

Aging: is it a disease?



Aging: is it a disease?

V Forum

27 September 2014, Frankfurt am Main

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Presentation

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The Forum “Aging: is it a disease?” was hosted at the Goethe University (Frankfurt am Main) that supported and promoted the organization of this meeting. Of note, several pioneering studies in different field of Medicine originated from the Goethe University itself. For instance, Prof. Andreas Zeiher, Director of Internal Medicine Clinic III, Chief of Department of Cardiology, Goethe University, really pioneered the field of tissues regeneration in particular the regeneration of human heart through application of adult stem cells. Today, several international scientists that impacted the field of aging in the past decades attended the meeting: Prof. Edward Lakatta for vascular science, Prof. Silvia Bacchetti for studies on telomeres and telomerase activity and, among others, Prof. Judith Campisi that really founded the field of aging.

The topics of the Forum is the key question “Is aging a disease?”. This is a very complex issue requiring comprehensive approaches. Western countries are rapidly aging, but although people live longer the good health is not ensured. So, there is the need to improve interventions ameliorating the aging process and to prolong the healthy aging.

Tissues and organs of our body are impacted in different way by aging and any functional impairment can affect evolution of disorders. Today, some interesting questions are addressed about the possibility, or better the need, to switch our modern medicine from controlling risk factors of aging toward a “functional restoration” counteracting directly the aging process. During this forum, several studies unveiled novel mechanisms and strategies aimed to develop realistic and practical methods for maintaining health through lifespan.

Introduction

Antonella Farsetti

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The theme of Aging was the subject of much debate in the ancient world, “*Senectus ipsa est morbus*” (Aging *per se* is a disease) is a famous sentence from the roman playwright Terenzio (in his comedy *Phormio*, 160 BC) and “*Senectus enim insanabilis morbus est*” (Old age is an incurable disease) wrote the great roman philosopher and politician Seneca at the end of his life (from *Epistulae morales ad Lucilium*, 62-65 AD). Thus for a long time, old age has been considered an incurable and untreatable disease. A different point of view has emerged from the Symposium on Aging in the attempt to answer the question whether aging itself is a disease. Aging of biological systems occurs in spite of numerous complex pathways of maintenance, repair and defense, but although aging may be seen as the common cause of all age-related diseases, perhaps aging *per se* cannot be considered a disease. In this regard we disagree with Terenzio and Seneca who had a quite skeptic vision of the problem.

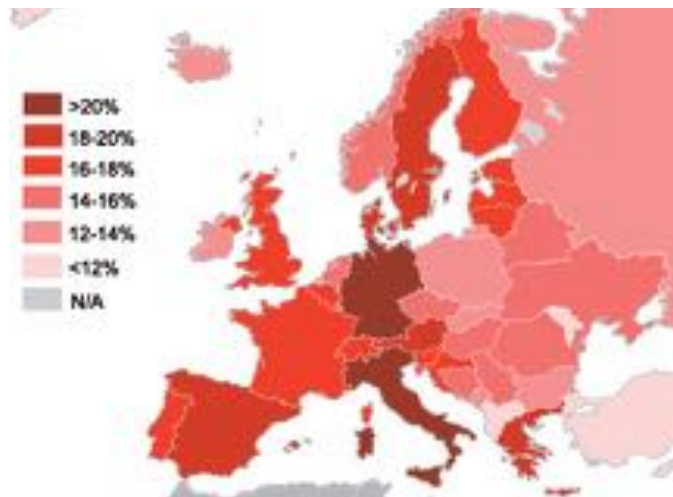
Nevertheless the worsening problem of an aging population is indeed an highly relevant issue in modern society, particularly in the industrialized/developed world for two main reasons: 1. the population in Western countries is aging rapidly; 2. people are living longer, but their chances of spending their later years in good health and well-being vary within and between countries.

The Forum brought into focus Aging and its relations with others fields (such as epigenetic and genomic regulation, cell transformation, cancer progression, reprogramming of cell fate and stem cells) and sought the interaction among people devoted to take the scientific challenges facing the fields of Aging under different points of view. It is important here to stress that aging is not merely a collection of diseases. With age, we become more disease-susceptible and endure a number of *physiological changes*, not all of which lead to pathology. The goal of the research in this field is to urgently improve our possibilities of interventions aimed at ameliorating the hu-

man aging process and to prolong “healthy aging” during a longer lifespan, acquiring deeper knowledge about the aging process. Indeed there are urgent and unmet needs to *understand the aging process* in order to identify novel, specific and sensitive targets, risk prediction factors and future treatments for diseases common in the elderly population.

The ageing of Europe is challenging our health care systems and economic growth. It is estimated that by 2050, the number of people aged over 60 in Europe will double from current 20% to 40% of the total population (Eurostat, *Population Projections*, European Commission, 2012; see also • **Figure 1**). Therefore, strategies to improve population health and to increase healthy life years must be a keystone for a sustainable Europe. Cardiovascular diseases are by far the leading cause of morbidity and mortality in industrialized nations, and they soon will become the most prevalent cause of death worldwide. Due to remarkable progress in prevention and acute cardiac patient care, cardiovascular diseases nowadays manifest significantly later in life. As such, the incidence of coronary artery disease, myocardial infarction and heart failure increases nearly exponentially with age (• **Figure 2**).

• **Figure 1.** Ageing of Europe: distribution of the European population above 60 years of age



According to this study, European countries are not ageing at the same rate. Italy, Germany, England, Sweden, Spain and Greece are ageing fast. A number of other central and northern European countries are ageing at a slower rate. However, the average trend indicates that Western Europe has one of the eldest populations in the world.

Source: Central Intelligence Agency-World Factbook 2010.

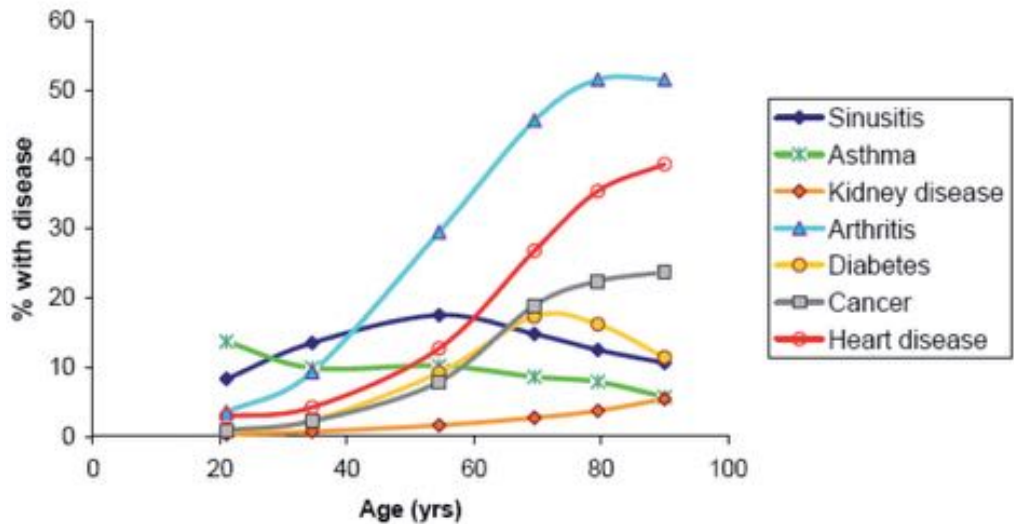
To identify those changes associated to healthy ageing and to determine their impact on the physiopathology of ageing-associated illnesses, it is the most important present and future area of investment for the European Community.

To address the complex issue of aging we ask how to fight the challenge posed by this biological process. To live up to this challenge it is crucial that *medicine shifts its approach*, accelerating the transition from control of the risk factors (risk factors management) to damage repair and function restoration by exploiting eventually the regenerative capacity of stem cells to counteract the aging of tissues and organs, in other words *towards a regenerative medicine*.

In this scenario, understanding of aging as a process should transform our approach towards interventions from developing illusory anti-aging treatments *to developing realistic and practical methods for maintaining health throughout the lifespan*. Moreover, since “healthy aging” could provide a solution to the problems associated with aging, various disciplines (e.g. social sciences, biomedical sciences and public health) are engaged in active research on this topic.

In conclusion, we rather favor the view of aging as that described already by another famous latin writer and orator, Cicero, who made a passionate defense of aging

• **Figure 2.** Prevalence of selected chronic conditions, expressed in percentages, as a function of age for the US population (2002-2003 dataset)



Source: Centre for Disease Control, Atlanta, USA.

in his work *De senectute*, 44 BC. Here he celebrated the figure of Cato the Censor, a forerunner of the concept of “healthy aging”, whose flourishing and very active late age was the best disproof of the charges against the elderly (the inactivity associated with physical weakness, the deprivation of the pleasures and the approach of death). Cicero cites numerous examples of shining figures of old people as Sophocles, who, as an old man, continued to write tragedies like *Oedipus at Colonus*, or Plato, Pythagoras, Isocrates and many others who have continued their activities even in old age.

