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The Basics of Biogerontology

Mark Rinnerthaler and Klaus Richter

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Abstract

Aging is an enormously complicated process. Despite a great many of theories (among them “Program Theories”, “Combined Theories”, “Damage Theories”, “Inflamm-Aging”, “Garb-Aging” and the “Rising Deleterium”), so far there is none which is able to explain this phenomenon satisfactorily and completely. A different approach to address the complexity of aging is to characterize the major “Hallmarks of Aging”. These are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and an altered intercellular communication. From research on these hallmarks, new avenues were opened on how to interfere with the aging process. Some of these possible therapeutic interventions are described here too.

Keywords: aging, aging theories, hallmarks of aging, senescence, healthy aging, anti-aging interventions

1. Introduction

Aging has ever been a puzzling phenomenon for mankind. It is already an important topic in ancient Greek mythology: Eos goddess of the dawn did fall in love with Tithonus son of Laomedon king of Troy. Eos asked Zeus to grant Tithonus immortality, she did forget to ask for eternal youth too, however. Therefore Tithonus was living happily, but the process of aging continued inexorably. Over time, there was not much more left of him than a croaking voice and finally, he was transformed into a cicada.

The question of then still remains today: Is aging really unavoidable? Not necessarily, as some organisms do not seem to age. Most prominent of them is the tiny fresh water polyp *Hydra*. It does already have a simple nervous system and does not display any signs of aging.

This remarkable feature of *Hydra* is due to the fact that its stem cells have an unlimited capacity for self-renewal. In this respect, the transcription factor FoxO plays a crucial role [1]. In contrast to *Hydra*, all vertebrates are aging although at a very different rate. The lifespan of the mouse is between 2 and 4 years, whereas the lifespan of the Greenland shark is beyond 400 years [2]. If we look at mammals, the lifespan can vary up to 100-fold: The Etruscan shrew has a lifespan up to 2 years, whereas for the bowhead whale a lifespan beyond 200 years has been estimated [3]. In general, large animals are living longer than small ones. A dog lives considerably longer than a mouse and an Elephant lives much longer than a dog. There is, however, a great variety in lifespans: as already mentioned, the lifespan of the mouse is in the range of 2–4 years, whereas the related naked mole rat which is roughly the same size lives up to 30 years [4]. Some organisms are aging very fast and some are aging quite slowly. Nevertheless for all of them is true: Aging is characterized by a reduction of fitness, an increase of age-related diseases and a massive increase of the risk of dying. Considering humans, the risk of dying of an 80-year-old is 300 times higher than for a 20-year-old person [5]. With this chapter, we want to give a short overview on major topics of current aging research. Furthermore, we want to point out possibilities that are arising from this research field which will probably help to increase the healthy lifespan quite considerably.

2. Theories of aging

In the quest to explain aging, many theories have been developed. In 1990, Zhores Medvedev collected all these different theories and their number exceeded 300 [6]. In the meantime, a number of new ones have been presented. Considering only this fact, one can easily imagine that aging is a very complex process. Accordingly, aging is far away from being understood completely. In a recent publication, the different theories are divided into “Program Theories”, “Combined Theories” and “Damage Theories” [7]. The program theories are based on the assumption that aging is genetically programmed. Among these theories is the theory of replicative senescence [8]. Already in 1965, Hayflick has observed that cells in cell culture divide about 40–50 times after which they enter a permanent cell cycle arrest from which they cannot escape any more. This phenomenon has been explained by the shortening of telomeres taking place at each cell division. If telomeres are becoming too short, the shelterin complex is lost and the cell recognizes this as a DNA double-strand break triggering a permanent DNA damage response [9]. Furthermore, it turned out that cellular senescence can be triggered by a variety of different stress factors, among them oxidative stress or overexpression of cellular oncogenes. Cellular senescence is a potent anti-tumor mechanism preventing damaged cells from dividing any further. Senescent cells undergo dramatic phenotypic changes and start secreting many proteins (among them cytokines and chemokines), this is known as the senescence-associated secretory phenotype (SASP). These proteins attract immune cells like macrophages, neutrophils and natural killer cells, which are supposed to remove the senescent cells. Nevertheless, senescent cells are accumulating with increasing age and triggering pathological changes in the particular organ. In a mouse model, it has been demonstrated that pathological changes in lung [10], kidney, heart and adipose tissue [11] can be prevented if senescent cells are cleared from the organism. There is convincing evidence that senescent cells play a major role in reducing the function of old organs and the emergence of age-associated pathologies [9, 12].

The formation of senescent cells might also be seen as kind of antagonistic pleiotropy. This “program” aging theory claims that during evolution mutations are taking place which provide an advantage for early stages of life but are detrimental late in life [13]: Damaged cells becoming senescent prevent tumor formation early in life but they produce a number of adverse effects late in life. The “disposable soma theory” [14] also a “program” theory claims that resources for an organism are limited and they have to be allocated between maintenance of the organism and reproduction. Furthermore does the “neuroendocrine theory of aging” [15] belong to the “program theories”. It states that aging is regulated by hormones similar to puberty and menopause. As a matter of fact, animals that reach sexual maturity in a short period of time have also a short lifespan. The mouse reaches sexual maturity within 6–8 weeks and has a lifespan of 2–4 years, whereas for the Greenland shark it takes some 150 years to reach sexual maturity but then its lifespan exceeds 400 years [2]. Interesting in this respect is that in parabiosis experiments, it was demonstrated that blood from young mice has a rejuvenating effect on old mice [16]. Recent research furthermore points to the fact that changes in the regulation of gene expression during aging are predominantly affecting genes which are responsible for growth and development [17]. The age an organism is able to live up to is without any doubt depending on its genome. Even under identical environmental conditions, a human will be living much much longer than a mouse. But up to now, there has been no gene identified that would actively regulate the aging process.

