/insulin growth factor I signaling (IIS), both in rodents and human. Circulating IGF-1 levels were decreased during CR in rodents$^{80}$, whereas in human the effect was not direct. Long-term CR results in a persistent increase in serum IGFBP-1 leading to a decrease in IGF-1:IGFBP-1 ratio levels, which can probably inhibit IGF-1 signaling by decreasing free IGF-1 in circulation$^{81}$. Notably, prolonged exposure of cells to IGF-1 can induce premature senescence through the regulation of SIRT1-p53 pathway$^{82}$. Accordingly, the decrease in IGF-1 activity, as a consequence of CR, might help in preventing premature senescence.

Finally, the mechanistic target of rapamycin (mTOR) is also negatively affected by CR. A large portion of the senescence associated secretory phenotype components is regulated by mTOR$^{28}$, including factors that can induce secondary senescence$^{83}$. In addition, mTOR can promote geroconversion, the conversion from a proliferative arrest to irreversible senescence, and mitigation of its activity favors quiescence$^{84}$. For these reasons, CR could also prevent the activation and spread of senescence by inhibiting mTOR.

**Conclusion**

CR is a well-established intervention for reducing age-associated chronic diseases and enhancing lifespan. In this review, we have summarized some of the mechanisms by which CR exerts its beneficial effects, highlighting their complexity and heterogeneity. However, reduction of cellular damage might well be related, at least in part, to prevention of cellular senescence. In the next few years, it will be key to monitor the effects of long-term CR on cell senescence in various tissues to determine whether there is...
any organ-specificity for damage protection. It will be also of high interest to treat animals under CR with senolytic drugs to prove any synergistic effect of lowering cellular damage and eliminating senescent cells. Another important question is whether the absence of senescent cells during CR might trigger side effects – for example promoting longer kinetic of wound healing, a known issue for animals under CR. Finally, similar studies on the prevention of senescent cells should be directed towards alternative and less invasive dietary interventions with anti-aging properties, such as intermittent fasting and/or protein restriction.

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The author apologizes for the omission of relevant work owing to space constraints.

Competing interests

M.D. is co-founder of Cleara Biotech, a company devoted to develop senolytic interventions. However, he did not receive any compensation from the company related to this work.

Bibliography