Targeting stem cells to improve regeneration and homeostasis of aging tissues

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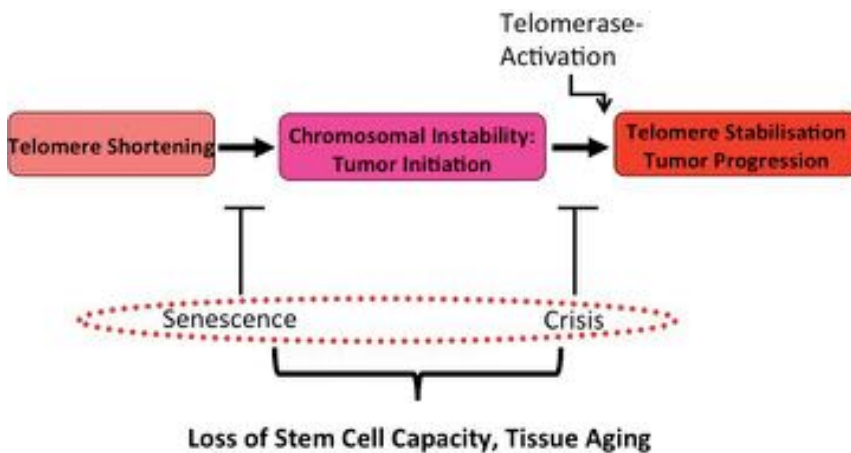
Aging leads to an increase of risk of developing tumors. Recent studies in cancer biology suggest that, in various malignancies, tumor-initiating cells are represented by adult stem cells. The stem cell compartment persists through our life and, by self-renewal, they are the most living cells of our organism. Stem cells accumulate point mutations and, as shown by whole exome sequencing in Hematopoietic Stem Cells (HSCs), an increased mutation rate with exponential acceleration occurs during aging. Of note, these mutations are the same observed in Leukemia suggesting that the accumulation of mutations in aging stem cells may occur early and prevents a ground stage for stem and progenitor derived cancers [1, 2].

Telomere shortening represents a molecular mechanism that, among others, can contribute to the accumulation of DNA-damage and mutations in aging cells. Telomeres are important structures needed to maintain chromosomal integrity. Telomeres are de novo synthesized by the enzyme telomerase that is silenced at birth in somatic tissues and reactivated in almost 90% of human tumours. The generation of telomerase knockout ($mTerc^{-/-}$) mice [3] allowed to study the consequences of telomere shortening on organismal aging. Due to long telomeres in laboratory mice, $mTerc^{-/-}$ mice of the first generation (G1 $mTerc^{-/-}$) do not exhibit strong phenotypes compared to $mTerc^{+/+}$ mice. However, when $mTerc^{-/-}$ mice were crossed to each other, severe telomere shortening and telomere dysfunction occurred in the third and fourth generation of the knockout (G3 and G4 $mTerc^{-/-}$). Interestingly, these mice exhibited a premature aging phenotype specifically affecting organ systems with higher rates of cell turnover like skin, intestinal epithelium and blood. In addition, G3 and G4 $mTerc^{-/-}$ mice showed an impaired regeneration and a decreased overall lifespan. Telomere shortening also led to an increase in chromosomal fusion and chromosomal instability. Of note, the transient induction of telomere dysfunction resulted in an

increase in cancer formation thus providing a molecular explanation for the increased cancer risk in aging human tissues affected by chronic diseases and telomere shortening [4] (● Figure 1).

In differentiated human cells (such as fibroblasts) it was shown that telomere shortening activates a p53/p21-dependent checkpoint limiting the replicative lifespan of the cells by induction of a permanent cell cycle arrest, which is known as replicative senescence. Telomere shortening occurs also in stem cells despite low level of telomerase activity [5]. Studies on late generation telomerase knockout mice were instrumental to characterize the induction of checkpoints in response to telomere shortening at the level of tissue stem cells. These studies revealed that p53 induces p21-dependent defects in self renewal and functionality of stem cells as well as Puma-dependent defects in the survival of stem cells in response to telomere shortening [6, 7]. Interestingly, the deletion of either Puma or p21 improved stem cell function, tissue maintenance and lifespan of telomere dysfunctional mice. In contrast, p53 deletion led to aberrant survival of genomically unstable stem cells and accelerated tissue aging [8]. Together, these data supported a new concept indicating that the selective inhibition

● Figure 1. The role of telomeres in cancer and aging



Telomere shortening limits the maintenance of functional stem cells during aging by induction of DNA damage checkpoints such as p53-dependent senescence and p53-independent crisis. These checkpoints limit the survival of stem cells with critically short telomeres thus preventing the induction of chromosomal instability and cancer initiation, which can lead to immortal tumour clones when telomerase or telomerase-independent mechanisms of telomere maintenance are activated. As a downside, the activation of DNA damage checkpoints in stem cells with critically short telomeres can limit the maintenance of functional stem cells and can thus aggravate the evolution of tissue dysfunction during aging.

of downstream targets of p53 (Puma or p21) may improve the maintenance of stem cells and tissue functionality in aging tissues.

Hematopoietic stem cells (HSCs) from telomere dysfunctional mice were also employed to conduct a stable RNAi *in vivo* screen to identify new checkpoints that limit the maintenance of stem cells in response to telomere shortening and DNA damage. These experiments revealed a BATF-dependent checkpoint that limits HSC self renewal by inducing differentiation of damaged HSCs in response to DNA damage. BATF (Basic leucine zipper transcription factor, ATF-like) is a protein that was known to regulate the differentiation of peripheral blood lymphocytes but it was not implicated to act at the level of HSCs. Interestingly, BATF specifically induces differentiation of lymphoid biased HSCs in response to DNA damage and therefore this differentiation inducing checkpoint could contribute to the loss of lymphoid biased HSCs and the reduction in immune-functions during aging [9]. Here, a new concept emerges about the evolution of imbalance in the HSCs pool during aging rather than simply a functional decline in the entire pool of HSCs.

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What is the relationship between aging and development? Insights from transcriptome analysis

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As a classical definition, aging is a progressive deterioration of physiological functions increasing mortality risk. But on the other hands, aging is also an adaptive process allowing the organism to remain functional despite age-dependent damage. In this view, aging is a prosecution of differentiation and maturation.

To really studying aging, a shorter lives animal model is needed as *Notobranchius furzeri*: it is a african annual fish characterized by a fast developing and sexual maturation in only 18 days and mostly by a very short lifespan, mediam 6 months and maximum one year [1].

Number of aging phenotypes are shared by most used animal models including *N. furzeri*. Analysis of transcriptome by RNA sequencing, performed from three different tissues (liver, brain and skin) at five time points, revealed a common signature of age among different tissues encompassing up-regulated and down-regulated pathways. Notably, these pathways are retained in aged prefrontal cortex of human brain with conserved regulation [2, 3]. So, *N. furzeri* can be considered, with good confidence, a good system to model transcriptional component of human brain aging.

Metanalysis on multi-tissues and multi-species revealed a relatively small number of common biological processes during aging. Focusing on conserved regulation between *N. furzeri* and human brain during aging, genes can be clustered according their kinetic profile, with fast or slow decay. Surprisingly, a set of genes present a bell- or U-shaped temporal expression attesting an inversion of regulation in oldest animals, with a specific time points of inversion at median lifespan.

The following question is whether different profile correspond to different biological functions. Of note, genes with fast or slow downregulation during aging are genes regulating cell cycle or DNA replication and axon-guidance or extracellular matrix receptors, respectively. Altogether, these genes correspond to age-dependent decline of

stem cells function and reduction in adult neurogenesis, with implication in synaptic plasticity and learning capacity. Surprisingly, cytoplasmic ribosomal proteins are novel age-markers with expression levels increasing during aging. Actual ongoing studies are focused on longitudinal analysis of gene expression, in particular on longest lived population compared to animals died before or at median lifespan.

In parallel, regulation of aging involves also microRNA signaling. In *N. furzeri*, the network of age-dependent microRNAs specifically targets either cMyc or p53 [4]. Among several miRs, particular attention was focused on both miR-29 and miR-101 sharing a conserved regulation in vertebrates: they accumulate in brain of *N. furzeri*, mouse and humans during aging. In agreement, levels of miR-29a, miR-29b and miR-101 direct target genes decrease during aging.

The open question is: when you block miR-29, can you stop aging? Experiments with inhibitors of miR-29a are actually ongoing. But, at the same time, the accumulation of miR-29a during aging may have a protective effect as happening in several pathological condition. Indeed, miR-29a is capable to counteract effects of forebrain ischemia in hippocampus area [5].

In conclusion, the age-dependent increase of miR-29a in the brain seems to be a compensatory mechanism to contrast the age-dependent neurodegeneration.

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Stem cell renewal: a link between thyroid hormone and aging?

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A number of observations link thyroid hormones with longevity. Not only does thyroid hormone control metabolic rate, but also metabolic rate regulates longevity and there is a negative correlation of thyroid hormone levels with longevity in both humans and mice [1].