On the other end of the spectrum of aging theories are the damage theories. Among them the most prominent is the free radical theory of aging [18, 19]. According to it, reactive oxygen species (ROS) a byproduct of oxidative phosphorylation in mitochondria, damage DNA, lipids and proteins. They will be briefly discussed as follows:

1. DNA damage: It is of utmost importance that DNA damage will be repaired efficiently. If not this causes a rapidly accelerated aging process as can be observed in patients with a progeria syndrome (Werner Syndrome). Therefore we have a number of DNA repair systems (direct reversal pathway, mismatch repair, base excision repair, nucleotide excision repair, homologous recombination and non-homologous end joining) which repair DNA damage very efficiently [20]. Altogether there are more than 100 DNA repair enzymes known [21] which take care that DNA damage is not a major cause for aging until their activity diminishes during aging. Most probably contributing to the aging process are DNA double-strand breaks [22].
2. Lipid peroxidation: ROS predominantly attack unsaturated fatty acids in the membranes of a cell. By this lipid peroxidation fatty acids literally become chopped down producing chemically very active aldehydes [23]. These aldehydes react with DNA and proteins resulting in irreversible modifications. Proteins modified this way often lose their function and form aggregates which are not degradable by the cell which is in particular problematic in the brain. It has been demonstrated that lipid peroxidation triggers neurodegeneration [24].
3. Protein oxidation: Proteins are also attacked by ROS. In particular, sulfur-containing amino acids are being oxidized. The first stage of oxidation can be reversed enzymatically but later stages not any more [25]. Oxidatively damaged proteins too have the tendency to denature and to form aggregates which the cell is not able to degrade. Such aggregates may

cause death of the cell eventually as is the case in Alzheimer patients. ROS are damaging cells in many locations in particular if they are produced excessively. On the other hand, ROS are important signaling molecules, therefore their complete removal by antioxidants is definitely counterproductive. In recent years, a modification of the free radical theory of aging gained attention: It claims that not ROS are driving the aging process but the disturbed redox homeostasis is a major culprit [26, 27].

Also a damage theory is the theory of “Inflamm-Aging” which claims that aging is caused by ongoing low level sterile inflammatory processes [28]. In fact all degenerative diseases in aging have an inflammatory component as there are: Alzheimer, Parkinson, arteriosclerosis, arthritis, multiple sclerosis, osteoporosis and diabetes type II [29]. Responsible for many inflammatory reactions are debris of dead cells and un-degradable protein aggregates which have not been removed completely. This was the basis to coin the term “Garb-Aging” (from garbage and aging) as an addition to inflamm-aging [30]. None of the so far mentioned theories, however, is able to explain all facets of the aging process. Therefore a new perspective was presented recently: “Aging: progressive decline in fitness due to the rising deleteriome” [31]. According to this theory neither the synthesis of biomolecules nor the repair systems in a cell are working absolute flawlessly. Furthermore there are chemical reactions between many biomolecule. The resulting compounds cannot be removed completely. That means that unwanted reaction products as well as non-repaired damage (= deleteriome) increases with time and causes the aging process.

3. Hallmarks of aging

A completely different approach than to explain aging by theories is to describe it by its major characteristic features: “The Hallmarks of Aging” [32]:

3.1. Genomic instability

DNA is permanently exposed to a great variety of damage caused by ROS, lipid peroxidation products, environmental mutagens, hydrolytic reactions, UV irradiation and many more.

Besides chemical modifications to DNA, there can be single- and double-strand breaks, depurination as well as cross links between bases. Addition, deletion or substitution of bases is causing mutations. All together, these changes can also lead to epigenetic alterations causing changes in gene expression. To repair this damage efficiently is of utmost importance as can be seen in progeria or premature aging syndromes. In these diseases, defects in DNA repair systems are causing a dramatically accelerated aging process [33].

Usually, DNA damage is repaired very efficiently, however, the activity of the repair systems declines during aging [34].

3.2. Telomere attrition

Telomeres are protective caps on the ends of chromosomes preventing degradation or fusion of chromosome ends. At every cell division, telomeres are getting shorter and if they reach a

critical length the cells, are not able to divide anymore. This telomere attrition eventually leads to replicative senescence. The degree of shortening is proportionate to risks of a number of aging diseases [35]. In particular, short telomeres lead to bone marrow failure causing anemia and immune senescence and to enterocolitis in the intestinal epithelium. Furthermore, short telomeres are causing premature onset of emphysema and pulmonary fibrosis in the lung, fibrosis in the liver and also osteoporosis [36]. In addition, short telomeres have an impact on gene expression through telomere position effects on nearby genes [37]. Through the expression of telomerase in mice, the normal aging process could be delayed [38]. In humans, 11 mutations inactivating a single gene are known which directly affect telomere maintenance and they lead to age-related diseases and accelerated aging [35]. Also a telomere biology disorder is the Hoyerlaal-Hreidarsson (HH) syndrome which is caused by mutations in genes with telomeric functions. It is characterized by very short telomeres and affected individuals die in childhood mostly due to bone marrow failure [39]. Interestingly, it has been shown that in wild animals, telomeres shorten more slowly in slow-aging than in fast-aging ones [40].

3.3. Epigenetic alterations

Epigenetic changes are comprising alterations in histone marks, DNA methylation, nucleosome positioning and non-coding RNAs [41]. Histone modifications and methylation patterns of CpG islands have a tremendous influence on gene expression. For a set of 353 CpG islands, a clear correlation with age could be demonstrated. Of these, 193 CpGs get hypermethylated and 160 get hypomethylated during aging [42]. According to its reliable changes in methylation status, this set has been termed the epigenetic clock [27, 42, 43]. With this epigenetic clock, it is possible to predict the biological age and an age-related functional decline. Furthermore, it has been demonstrated that lifestyle factors like diet, exercise and education have an influence on this epigenetic clock [44]. In addition to DNA, the histones are subject to modifications (acetylation, methylation, phosphorylation and more). There are also a number of methylation marks that are changing with age. But the present picture is less clear than with DNA methylation [41]. A lot of attention has been focused on sirtuins. Sirtuins are class III histone deacetylases, which need NAD^+ as a cofactor and remove acetyl groups from previously modified lysines in the histone N-terminal tails. By removing the acetyl groups, lysines regain their positive charge and bind more tightly to DNA. The result is a more compact chromatin structure and down-regulation of gene expression. The removal of histone acetyl groups by sirtuins results in an extension of lifespan [45]. In total, seven sirtuins are known, of which SIRT1, SIRT6 and SIRT7 are localized in the nucleus. SIRT2 is predominantly found in the cytoplasm and is only localized to chromatin during the G/M phase of the cell cycle. SIRT3, SIRT4 and SIRT5 are the three mitochondrial deacetylases. Sirtuins do not only deacetylate histones but regulate the activity of a number of other proteins too. This way they play a central role in regulatory networks important for aging and longevity [46]. Mutant mice where single sirtuin genes have been deleted show a number of different pathologies connected to metabolism, cancer and inflammation [47].

3.4. Loss of proteostasis

Proteins not only have to be synthesized but they have to be removed and degraded eventually. Among many others, there are two major ways to remove damaged proteins: either to degrade

them by the proteasome or via autophagy [48, 49]. In addition, the cell has chaperones. These proteins help to fold proteins correctly or enable the renaturation of already denatured proteins. If refolding is impossible, chaperones are also able to target misfolded proteins to the proteasome. Therefore proteostasis, the maintenance of an intact proteome, includes not only synthesis and degradation of proteins but also folding and conformational maintenance. The disturbance of proteostasis is considered to be a major cause of aging [50, 51]. Not only the amount of chaperones is decreasing [52], but also the proteasomal activity and autophagy are declining during aging. This decline causes an accumulation of denatured proteins which have the tendency to form aggregates that cannot be removed by the cell anymore. These aggregates are detrimental to the cell and can even cause death of the cell (e.g. nerve cells in Alzheimer and Parkinson patients) [53]. Furthermore, it has been demonstrated that long-living animals have less denatured proteins than short-living ones [54]. In addition, the activity of the proteasome is remarkably higher in the long-living naked mole rat than in the short-lived mouse [55]. Particularly interesting in this respect is that experimental interventions which reduce the aging process are stimulating autophagy like caloric restriction, rapamycin, metformin, resveratrol and spermidine [56].

3.5. Deregulated nutrient sensing

Nutrient sensing is of utmost importance for every cell. The major nutrient sensing pathways that are also longevity pathways are [57]:

- IGF-1 and insulin signaling pathway
- mTOR pathway
- AMP-activated protein kinase (AMPK) pathway
- NAD⁺ dependent sirtuins

IGF-1 is like insulin a growth factor for many cells and acts via the insulin and IGF-1 signaling (IIS) pathway. The down-regulation of this pathway leads to a prolonged lifespan [58]. IGF-1 but also EGF and high amino acid levels are activating the mTOR pathway which stimulates protein synthesis and growth in general but down-regulates autophagy [59]. AMPK is the sensor and regulator for energy metabolism and homeostasis of the cell. AMPK activity can extend the lifespan of yeast, *C. elegans* and drosophila and the healthspan of mice [60].

NAD-dependent sirtuins are a family of deacylases which not only deacetylate histones but modify a large number of non-histone proteins too. They show impressive activities to prevent diseases and some aspects of aging [61].

During aging, the synthesis of these sensor proteins is reduced however [62].

The different signal transduction pathways that are sensing the availability of nutrients are deregulated during aging by metabolic diseases [63].

3.6. Mitochondrial dysfunction

As mitochondria are not only the power plants of the cell but also important signaling centers they play a central role in the aging process. For energy production in form of ATP, they reduce

oxygen to water. If accidentally oxygen gets only one electron, it leads to the production of ROS instead of water. This has inspired Harman already in 1956 to present his “Free Radical Theory of Aging,” which he has repeatedly improved [18]. Furthermore, it has been demonstrated that ROS are not only causing damage but also are important signaling molecules, which are able to regulate many pathways. For example, ROS can induce autophagy (mitophagy) [64].

The events of biogenesis, fusion, fission and mitophagy are collected under the term mitochondrial dynamics [65]. Function as well as quality of mitochondria is regulated by mitochondrial dynamics. Nutrients in excess cause fragmentation (fission) of mitochondria and a low level of nutrients leads to fusion and elongation [66]. Mitochondrial dynamics is also influenced by external signals like hormones, nutrients and physical exercise. A number of pathways are involved like mTOR, AMP-activated kinase and sirtuins [67]. In particular, sirtuins play an important role as they do not regulate a few target enzymes but regulate functional clusters (e.g. TCA cycle, fatty acid metabolism, electron transport chain and others). This way they are involved in the regulation of ROS-mediated signaling pathways as well as in the detoxification of damaging ROS. Furthermore they regulate metabolic plasticity. SIRT3, for example, promotes switching to fatty acid oxidation upon caloric restriction [68]. The modulation of metabolic changes plays a crucial role in senescence too. In addition, defect mitochondria are stimulating inflammatory reactions which are triggering inflamm-aging [69]. Altogether, mitochondrial dysfunction leads to a number of age-related diseases including metabolic, cardiovascular and neurodegenerative pathologies, sarcopenia and fibrosis in different organs [65].