For instance, the Leiden Longevity Study demonstrated that, in both men and women with a familial predisposition for longevity had slightly but significantly lower tri-iodothyronine (T3) or higher thyroid-stimulating hormone (TSH) levels than controls [2, 3]. One of the key hypothesis that we are currently investigating is that the effects of thyroid hormone action on metabolism and aging could be played out at the level of adult stem cells, thereby affect tissue regeneration. Interestingly, at the cellular level thyroid hormone can be seen as activating an “epigenetic switch”. This certainly occurs during metamorphosis and could well occur in stem cell populations as two daughter cells differentiate into completely different phenotypes.

Our focus is on thyroid hormone action on the neural stem cell (NSCs) niche. In adult humans and mice the two principal adult neurogenic niches are found in the hippocampus, a brain area critical for memory formation, and the subventricular zone (SVZ), from which new neurons migrate to the olfactory bulb, maintaining olfaction. Several studies link thyroid status to problems with olfaction and with memory both in animal models and humans. So, what about of the effects of thyroid hormone on these stem cell populations? Stem cells undergo self-renewal by asymmetric division and, at same time, produce transit-amplifying progenitors cells that can differentiate into neuroblasts and potentially neurons. Early *in vivo* experiments showed first, that hypothyroid mice displayed reduced proliferation in adult stem cell niches, as reflected by decreased BrdU incorporation and second, that both T3 and thyroid hormone receptor-alpha1 (TR α 1) are required for full proliferative ca-

capacity of NSCs [4]. Interestingly, one of the well-known pluripotency genes, the sex determining region Y box-2 (*Sox-2*) gene is a direct target of thyroid hormone in the NSC niche. TR α 1 loss of function induces upregulation of SOX-2 and a series of neural stem cell markers, including cyclin D1 and nestin, without affecting markers of neuronal differentiation. These findings suggest that neural stem cells are retained in “a stem cell phenotype” in the absence of T3/TR α 1 stimulation.

We also know that the T3-dependent repression of *Sox-2* is exerted at transcriptional level [5]. The regulatory region of *Sox-2* gene is characterized by the presence of several Thyroid hormone Response Elements (TREs) and Chromatin Immuno-Precipitations (ChIPs) revealed a specific recruitment of TR α 1 in the presence of T3 on a specific TRE site. A take home message is that, the *Sox-2* gene is a direct negative T3 target. Thus, in a physiological context, T3 acts as a commitment factor in the neural stem cell population by directly regulating *Sox-2* pluripotency factor and its downstream regulatory network. In line with this finding, TR α 1 *in vivo* overexpression commits progenitors to a migratory neuroblast phenotype.

These observations lead to a number of questions. Given that thyroid hormone and its receptor TR α 1 are proliferation and commitment signals in the neural stem cell niche, we can hypothesize that excess thyroid hormone in the niche will rapidly exhaust the stem cell population, driving them to differentiate. So the first question that arises is how is thyroid hormone availability controlled within the stem cell niche? A second question is whether these observations on the differentiating role of thyroid signalling in the NSC also extends to stem cell niches in other adult tissues, such as skin, muscle or bone.

As regards the first question, we know that two main factors govern local supply of thyroid hormone in target cells, activation and inactivating deiodinases and membrane transporters. We are currently examining the distribution of the deiodinase in the NSC, focussing on the inactivating deiodinase, D3. As the niche contains the precursors of the two main type of cells in the brain, neurons and glial cells we are examining how D3 expression correlated with cell markers for neuronal precursors and glial (more specifically, oligodendrocyte) precursors, respectively, T doublecortin (DCX) and epidermal growth factor receptor (EGFR). Currently, we have established that T3 and TR α 1 are needed for the commitment to neuroblast phenotype and neuronal differentiation and the analysis of how T3 affects the oligodendrocyte lineage is ongoing.

Neurodegenerative disease in aging can affect neurons, as in Alzheimer and Parkinson, or oligodendrocytes as in multiple sclerosis. Our current hypothesis is that therapeutic modulation of thyroid hormone signalling should be feasible, thereby redirecting adult neurogenesis to one or another of the main neural populations. Furthermore, controlling thyroid hormone availability in stem cell niches could be harnessed to improve tissue regeneration during aging.

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Aging, mitochondria and epigenetics

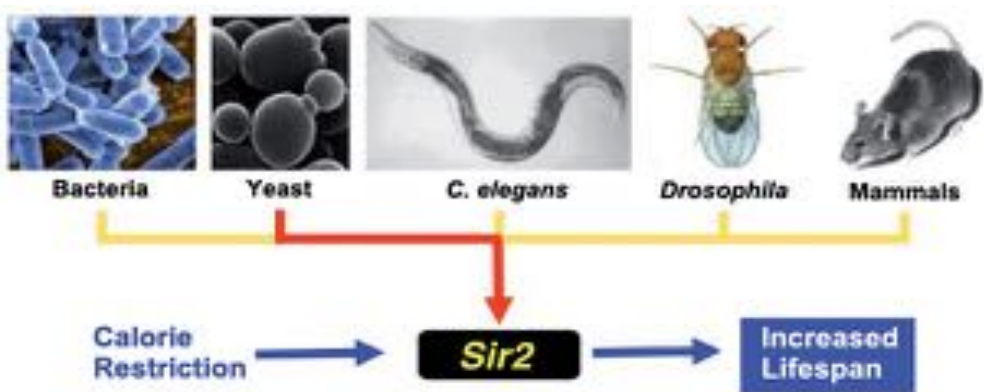
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Several modifications of the environment, mainly the diet, may impact lifespan and prevent the development of some chronic diseases associated with aging. Unique metabolites in specific metabolic networks are capable to modulate the activity of master regulators such as sirtuins and histone-deacetylase (HDACs).

Sirtuins are a family of a conserved proteins found in all forms of life. They exhibit NAD-dependent protein deacetylase activity. In yeast, Sir2 has been shown to increase lifespan and to be necessary for the increase of lifespan induced by caloric restriction. So, sirtuins are metabolic sensors of the environment and nutrition and influence cellular function by protein deacetylation (• Figure 1).

• **Figure 1.** Sirtuins are conserved from bacteria to mammals and are critical players in the response to calorie restriction

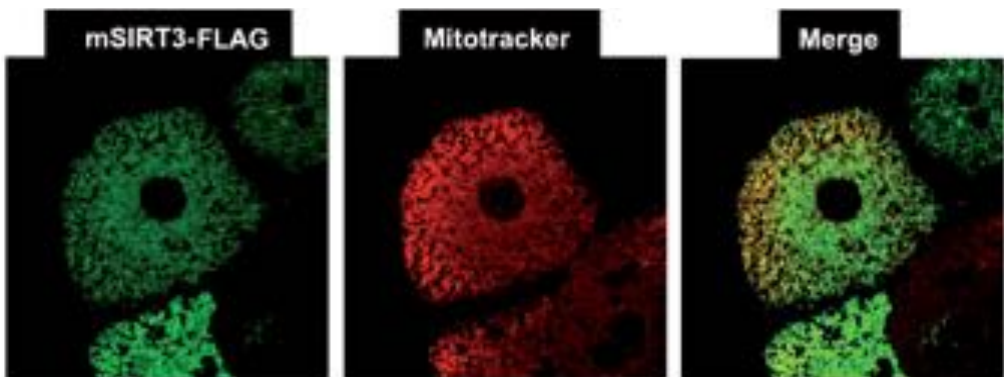


Seven sirtuins, SIRT1 to SIRT7, have been identified in mammals and are characterized by a conserved catalytic domain but variable N- and C-terminal extensions that contribute to different biological activities. Notably, SIRT3, SIRT4 and SIRT5 are localized in mitochondria in both human and mouse (• **Figure 2**).

SIRT3 exerts its deacetylase activity in mitochondria where it is the major regulator of mitochondrial protein deacetylation. Indeed, SIRT3-deficient animals exhibit striking mitochondrial protein hyperacetylation and no mitochondrial hyperacetylation is detectable in mice lacking SIRT4 or SIRT5 [1]. Interestingly, SIRT3 expression increases in liver and other tissues during fasting leading to the deacetylation of unique mitochondrial target. To characterize the SIRT3-dependent acetylome, a novel label-free quantitative mass spectrometry approach, able to quantify post-translational modifications, was developed [2]. Analysis of lysine acetylation from mouse liver mitochondria in presence or absence of SIRT3 revealed that 16% of total lysines are acetylated and 12% of these are regulated by SIRT3. SIRT3 targets include proteins across several metabolic pathways such as fatty acid oxidation, ketogenesis, amino acid catabolism and the urea cycle [3].

Particular attention was focused on ketone body synthesis. During fasting, fatty acids are oxidized in mitochondria into acetylcoenzyme A which serves as a precursor to the synthesis of acetoacetate and β -hydroxybutyrate (β OHB), the two ketone bodies. Both are released into the bloodstream and circulated to extra-hepatic organs as diffuse form of energy. SIRT3 regulates ketone body production via deacetylation of the rate limiting enzyme in ketone body synthesis, hydroxymethylglutarylcoenzyme A synthase 2 (HMGCS2), and mice lacking SIRT3 show a decrease of β OHB and cold intolerance during fasting condition [4]. Another finding, that places SIRT3 as key regulator of caloric restriction response, is that prevention of age-related hearing

• **Figure 2.** SIRT3 is a mitochondrial sirtuin. The pattern of expression of an exogenous SIRT3 protein (FLAG-tagged) is similar to the mitochondrial marker mitotracker



loss under caloric restriction requires SIRT3 activity on mitochondrial glutathione antioxidant defense system [5].

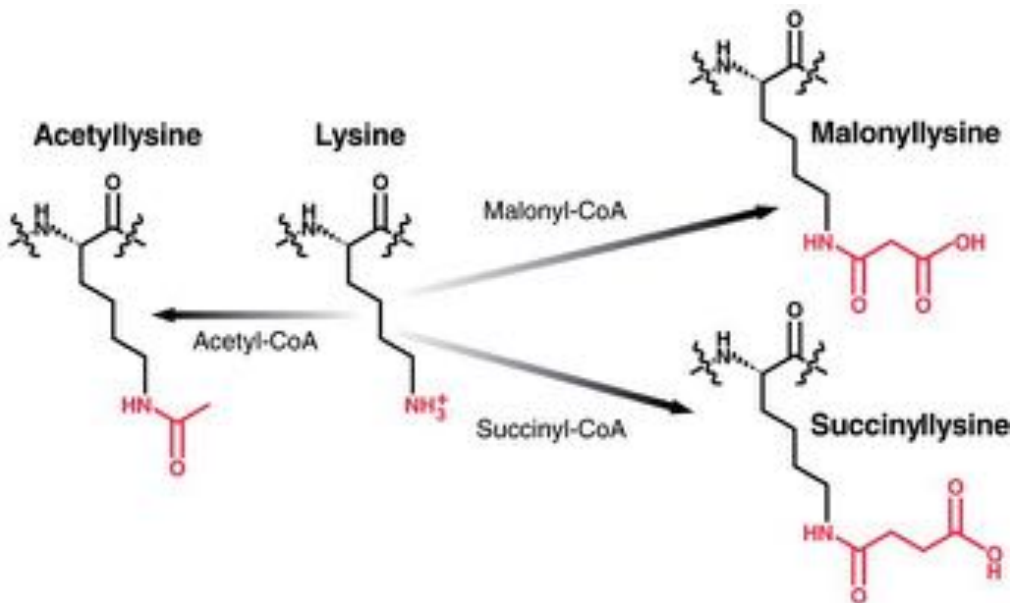
Recently, it was shown that lysine residues within proteins can be reversible modified not only by acetylation but also by malonylation and succinylation (• Figure 3).

Interestingly, the other mitochondrial sirtuin SIRT5 has both demalonylase and desuccinylase activity *in vitro*. Lysine-succinylated proteins were detected in mitochondria of several mouse tissues (liver, skeletal muscle) and protein hypersuccinylation was observed in mice lacking SIRT5. Characterization of SIRT5-targeted mitochondrial succinylproteome through a label-free quantitative proteomic approach revealed that 140 out of 252 identified succinylated proteins are SIRT5 targets. Several metabolic pathways are regulated by SIRT5 including fatty acid β -oxidation and ketone body synthesis [6].

Remarkably, SIRT3 and SIRT5 target the same lysine residues modified either by acetylation or succinylation and both SIRT3- and SIRT5-deficient mice exhibit defective ketone body production. Focusing on the rate-limiting ketogenic enzyme HMGCS2 and its modifications in both SIRT3- or SIRT5- deficient mice, i.e. acetylation and succinylation, respectively, a strong overlap is observed in key lysines.

Lastly, class I and class III deacetylases, HDACs and sirtuins, are interestingly linked in controlling lifespan: i) treatment of mice with β OHB, an endogenous in-

• **Figure 3.** Two novel lysine acyl modifications, succinylation and malonylation, target lysine residues in proteins

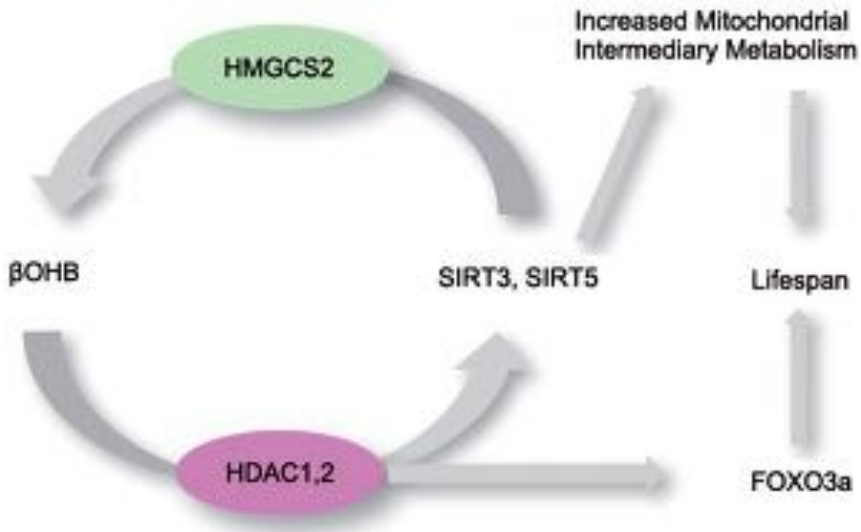


hibitor of class I HDACs, leads to a strong increase in FOXO3A gene expression. Importantly, FOXO3a has been associated with increased longevity in humans [7]; ii) in drosophila, calorie-restriction operates through a genetic pathway that includes Rpd3 and Sir2 (orthologous of class I HDACs and sirtuin, respectively). Rpd3 is an inhibitor of Sir2 and inhibition of Rpd3 by phenylbutyrate significantly increase lifespan [8, 9].

These observations suggest that a diet leading to increased β OHB production will increase lifespan in mice and possibly in humans. Interestingly, comparison between the ketogenic diet and caloric restriction shows several common parameters: increase in β OHB, decrease in insulin and mTOR activity.

Preliminary analysis of gene expression in mice on the ketogenic diet shows change in gene expression, one of the most upregulated gene is the 3-hydroxybutyrate dehydrogenase (Bdh), a key enzyme in ketogenesis. Interestingly, a strong induction of mitochondrial sirtuin expression SIRT5 was observed together with FOXO3a, SIRT4 and SIRT3. In conclusion, it is possible to delineate a network in which sirtuins and ketone bodies intersect with a possible role in lifespan: both SIRT3 and SIRT5 modulate mitochondria metabolism including ketone bodies production. Increased ketone body production, including β OHB, inhibit the activity of class I HDACs leading to an increase in FOX3A and sirtuin expression. Experiments are underway in mice to directly test this hypothesis (• Figure 4).

• **Figure 4.** Model for the reciprocal regulation of ketone bodies, FOXO3a and sirtuin regulation and aging (see text for details)



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The what and why of aging and arterial disease

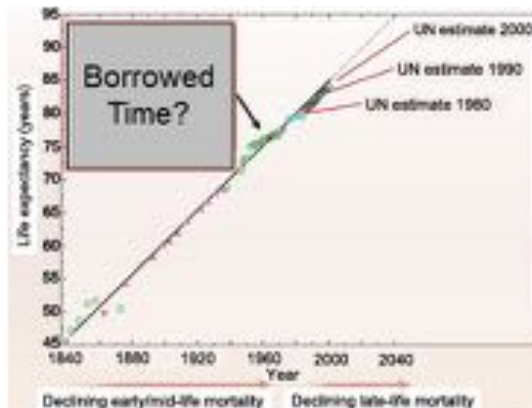
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The demographic imperative and age-associated risk for cardiovascular disease

Life expectancy around the world has increased steadily for 200 years. Thus, the world population in both industrialized and developing countries is aging (• **Figure 1**). The clinical and economic implications of this demographic shift are staggering [1] and lead to the idea that adding years to life by reducing late life mortality (• **Figure 1**) creates a scenario of living on “borrowed time”, when chronic age-associated arterial diseases become rampant.

- **Figure 1.** Life expectancy around the world has increased steadily for 200 years



Source: Kirkwood, 2008 [1], adapted.