All these facts demonstrate that the quality of mitochondria has a tremendous impact on the aging process [70, 71].

3.7. Cellular senescence

Cellular senescence is characterized by an irreversible arrest of the cell cycle. This means that cellular senescence can only affect cells that are able to divide like stem cells, progenitor cells or cells that are not yet terminally differentiated. This phenomenon has been discovered with cells in cell culture. It turned out that they cannot divide without limits but stop growing after about 40–50 cell divisions [72].

The irreversible cell cycle arrest can be induced by erosion of telomeres, substantial DNA damage, oxidative stress, overexpression of oncogenes, mitochondrial dysfunction and proteotoxicity [73]. Cells in the state of senescence change their morphology, they are getting larger and there are massive changes in the organization of chromatin [74]. Furthermore, they start to secrete a large number of proteins. The sum of all these proteins is called the senescence-associated secretory phenotype (SASP). Among these proteins are pro-inflammatory cytokines, chemokines, growth factors and matrix metalloproteases (MMPs). The pro-inflammatory cytokines are causing local sterile inflammations which contribute substantially to “inflamm-aging”. They attract cells of the immune system which are killing senescent cells. The overproportional increase of senescent cells during aging is probably due to a decline in immune function [75]. An important additional feature of senescent cells is the active suppression of apoptosis [76]. At the level of gene expression, permanent cell cycle arrest is mediated by the protein p16^{Ink4a} which is an inhibitor of the cyclin dependent kinases 4 and 6 (CDK4 and CDK6).

In healthy young cells, p16^{Ink4a} expression is low or undetectable but increases dramatically in senescent cells [9]. This way it is evident that an essential function of senescence (maybe the most important one for the organism) is to pull the emergency brake to prevent uncontrolled cell division which otherwise could cause tumor formation. Senescent cells have also an important additional function in wound healing. After a wound has been inflicted many cells are produced in excess to close the wound. During the subsequent remodeling process, the surplus of cells is entering senescence and will be removed by the immune system. For years it has been discussed by researchers if cellular senescence has any influence on the aging process itself. During the past few years, scientists came to the conclusion that cellular senescence is one of the major causes of aging [77]. In genetically modified mice, it was already possible to delete senescent cells (p16^{Ink4a} positive cells). These animals showed less age-related pathologies, an improved healthspan and a prolonged median lifespan [9, 11]. Therefore, there are already a number of different interventions under investigation how senescent cells can be removed from the human body [78, 79]. To succeed in this respect could dramatically improve human healthspan. Senescent cells are detrimental to the function of organs they are residing in and this way they have a tremendous impact on age-dependent degenerative diseases [12, 78].

3.8. Stem cell exhaustion

Stem cells are of utmost importance for tissue homeostasis and regeneration and stem cell exhaustion is among the most significant hallmarks of aging. Stem cell exhaustion is leading to a reduced regenerative capacity during the aging process. Premature stem cell exhaustion is also seen in age-related diseases [80]. Stem cells are usually very small remaining in a state of quiescence. This state is characterized by low metabolism and the presence of few mitochondria. From dormancy, they can be activated to replace lost stem cells or to produce transit amplifying cells which will provide many cells for repair or regeneration of their particular tissue. During this differentiation process, they are going through a developmental program which is tuning them precisely to their new function [81].

There are tissues with a very high turnover of cells like bone marrow, intestine and the epidermis of the skin. There are also tissues where stem cells get activated rarely like muscle and brain. Essential for survival and quiescence of stem cells is their immediate environment which is defined as the stem cell niche. The stem cell niche comprises proteins of the extracellular matrix and surrounding cells which secrete a number of growth regulating proteins (Wnts, BMPs, EGF and Notch). It is essentially regulating the state of quiescence [82, 83]. Different drivers of aging (telomere attrition, cellular senescence, DNA damage, epigenetic alterations, nutrient sensing and disturbed proteostasis) have their impact on stem cells too and are responsible for stem cell aging [84]. As stem cells usually stay in the state of quiescence and divide rarely, many pro-aging impacts affect the stem cells via their niche. Muscle stem cells, so called satellite cells, rarely divide, but proliferate massively upon demand. They produce myoblasts which are the precursor cells necessary for the regeneration process of the muscle. If old satellite cells are transplanted into young muscle tissue their regenerative capacity increases which demonstrates the influence of the young niche [85, 86]. The opposite is true for transforming growth factor beta (TGF-beta1). This factor is produced by the niche and reduces the proliferative potential of satellite cells. During aging under certain circumstances the niche increases the production of fibroblast growth factor 2 (FGF2). This triggers the stem cells to leave quiescence and start to divide which eventually leads to a reduction of satellite