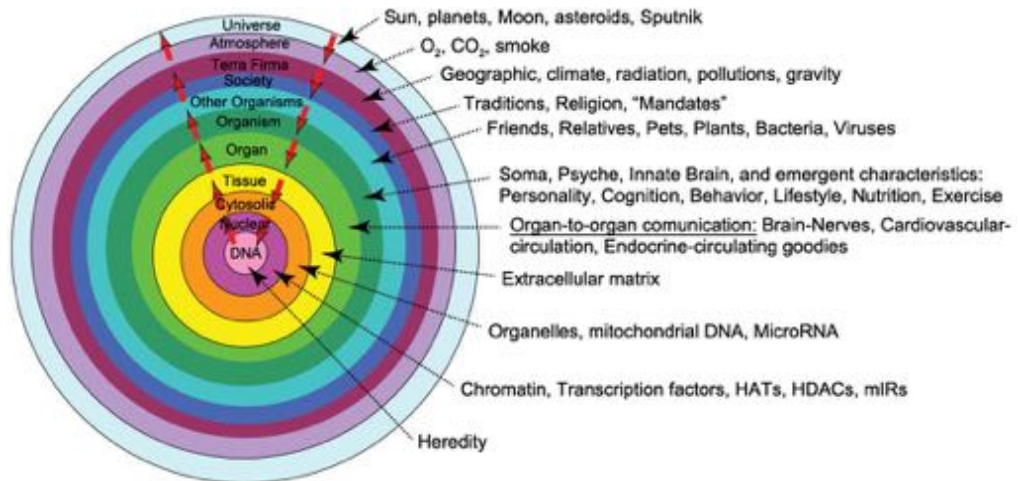
The reality of aging

No discussion about arterial disease can beg the question of: what is aging? This is a tough question, about which there have been, and continue to be numerous different perspectives. Even most scientists who participate in aging research have never stopped to think about asking, “What is aging?” because there is no definitive answer. To begin to understand aging, we need to address numerous facets of life that change over time and thus to appreciate how organisms, not just cells, tissues or organs, change over time. My view is that “Aging is a shift in an organism’s reality” [2].

So what’s reality? This is another tough question. My view is that reality can be comprehensively defined as a system of “mutual enslavement” of DNA and its environment [2]. If this appears to be a naïve assessment of reality, check out what constitutes the DNA environment (• **Figure 2**).

The intracellular DNA environment has nuclear, organelle and cytosolic components. Tissues constitute environments for cells, and comprise organs that define the organisms, with somas, psyche’s innate brain function, and from these interactions, cognition and personality emerge (• **Figure 2**). Organisms differ in the development of their cognitive and stress coping mechanisms, in part, due to differences in personality characteristics, which give rise to the development of distinct behavior lifestyles, e.g. what and how much food we consume, how much we exercise, etc. And there are other organisms in our reality: friends, relations, plants, bugs, etc. As organisms, we are all immersed societies, which issue mandates, traditions, and religion, etc. And

• **Figure 2.** Reality is a mutually enslaved “system” of DNA and its environment



Source: Lakatta, 2013 [2].

then beyond that, we are surrounded by geographical realities of climate, radiation, pollution, etc., and of course, gravity (● **Figure 2**). So, the integrated constellation of different environments that surround our DNA and its function, in my opinion, constitutes our reality. “Epigenetics”, therefore, embrace the entire concentric series of environments depicted in ● **Figure 2**, not just chromatin, HATs, HDACs and microRNAs, as narrowly preached by most scientific cognoscenti. The arrows in the diagram in ● **Figure 2** indicate continual bidirectional signaling that must occur to sustain our existence. This continual signaling back and forth across each of these environments, confers “mutual enslavement” of the components within the coupled DNA-environment system. Different signals across these environments are transmitted with different kinetics and vary in acute or chronic impact on the coupled system.

Aging is a manifestation of time-dependent failures of signaling within the DNA-environment system

*“Inside every old person is a young person
wondering what happened”
(Anonymous)*

Aging can be construed as a series of failures in the signaling within the DNA environmental system that occur over time. Because signaling within the system must operate in a rigorously ordered manner for an organism to function properly, failure of the system can be perceived to indicate a generalized disorder among molecular and cellular mechanisms and their interactions. Thus, the aging phenotype is a manifestation of time-dependent failing interactions that emerge within system of DNA and its environment.

Impairment of nearly all aspects of the DNA-Environment system occurs with advancing age and characterize the “reality of aging”. As we age, signals change, as does sensing of the signals, transmission of signals, responses to signals (● **Figure 3**). Aging is characterized by changes in the proteome due to alterations in genomic transcription, mRNA translation, and the local protein environments (proteostasis). The density of some molecules becomes reduced and post-translational modifications, e.g. oxidation, nitration phosphorylation, etc., lead to disordered molecular interactions that alter the stoichiometry and kinetics of reactions that underlie optimal cell functions and robust reserve mechanisms. The system loses its robustness and flexibility. Physical and psychic energies dwindle as reality shifts with aging in the context of changes in the ticking speed of multiple clocks. Our entire bodies and minds become different. We’re not exactly the same organism that we were 10 years ago, or 10 years before that. As a result of changes in the environments in ● **Figure 2** and in their interfaces (● **Figure 3**), aged organisms appear different to younger members of society and vice versa.

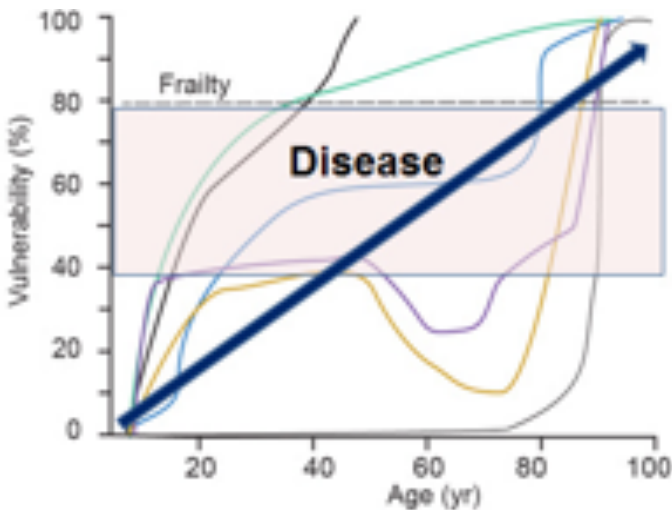
So, is aging, per se, a disease?

This question has been debated from the era of famous Greek philosophers. One opinion, and an opinion that has underpinned modern gerontology, the study of aging, is that “aging and disease are not synonymous. There are processes of aging and etiologies of disease. The relationship between the two are important, but not inevitable” (Nathan Shock, *Annual Review of Physiology*, 1961 [3]). This is not a unique opinion that has survived. Others would argue that “... to draw a distinction between disease and normal aging is to attempt to separate the undefined from the undefinable” (J. G. Evans, *Research and The Aging Population*, 1988 [4]).

Cumulative loss of our reserve over time functions leads to increasing organismal vulnerability (• **Figure 4**).

Increasing molecular and cellular disorder as we age leads to loss of tissue organ and system reserve functions. The cumulative loss of our reserve functions over time leads to age-associated increasing vulnerability to diseases (• **Figure 4**) from which we were protected at earlier ages. The rate of increased vulnerability of our various functions (thin lines in • **Figure 4**) is variable, and not always monotonic but sometimes biphasic or oscillatory due to compensatory mechanisms that occur among functions. The eigen vector for vulnerability, however (thick arrows in • **Figure 4**), progressively increases with advancing adult age and underlies phenomena presently referred to as diseases and frailty.

• **Figure 4.** The reality of aging: chronic inflammation changes the cardiovascular structure/function landscape and leads to the markedly increased risk for cardiovascular diseases in older persons



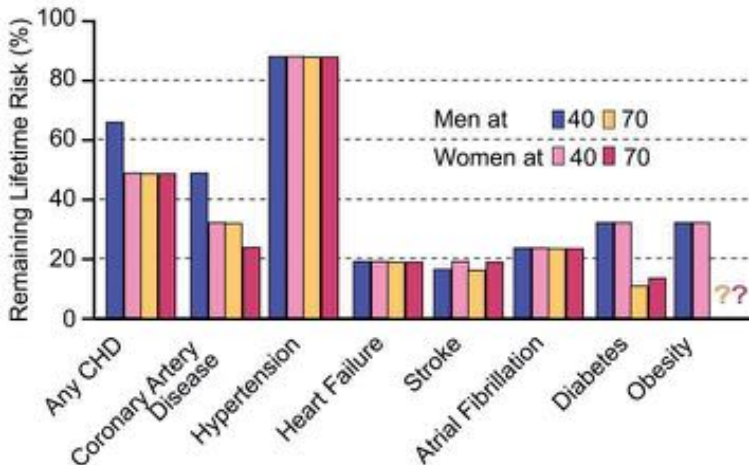
Age-associated vulnerability to cardiovascular diseases

Aging hearts and arteries operate “on the edge of disease”. The epidemic of cardiovascular diseases has taken on a global dimension and is no longer restricted to Western societies. Cardiovascular diseases now represent more than 30% of all deaths worldwide, and by the year 2020, they are expected to surpass infectious diseases as the leading cause of mortality and disability. According to the *World Health Report*, 1 cardiovascular diseases were responsible for 15 million annual deaths worldwide, of which 9 million were in developing countries and 2 million in economies in transition [5].

Both the incidence and prevalence of hypertension, coronary artery disease, congestive heart failure, and stroke increase exponentially with age. The remaining lifetime risk for CVD and other diseases among men and women free of disease at 40 or 70 years of age is staggering (● Figure 5): The odds of having a chronic CV disease are 50%, for hypertension 85%, and for chronic heart failure 20% [6].