cells available for the regeneration of muscle tissue [87]. In a similar way, the prolonged signaling of the growth hormone (GH)/insulin/insulin-like growth factors (IGF) axis is considered to cause a depletion of stem cells [88]. There are also areas in the adult brain where stem cells are residing: in the dentate gyrus of the hippocampus in the hypothalamus and in the subventricular zone of the lateral ventricles [89]. Like in other tissues, there are age-related changes in the stem cell niche and the number of neural stem cells is declining during aging. Not only the numbers of stem cells are decreasing during aging but also the proliferation of the developing precursor cells will be damped via an elevated concentration of TGF- β 1. This way the production of new neurons is reduced while the generation of oligodendroglia remains at about the same level [90]. Furthermore, sterile micro-inflammation in the hypothalamus can cause a reduction of neural stem cells which in turn leads to a reduction of cognitive functions [91]. In addition to the number of stem cells and the contribution of the stem cell niche, the regenerative capacity will be influenced by systemic factors. Via parabiosis experiments (connecting an old mouse to a young one), it was possible to correct malfunction of old satellite cells in muscle tissue. These satellite cells could be reactivated again. In a similar way, it was possible to improve the function of stem cells and neurogenesis in old brains. The proteins responsible for this activity could be identified as growth differentiation factor 11 (GDF11) and oxytocin [92]. There are numerous factors that regulate the biological function of stem cells. Presently, it seems that the most important ones are metabolism and epigenetic changes [80]. As excessive nutrient sensing leads to premature depletion of adult neural stem cells [89] and chronic activation of mTOR leads to loss of stem cells in the airway epithelium of the mouse [93].

3.9. Altered intercellular communication

The regenerative capacity of stem cells is independently influenced by intrinsic and extrinsic determinants [92]. An intrinsic determinant is the maintenance of autophagy. A failure of autophagy in old satellite cells leads to senescence eventually [94]. Among the extrinsic factors, growth hormone/insulin/IGF-1 (somatotrophic axis) is a center piece for the regulation of growth in the mammalian organism. Mouse mutants with defects in the biosynthesis of the growth hormone (Ames dwarf mice, Snell dwarf mice or GHRKO-mice (GH receptor deletion)) are considerably smaller than wild type mice but have an approximately 50% longer lifespan [95]. In humans, the amount of growth hormone and IGF-1 in the circulation is changing during aging. The highest level is reached during the second decade where growth is most prominent. Afterwards the concentration is going down continuously until it reaches a low plateau during the sixth decade. Humans with genetic polymorphisms resulting in a reduced activity of IGF-1 show a significantly increased lifespan. An elevated concentration of IGF-1 is correlated to a higher risk for some tumors [96]. There is also an altered communication between muscle stem cells and the environment. Growth hormone is important for the maintenance of muscle mass [97]. IGF-1 is modulating the differentiation of muscle progenitor cells (myoblasts) and influencing satellite cells [98]. An increased aging of muscle stem cells is caused by an elevated concentration of Wnt-proteins (e.g. Wnt3A) [99]. As an antagonist of Wnt/ β -catenin signaling acts the protein klotho. Unfortunately, the amount of klotho in the circulation is decreasing during aging. In the mouse, the silencing of the klotho gene triggered a rapid aging process [100]. Klotho is essential for the homeostasis of mineral metabolism (in particular phosphate) but it also modulates the signaling pathways of IGF-1 and Wnt. The deletion of the klotho gene in mice reduces their lifespan to 2–3 months which

is only about 10% of their regular lifespan [101]. A remarkable activity has also been demonstrated for GDF11 which improves regeneration in old organisms and serum levels of GDF11 are significantly lower in old individuals [102]. An increased regenerative activity has been shown for bone [103], brain [104], skeletal muscle [105] and heart [106].

Furthermore, a number of chemokines (CCL2, CCL11, CCL12 and CCL19) have been identified via parabiosis experiments and they have been correlated with impaired neurogenesis in old individuals [107]. Other potential pro-aging factors that increase during lifetime are TGF-beta1, IL-6 and TNF-alpha [107]. Beta2microglobulin too is a systemic pro-aging factor triggering age-related cognitive impairment [108].

Another pro-aging factor is the plasminogen activator inhibitor 1 (PAI-1) which is secreted by senescent cells. It induces the accumulation of p16^{Ink4a} leading to cellular senescence [109]. An anti-aging factor is kallistatin which inhibits oxidative stress and inflammation. It is also able to down-regulate the miRNA synthesis of miR21 and miR-34a, thereby reducing vascular senescence and aging [110]. The protein tissue inhibitor of metalloproteinase 2 (TIMP 2) was isolated from human umbilical cord. It is an anti-aging protein which revitalizes the hippocampus, increases synaptic plasticity and improves cognitive function [111]. The intercellular communication is also altered by numerous pro-inflammatory cytokines which are released by senescent cells. These cytokines are causing inflammatory processes [112]. Furthermore, inflammasomes in the cells of the innate immune system can be activated by DAMPs (damage-associated molecular patterns) [113]. DAMPs are comprised of debris of necrotic cells, amyloide fibers, HMGB1, heat shock proteins, crystals of cholesterol and uric acid. Activated inflammasomes are causing the release of interleukins IL-1beta and IL-18 [114]. These interleukins trigger inflammatory reactions in the surrounding tissue which are causing age-related diseases [115], among them Alzheimer's disease [116].

Exosomes provide an additional possibility for intercellular communication. They are small lipid vesicles which are secreted by the cell and they carry proteins and functional RNAs [117]. They can contact nearby cells or they can be distributed via the circulation across the whole organism. They help the cell to get rid of toxic protein waste [118] or to contribute to intercellular communication [119]. In the latter case, predominantly miRNAs play an important function [120]. During aging, the amount of exosomes in the blood stay more or less constant. Their content, however, becomes more pro-inflammatory [121]. Recently, it has been shown that they also play a role in senescence and aging [122].