One way to conceptualize why the clinical manifestations and the prognosis of CV diseases worsen with age is that in older individuals, the specific pathophysiologic mechanisms linked to clinical disorders become superimposed on heart and vascular substrates that are modified by aging in the context of molecular and cellular disorder that accompanies “borrowed time” (● Figure 1). First, they lower the extent of disease severity required to cross the threshold that results in clinically significant

● Figure 5. Remaining lifetime risk for CVD and other diseases among men and women free of diseases at 40 and 70 years of age



Source: Lloyd-Jones et al., 2010 [6], adapted.

signs and symptoms. Age-associated changes may also alter the manifestations and presentation of common cardiovascular diseases can also influence the response to and therefore the selection of different therapeutic interventions in older individuals with cardiovascular disease. Thus, age-associated changes in the cardiovascular system might be construed as specific risk factors for the diseases that they relate to and thus might be targets of interventions designed to decrease the occurrence or manifestations of cardiovascular disease at later ages.

The reality of aging viewed from the arterial wall

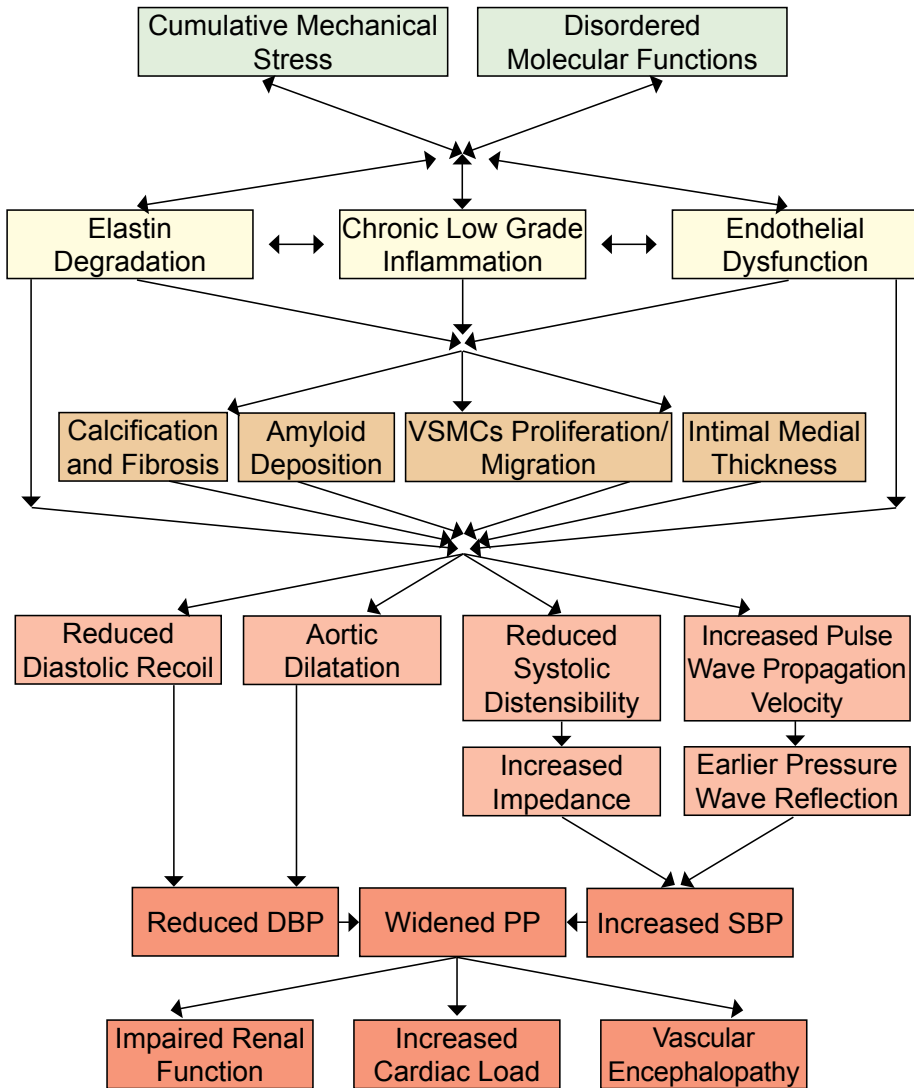
A view of the reality of aging from the arterial wall begins with the realization that arterial diseases, e.g. atherosclerosis and hypertension, are rampant in Western society, and increase exponentially with advancing age (• **Figure 5**). Because the risk for predominantly systolic hypertension and atherosclerosis increases in epidemic proportion among older persons (• **Figure 5**), it behooves us to examine specific mechanisms that underlie phenotypic alterations in the arterial substrate that accompany “aging” as may be intimately linked to the exponential increase in the likelihood for predominantly systolic hypertension and atherosclerosis to become manifest in older persons. Progressive changes in the structure and function of central arteries occur throughout life and include diffuse intimal and medial thickening, and increased stiffening and reduced distensibility of central arteries (• **Figure 6**).



Arterial aging consists of a myriad of progressive structural and functional changes that occur throughout life ranging from changes in molecules to cells to arterial tissue, the blood it transports, and the hormonal and neural factors that modulate molecules, cells, tissues, etc. that comprise our cardiovascular system. While our textbooks usually describe the characteristics of central arterial changes that accompany advancing age as “physiologic” arterial aging, these changes are far from being “physiologic” and are more aptly construed as pathophysiologic.

Characteristically, there is fragmentation and calcification of elastic fibers, increased deposition of collagen and collagen cross linking, amyloid deposition in the medial layer, and migration/proliferation of vascular smooth muscle cells (VSMC) leading to intimal and thickness. These events act in concert to reduce central arterial distensibility and render the arterial wall stiffer, which results in a more rapid pulse wave velocity and early return of the reflection wave to occur during systolic ejection (• **Figure 6**). As a result, the systolic blood pressure increases, diastolic pressure decreases and the pulse pressure increases with aging. The chronic increase in pulse pressure transmitted to the brain and kidney damage the arterial supply of those organs, leading to vascular encephalopathy and chronic renal failure (• **Figure 6**).

• **Figure 6.** Conceptual model of arterial aging



Age-associated molecular disorders and cumulative mechanical stress lead to a state of chronic inflammation, elastin degradation and endothelial and VSMC dysfunction. These products interact and lead to arterial wall calcification, fibrosis, amyloid deposition, VSMCs proliferation, and increased intimal medial thickness. These structural changes lead to functional alterations resulting in widened pulse pressure. The increase in pulsatility leads to increase left ventricular load, chronic kidney disease, and vascular dementia.

Source: AlGhatrif, Lakatta, 2014 [7].

A chronic arterial proinflammatory profile characterizes the aging arterial wall

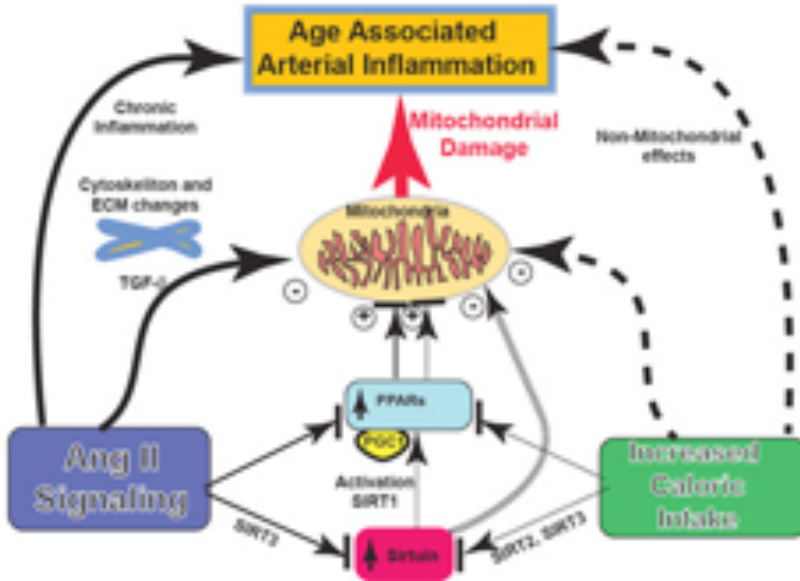
In order to determine whether or not to consider arterial aging a disease, we must understand the mechanisms that lead to age-associated changes in the arterial wall. There is a substantial gap between our knowledge about what's going on in the arterial wall under the microscope with respect to structure or function of the large arteries and what can be measured *in vivo*. Processes that lead to cellular and matrix structural and functional changes are driven by a proinflammatory microenvironment, mediated by mechanical and humoral factors (• **Figure 6**). These processes are driven by oxidative stress and low-grade inflammation.

Our body's initial response to stress is moderated by increased adrenergic signaling; the downstream receptor signaling cascade results in increased activation of renin-angiotensin-aldosterone, and endothelial dysfunction (• **Figure 7**), mechanisms that our body utilizes to respond to chronic stress. The proinflammatory profile of the central arterial wall features increased production of angiotensin II (Ang II) and increased vascular smooth muscle cell expression and secretion of downstream Ang II/ AT_1 , mineralocorticoid endothelin receptor signaling molecules (• **Figure 7**), e.g., matrix metalloproteinases (MMPs), calpain-I and monocyte chemoattractant protein (MCP-1), transforming growth factor β 1 (TGF- β 1), and NF κ b, TNF α , iNOS, and VCAM. Activation of calpain-I, MMPs, TGF- β , and NADPH oxidase within the arterial wall is increased, and nitric oxide bioavailability is reduced [8-12]. Invasive, proliferative and secretory capacities of early passage vascular smooth muscle cells (VSMC) isolated from the arterial wall are increased, and are linked to augmented Ang II signaling.

The aortic wall remodeling induced by aging, however, likely results from the concerted effects of numerous signaling proteins that have yet to be identified. The expression of one such recently discovered arterial wall protein, milk fat globule protein epidermal growth factor 8 (MFG-E8), increases 2.3-fold in abundance in aortae of humans, non-human primates and rats (• **Figure 7**). Milk fat globule E-8 (MFG-E8), aka lactadherin or SED I, colocalizes with both angiotensin II and monocyte chemoattractant protein (MCP)-1 within vascular smooth muscle cells (VSMCs) and matrix of the thickened aged aortic wall (• **Figure 7**).

Exposure of early passage VSMCs from young aorta to angiotensin II markedly increases MFG-E8 and enhances invasive capacity to levels observed in VSMCs from old rats. MFG-E8 not only induces VSMC invasion, but also affects VSMC proliferation, which is a salient feature of arterial inflammation (• **Figures 6, 7**). An MFG-E8 degradation product, medin, is an amyloid protein that accumulates within the aging arterial matrix wall. Thus, the increase in MFG-E8 is a novel pivotal relay element within the angiotensin II MCP-1/ERK, CDK4 VSMC signaling cascades.

• **Figure 8.** Age associated arterial inflammation

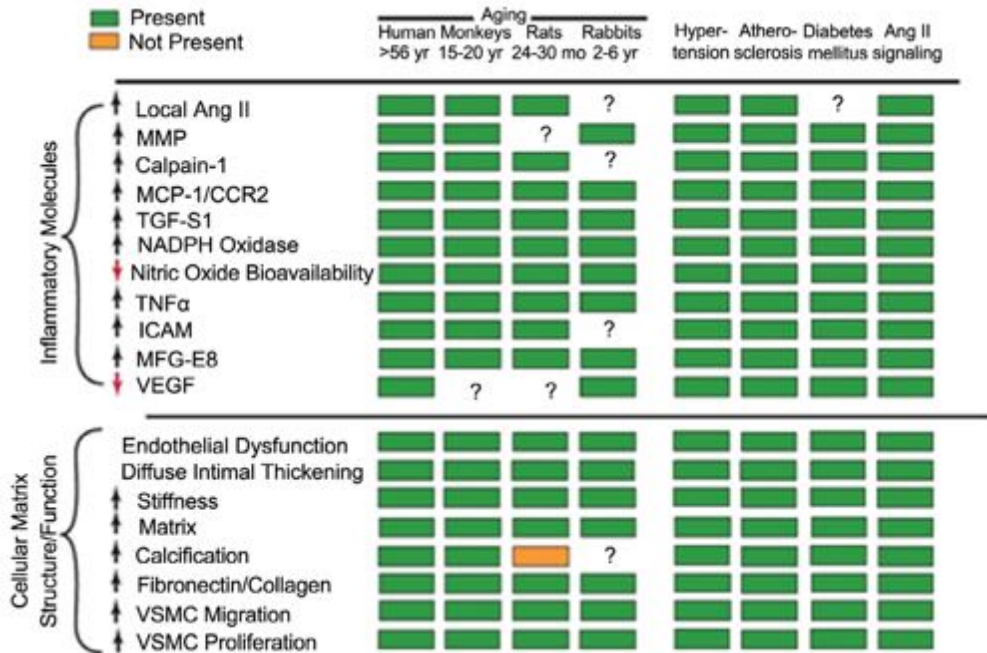


Source: de Cavanagh et al., 2011 [16], adapted.

of these signaling cascades results in additional oxidative stress and an “overshoot” in the chronic inflammatory response contribute to the progression of age-associated structural and functional arterial remodeling. The overshoot might be expected to occur because the molecular disorder that elicits the response cannot be resolved, resulting in chronic increase in chronic inflammatory signaling. A similar and well-known overshoot scenario occurs in the context of catecholamine signaling in chronic heart failure.

Arterial wall aging is quite similar in humans, non-human primates, rabbits and rats (• **Figure 9**) and involves inflammatory processes associated with oxidative stress. The inflammatory patterns are the same for most species that have been studied (• **Figure 9**). “Aging”-associated arterial changes and those associated with hypertension (and early atherosclerosis and diabetes) are fundamentally intertwined at the cellular and molecular levels (• **Figure 9**). Arteries of younger animals, in response to experimental induction of hypertension or early atherosclerosis or diabetes, parts of this proinflammatory profile within the arterial wall that have been studied to date are strikingly similar to the Ang II-mediated profile that occurs with advancing age (• **Figure 9**).

● **Figure 9.** Molecular and cellular remodeling observed with aging in different species, and various vascular diseases



A universal upregulation of Angiotensin (Ang II) pathway, increase in inflammatory molecules, reduction in NO bioavailability, and arterial wall ultrastructure are observed with aging in various species. Interestingly, similar alterations are observed in vascular diseases, e.g., hypertension, atherosclerosis, and diabetes.

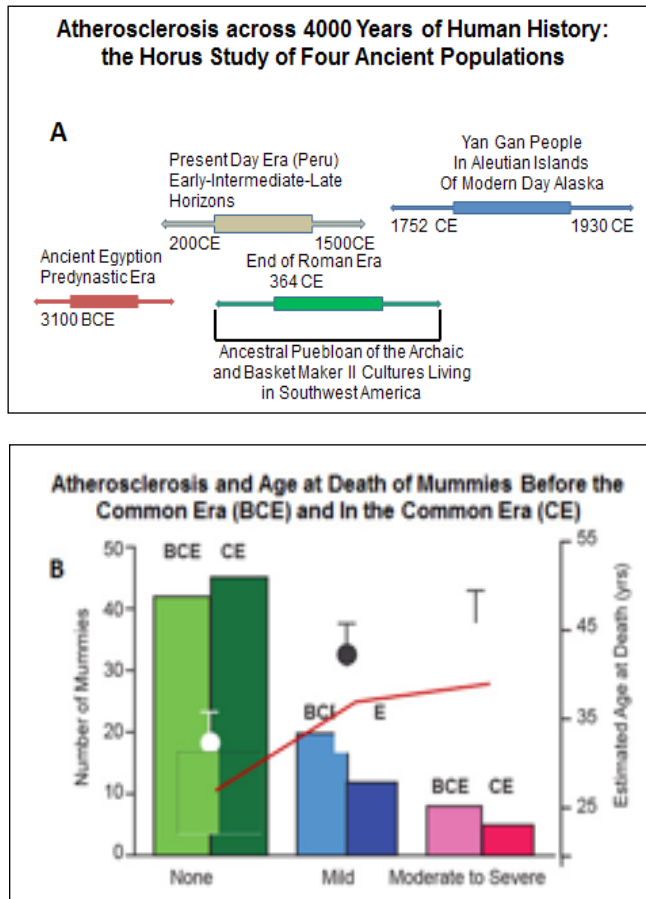
Source: Wang *et al.*, 2014 [17].

Are most arterial diseases, in essence, clinically relevant manifestations of advanced effects of aging on the arterial wall? Is atherosclerosis a manifestation of aging?

In humans, other well-known risk factors (e.g., excess food intake, altered dietary lipid and metabolism, smoking, and lack of exercise) likely interact with this arterial substrate that has been altered during aging, and that renders the aging artery a “fertile soil” that facilitates the initiation and progression of these arterial diseases.

New evidence (● **Figure 10**) that aging and atherosclerosis are intertwined is gleaned from a recent study that imaged arteries of mummies. This study imaged the arteries of mummies from over 4000 years of human history ranging from before the Common Era (BCE) to the Common Era (CE) (● **Figure 10A**) to detect the pres-

• **Figure 10.** Aging and atherosclerosis



Source: Thompson et al., 2013 [18], adapted.

ence and severity of atherosclerosis defined as calcified plaques within the arterial wall. The major finding was that although diet and lifestyle differed widely among the populations studied (• **Figure 10A**) the prevalence and severity of atherosclerosis in BCE was nearly identical to that of CE (• **Figure 10B**). However, the prevalence of atherosclerosis varied by age, regardless of the era (• **Figure 10B**). The conclusion of this study, “Atherosclerosis across 4000 Years of Human History: The Horus Study of Four Ancient Populations”, was that “the presence of atherosclerosis in modern human beings suggests that it is an inherent component of human aging and NOT characteristic of any specific diet or lifestyle”.