4. Possible therapeutic interventions

4.1. Physical exercise

The most simple and probably the most efficient way to attenuate aging is to perform physical exercise. A sedentary lifestyle with minimal physical activity on the contrary is detrimental for health comparable to smoking [123]. It is quite obvious that physical exercise is the best way to keep skeletal muscles in a healthy condition [124] and to prevent sarcopenia and frailty in old age [125]. Physical exercise does not only improve physiological parameters like

maximum oxygen consumption and reduced levels of cholesterol and triglycerides in the blood, but it also improves physical and psychical conditions in old age [126]. Although a number of physiological parameters can be improved considerably by physical training, the protective function for the cardiovascular system are about twice as high as can be explained by these parameters only. Therefore there are still many open questions concerning the molecular mechanisms which are activated by physical training [127]. Very well documented is, however, the positive effect on the brain and in particular on cognitive functions and the stimulation of neuronal growth in the hippocampus, an area critically important for memory processes [128]. Physical exercise increases hippocampal volume, functional connectivity and improved connectivity between the default mode network and the prefrontal cortex [129]. In this context, it should also be mentioned that physical exercise leads to a significant improvement of memory functions in Alzheimer patients [130].

4.2. Caloric restriction/dietary restriction

Already in 1935 it has been demonstrated on rats that reducing the amount of food intake can extend the lifespan by 30% [131]. This experiment has been repeated many times and it turned out that animals are not only living longer but they also show less age-related deficits. During the past years, it has been demonstrated that the amount of calories is less important than the amount of proteins. Therefore the term caloric restriction has been replaced in most cases by the term dietary restriction. In addition to the amount of food, the timing of food uptake is important. Animals getting their food evenly distributed during the day did not show positive effects but animals fed only once a day did show the positive effects. Also did fasting every second day result in an increase of lifespan by 30% [132]. Altogether a great many experiments have been performed concerning this topic and results are sometimes contradictory. Some authors are pointing out explicitly that it is necessary to test many different combinations of carbohydrates and proteins in a single experiment. It has been demonstrated that a relation of 1:10 (proteins:carbohydrates) results in the longest lifespan in mice. Remarkable in this respect is the fact that the traditional diet of the population of Okinawa consists of protein to carbohydrates in a relation of 9:85 and it is well documented that the people of Okinawa have the highest life expectancy worldwide [133]. It has to be mentioned that not only permanent dietary restriction is effective but intermittent fasting too. In rats and mice as well as in humans, there are profound health benefits. Results of intermittent fasting (2 days per week or every other day) decreased insulin levels, increased resistance to stress of heart and brain, reduced inflammation, enhanced autophagy, mitochondrial health and DNA repair [134]. Concerning DNA repair, the following experiment is really remarkable: mice lacking the DNA excision repair gene *Ercc1* are aging very fast with a lifespan of 4–6 months. If they are subjected to a dietary restriction of 30%, this treatment triples their lifespan [33]. The single cell senses the availability of nutrition via nutrient sensing pathways which are GH/insulin/IGF-1, mTOR, sirtuins and AMPK and via these pathways the metabolic influence on the aging process is regulated [57].

4.2.1. The somatotrophic axis (GH/insulin/IGF-1)

Attenuating the signaling of the somatotrophic axis results in an increased lifespan. This has been demonstrated in animal models, in genetic polymorphisms or functional mutations in

the IGF1R gene in humans [96]. Pharmaceutical interventions to block the signaling of this pathway are being tested but there are no drugs available yet to be used in humans [96].

4.2.2. *mTOR*

mTOR is a serine/threonine kinase which is “the grand conductor of metabolism and aging” and is either part of the multiprotein complex mTORC1 or mTORC2 [135]. Growth factors, insulin, IGF-1, amino acids and glucose are activating mTOR which in turn stimulates growth and inhibits autophagy.

Rapamycin binds to FKBP12 in this way inhibiting mTOR. Blockage of mTOR increases the lifespan in different organisms among them mice. But unfortunately there are numerous side effects which prohibit the use on a daily basis for healthy individuals [135].

4.2.3. *Sirtuins*

Sirtuins interact with IGF-1, mTOR and AMPK signaling pathways and regulate many other proteins involved in energy metabolism, DNA repair, cell survival, inflammation and tissue regeneration. SIRT1, for example, besides deacetylating histones H1, H3 and H4 modifies more than 50 other proteins [61]. Sirtuin-activating compounds (STACs) have gained much attention since their discovery 2003 and more than 14,000 STACs have been identified since then [61]. Essentially there are two different classes: sirtuin activators and compounds that raise NAD⁺ levels. Using rodents numerous studies have shown that STACs promote health during aging involving protection against cardiovascular disease, diabetes type 2, neurodegeneration and even cancer [61].

4.2.4. *AMP-activated protein kinase (AMPK)*

AMP-activated protein kinase is a heterotrimeric protein and a key enzyme in cellular energy sensing. The alpha subunit kinase domain contains a conserved threonine which is phosphorylated by upstream kinases activating AMPK. The beta subunit binds the alpha and gamma subunits and has an additional domain to sense glycogen. The gamma domain has four sites that can bind AMP, ADP and ATP which provides AMPK with the ability to sense AMP:ATP and ADP:ATP ratios [136, 137]. These features make AMPK the centerpiece of “an energy-sensing pathway with multiple inputs and outputs” [136].

AMPK turns on glucose uptake, fatty acid oxidation, autophagy and mitochondrial biogenesis and it inhibits mTOR and the synthesis of lipids and proteins. It is therefore a central regulator of metabolic pathways including their effects on age-related diseases. It is also involved in the process of inflamm-aging via the regulation of the NLRP3 inflammasome during aging [138]. The capacity of AMPK signaling declines with aging which has a negative effect on the maintenance of cellular homeostasis [60]. Considering these facts, it is obvious that there is extensive research going on how to restore or boost AMPK activity by metformin [139] or by other nutraceutical compounds in particular polyphenols like resveratrol [140].

4.3. Pharmacological substances

4.3.1. Metformin

Metformin is in use to treat diabetes type 2 since a long time already. The inhibitory effect on the synthesis of glucose in the liver is due to the activation of AMPK [141, 142]. In addition metformin inhibits mTOR and complex I of the mitochondrial electron transfer chain resulting in a reduced production of ROS. In addition metformin stimulates autophagy, dampens inflammatory processes and senescence and increases the lifespan in animal models [58, 143, 144]. There are reports claiming that metformin does not only improves the healthspan and lifespan but also reduces the risk of some cancers and shows positive effects with congestive heart failure, chronic liver disease, chronic kidney disease and multiple sclerosis (summarized in [145]). This had led some researchers to call metformin “the aspirin of the twenty-first century” [145].

4.3.2. Rapamycin

This substance has been isolated from the microorganism *Streptomyces hygroscopicus* which has been found on the island of Rapa Nui, hence the name rapamycin. It is widely used as an immunosuppressant to prevent rejection after organ transplantation. The protein complex inhibited by this substance has been termed “Target of rapamycin” or TOR and it has been demonstrated that it leads to a significant increase in lifespan when applied to mice and most other “aging” model organisms [146].

4.3.3. Resveratrol

Polyphenols are comprising a large group of plant secondary metabolites. They are classified into phenolic acids, lignans, flavonoids and stilbenes [147]. The most prominent member of stilbenes is resveratrol which is synthesized by many plants in particular in wine. Resveratrol activates SIRT1 which mediates the effect of caloric restriction [148]. It could be demonstrated that resveratrol increases the lifespan of some organisms, in mice only if they are fed a high-fat diet [149]. In addition, resveratrol causes a number of positive effects in the cardiovascular system, cancer, diabetes type 2 inflammation and neurodegeneration [150]. As resveratrol is also stimulating autophagy and together with its neuroprotective effects, there are indications that resveratrol might also be applicable to treat Alzheimer’s disease [151]. It has to be mentioned that resveratrol not only activates SIRT1 but also AMPK which explains many of its anti-oxidant and anti-inflammatory activities [152]. Furthermore resveratrol activates a number of stimulus-responsive transcription factors and inhibits cAMP-degrading phosphodiesterases which helps to understand its many effects [153–155].

4.3.4. Spermidine

Like rapamycin and resveratrol, the polyamine spermidine also stimulates autophagy although via a different molecular mechanism. Similar to resveratrol, the stimulation of

autophagy is achieved by a change in the acetylation status of several proteins, but this occurs in a SIRT1-independent manner (most probably due to an inhibition of acetylases) [156]. For spermidine too, it has been demonstrated that it is increasing the lifespan of mice and all “aging” model organisms [157]. Surprisingly, the amount of this substance that is present in all cells dramatically decreases with aging [158]. In addition spermidine has neuroprotective capacities [159] and reduces the risk for cardiovascular diseases [160].

Spermidine also stimulates the synthesis of anti-inflammatory cytokines and has a positive influence on lipid metabolism [161].

4.3.5. *Vitamin D*

Muscle and bone are forming a physiological unit whereby both partners are regulating each other via endocrine signals [162]. Vitamin D has an essential function within this regulatory network. A sufficient supply of vitamin D prevents loss of muscle mass (sarcopenia) and age-dependent deposition of fat in muscles [163, 164]. In addition vitamin D shows a positive effect on cognitive function in old age [165]. Mice lacking the vitamin D receptor do age prematurely and the animal model for Alzheimer’s disease show better memory performance and a reduction of some markers for Alzheimer’s pathology after vitamin D supplementation. Humans with Alzheimer’s disease show very low blood levels of vitamin D. Altogether vitamin D is a neuroprotective substance [166].

4.3.6. *Soluble proteins/growth factors*

Treating age-related ailments with soluble proteins is particularly attractive because it can be performed via simple infusions. Among the best candidates, GDF11 and oxytocin have demonstrated a rejuvenating effect in old mice [92]. In a study comparing very old healthy individuals (beyond the age of 100) with 70–80-year-old persons, a set of proteins have been identified whose levels were elevated in the serum of the 100+ probands. This study correlates “successful aging” with these four proteins: Chemerin, Fetuin-A, FGF19 and FGF21 [167]. In particular, FGF21 is a “systemic enhancer of longevity” [168]. It is involved in the coordination of glucose and lipid metabolism and maintains tissue homeostasis under stress conditions. FGF21 can enhance autophagy and mice overexpressing it live up to 40% longer [168]. Another good candidate to provide a healthy lifespan is adiponectin. Adiponectin is expressed in and secreted from small adipocytes. It increases insulin sensitivity, shows anti-atherosclerotic effects and improves metabolism in skeletal muscle, liver and adipose tissue. Adiponectin activates AMPK and SIRT1 and this way it acts like an exercise mimicking factor [169]. Finally, it has to be mentioned that it also turns on catalase and superoxide dismutase reducing oxidative stress in metabolically active organs (summarized in [169]). A further “pro-youthful” factor is follistatin-like 1 (FSTL1) which together with GDF11 supports heart regeneration, as it increases the survival of cardiomyocytes [170]. Another good candidate is the soluble isoform of Klotho which increases the lifespan of mice and shows a neuroprotective function making it a good candidate for the treatment of Alzheimer’s and multiple sclerosis [171].

4.3.7. *Acetylcholinesterase inhibitors*

Alzheimer's disease (AD) is the most devastating aging disease. For 2013, it was estimated that more than 44 million people were affected worldwide and this number is expected to be beyond 135 million by the year 2050. Although there is presently no cure, there are a few drugs available that make life easier for AD patients. Most prominent are acetylcholinesterase inhibitors which show modest effects on improving cognitive function. The degeneration of cholinergic neurons which is seen in AD as well as Parkinson's disease dementia (PDD) leads to a reduction of acetylcholine levels. Furthermore, cholinergic pathways are not only important for the brain but also for skeletal muscle and the autonomous nervous system [172]. The increase of acetylcholine levels via inhibition of acetylcholinesterase improves cognitive function and has also beneficial effects on some of the comorbidities that usually affect AD and PD patients [173].

4.4. **Selective elimination of senescent cells**

If senescent cells are not removed by the immune system they are causing organ dysfunction and are a major cause of age-related diseases [174]. The removal of senescent cells in mice has improved their health conditions considerably. The elimination of senescent cells via drugs (senolysis) [175] or to trigger apoptosis (senoptosis) is also a realistic possibility in humans. From the observation that senescent cells do not respond to their own pro-apoptotic SASP, it was concluded that they have senescent-cell anti-apoptotic pathways (SCAPs). Six such SCAPs have been identified and these SCAPs were then screened for targets sensitive to senolytic drugs [176]. A number of senolytic drugs synthetic ones as well as of plant origin have been identified in the meantime. Prominent among them is quercetin which demonstrates promising activities [166]. It has to be mentioned that not every senolytic drug is effective on each senescent cell type and often is the combination of two or three drugs much more effective. An advantage over other medications is that senolytic drugs need not be taken continuously to exert their effect but just administered intermittently [176]. The use of senolytic drugs increase hope for the treatment of diseases for which there are hardly any other therapeutic options like idiopathic pulmonary fibrosis (IPF) a devastating lung disease [177]. Another elegant approach to trigger apoptosis in senescent cells has recently been demonstrated using a synthetic peptide. This cell penetrating peptide (CPP) was deduced from the sequence of the transcription factor FOXO4 and it excludes p53 from the nucleus. Instead of residing in the nucleus p53 is docking on to mitochondria to trigger apoptosis [178]. An additional possibility is to attack senescent cells with specific antibodies or by modified T cells [9].

4.5. **Transplantation of stem cells**

Stem cells are of utmost importance for regeneration and function of all organs. Transplanting stem cells into target tissues opens the possibility to repair major defects. Here we are at the brink of breathtaking possibilities for regenerative medicine. In particular, multipotent mesenchymal stem cells offer a wide spectrum of applications however these cells are losing a lot of their regenerative capacity during aging [179]. Substantial progress has been made by

the discovery that only four transcription factors (OCT3/4, SOX2, KLF4 and MYC) can induce reprogramming to pluripotency. Somatic cells can be transformed into young embryonic stem cells (induced pluripotent stem cells = iPSCs) [180]. From human fibroblasts such iPSCs could be generated and after specific differentiation processes used in different tissues [181]. In clinical trials, specific cells have been differentiated from iPSCs to treat Alzheimer disease, Parkinson disease, spinal cord injuries, diabetes or congestive heart failure [182]. Such a strategy for rejuvenation of old organs via stem cell therapy offers possibilities almost without limits for the future [183]. There is, however, a number of points that critically affects the success of stem cell transplantation. No matter how the replacement cells have been generated either as induced pluripotent stem cells (iPSCs) and subsequent differentiation steps or by direct transdifferentiation of somatic cells the condition of the stem cell niche is of utmost importance for regenerative success [183]. Also protein factors of the circulation effect transplanted cells massively [184]. Furthermore inflammatory processes which are often increased during aging effect stem cells dramatically as has been demonstrated for satellite cell function [185]. This demands the inhibition of inflammatory signaling as absolutely necessary.

5. Conclusion

Since the turn of the century there has been enormous progress in aging research in many fields. In this book chapter, we made a selection of aging theories and pathways that in our opinion are of great importance. To name them all would by far go beyond the scope of this article. It also has to be stated that of all the organelles in the cell we did just name mitochondria and their role in the aging process. But there is rising knowledge that all organelles have their specific share to aging. In the focus of this article were especially pathways and mechanisms on the cellular level. We did neglect that basically each organ and tissue has its private aging mechanisms [186, 187]. Therefore we believe that aging research will move on from cells toward tissues/organs and whole organisms. The possibilities that epigenetics will provide to increase health span look breathtaking, however, they cannot be really estimated to their full extend today yet. Much more realistic seems the application of stem cells which will provide regenerative medicine with fabulous opportunities. A very positive effect for an increased healthspan for almost all people will be possible if we will be able to boost autophagy without side effects. A similar effect on health span and the prevention of age-related diseases can be expected if it will be possible to eliminate senescent cells. Taken together there are really good chances that in the near future it will be possible to help many humans to live a healthy aging.

Author details

Mark Rinnerthaler and Klaus Richter*

*Address all correspondence to: klaus.richter@sbg.ac.at

Department of Biosciences, University of Salzburg, Salzburg, Austria

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