Clinical implications of cardiovascular aging

Because many of the age-associated alterations in cardiovascular structure and function, at both the cellular and molecular levels (• **Figures 6-9**), are implicated in the pathophysiology of arterial diseases (• **Figure 9**), there is an urgency to incorporate cardiovascular aging into clinical medicine. But in spite of the interest in the physiology of the age-associated changes in cardiovascular structure and function, cardiovascular aging has remained, for the most part, outside of mainstream clinical medicine!

The pathophysiologic implications of these age-associated changes in the arterial wall are largely underappreciated and are not well disseminated in the medical community because many of these manifestations cannot be detected in the blood, as is the case for “traditional” arterial disease risk factors. Thus, although age is the dominant risk factor for cardiovascular diseases (• **Figure 5**), most of the research efforts on prevention of these diseases have ignored focusing on the effects of aging of the arterial wall, and have instead focused on development of interventions that target “traditional” cardiovascular risk factors (such as hypertension and hyperlipidemia). An emerging school of thought, however, proposes that the effects of cardiovascular aging need to be recognized as accelerated or dysregulated age-associated alterations in the cardiovascular system, at the molecular, enzymatic, biochemical, cellular, histologic, and organismal levels, that constitute the risky components of aging for the subsequent emergence of clinical signs and symptoms of cardiovascular disease.

Conclusion

Aging is the dominant risk factor for cardiovascular diseases. As life expectancy increases, a systems-biology approach is needed to ensure that we have a healthy old age.

We must realize that, in reality, age, disease, lifestyle, genetics and environmental factors are intertwined and interventions vary over time (• **Figure 11**). To understand aging, we must discover failures that occur in cardiovascular tissue and define their underlying mechanisms, and integrate and translate these discoveries. But, instead of integrating discoveries, we (mortals) usually fragment knowledge: organisms into systems (e.g. CNS CVS, etc.), losing the site of the organism; systems into cells – losing site of organs, systems, and organisms; cells into “departments” (e.g. biochemistry, physiology, etc.) – losing site of cells, organs, systems, organisms.

Nevertheless, a steady stream of incremental knowledge, derived from both animal and human studies, has established that several of the aging-associated changes within the walls of the central arteries are themselves potent and independent risk factors for cardiovascular diseases. Changes in the perspectives of the reality in the clinical prac-

tice of medicine are long overdue. Our present understanding of the age-associated alterations in cardiac and arterial structure and function at both the system, cellular and molecular levels provides valuable clues that may assist in the development of effective therapies to prevent, to delay, or to attenuate the cardiovascular changes that accompany advancing age. This suggests that these age-associated alterations in arterial structure and function could represent the link that explains, at least in part, the risky component of aging.

Policymakers, researchers, and clinicians should intensify their efforts toward identification of novel pathways that could be targeted for interventions aiming at retardation or attenuation of changes in age-associated interactions depicted in • **Figure 11**, particularly in individuals in whom these alterations are accelerated. Future studies would then examine whether these strategies (i.e., those targeting cardiovascular aging) can have a salutary impact on the adverse cardiovascular effects of accelerated cardiovascular aging, and attenuate the impact of age as the dominant risk factor for cardiovascular diseases. Importantly, this should help alter our view of the effects of aging from immutable risk factors to those amenable to modification and retardation. Cardiovascular aging is a promising frontier in preventive cardiology that is ripe for and in dire need of attention!

Acknowledgment. The author would like to thank Ruth Sadler for editorial assistance and Harold Spurgeon for work on figures.

• **Figure 11.** This is reality!



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Role of noncoding RNAs in cardiovascular aging

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Aging increases risk of developing cardiovascular diseases and heart failure. Recently, loss of myocardial cells is emerged a major pathogenic factor mainly due to apoptosis of cardiomyocytes: died cardiomyocytes are replaced by fibroblasts and survived cardiomyocytes become hypertrophic, both mechanism lead to a decrease of heart function. To address the role of noncoding RNA in these processes, microRNA profiling was performed from total heart of young and old mice (6 months compared to 18 months animals). As main finding, miR-34a, known to be involved in apoptosis [1], is increased in aged hearts [2]. Interestingly, antagomirs specific to miR-34a efficiently knock down miR-34a *in vivo* and inhibit age-associated apoptosis in aged-hearts. Moreover, in Ku80^{-/-} progeria mouse model, that has an accelerating aging with decreasing heart function, cardiac dysfunctions are reduced after treatment with antisense oligonucleotide for miR-34a. Again, in natural/normal aging a recovery of cardiac contractile function is observed in miR-34a knockout mice compared to wild type mice.

Among known targets of miR-34a, SIRT1 was increased after inhibition of miR-34a but not in SIRT1 heterozygous mice and yet miR-34a inhibition still had therapeutic effects in SIRT1 heterozygous mice. So, to identify novel target of miR-34a in aged heart, an *in silico* prediction was performed using three public available software and only one target downregulated during aging emerged from this analysis. Here, PNUTS protein is directly regulated by miR-34a and specifically interacts with telomere repeat-binding factor TRF2. TRF2 protects telomeres from degradation and has a role in DNA damage response regulation. TRF2 loss is also linked to activation of Chk2 signalling in human heart failure [3]. To assess whether PNUTS has an anti-apoptotic role, PNUTS was overexpressed in cardiomyocytes and it was able to reverse miR-34a induced apoptosis by H₂O₂ and to reduce Chk2 activation. In ad-

dition, PNUTS overexpression inhibited DNA damage response (γH2AX staining) and telomere attrition.

To study the role of PNUTS in the heart, *in vivo* genetic deletion of PNUTS in mice was attempted but PNUTS^{-/-} was embryonically lethal and inducible cardiomyocytes specific PNUTS deletion mouse model is needed. In summary, aging increases miR-34a expression, this leads to SIRT1 and PNUTS decrease. Low levels of PNUTS result in telomere dysfunction and DNA damage signalling that in turn induce apoptosis, fibrosis and hypertrophy resulting in cardiac dysfunction occurring during aging.

Long non coding RNAs (lncRNAs) are emerging as important players in several biological process. Recently, it was shown that the lncRNA MALAT1 is required in endothelial cell proliferation [4]. To identify cardiomyocyte-enriched lncRNAs during aging, RNA-sequencing was performed on Langendorff heart preparations from young and old mice. Several lncRNAs were identified and independently validated *in vivo*. As functional assay, induction of apoptosis was tested in H₂O₂-treated HL1 cardiomyocytes cells after transfection of siRNA targeting lncRNAs. A discrete number of lncRNAs with anti- or pro-apoptotic function was identified with the lncCMI1 showing the strongest anti-apoptotic properties.

Some lncRNA are also enriched in endothelial compartment and particular attention was focused on the lncRNA H19 that is dramatically and specifically down-regulated in endothelium during aging. Of note, shear stress, a protective stimuli of endothelium, is able to induce H19 in endothelial cells and H19 silencing induces b-gal positive senescent phenotype. Thus, the lncRNA H19 is a promising tools to counteract the endothelial senescence during aging.

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Aging and cancer: rival demons or twin oracles?

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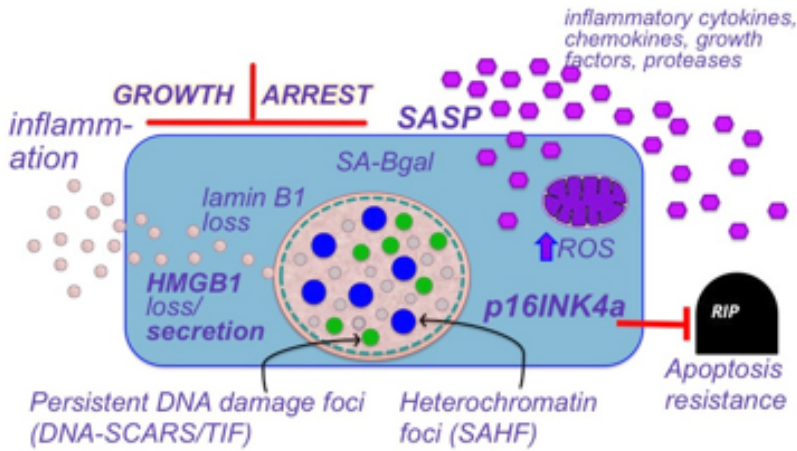
The susceptibility to several chronic diseases increases during aging and, although diseases like neurodegenerative disorders or cancer are characterized by different manifestations, they are driven by common “basic aging processes”. Of note, cellular senescence is an excellent candidate of such “basic aging processes”. The senescence response can be considered a stress response with two characteristics: an irreversible growth arrest and a multifaceted secretory phenotype. Moreover, the senescence response can be regarded as a balance between tumor suppression and aging, as well as between tissue remodelling and repair and aging related diseases.

Currently, there are no specific senescence markers, and so multiple markers are needed to define senescent cells. Among these markers are high expression levels of the tumor suppressor p16^{INK4a}, the p53-dependent loss of High Mobility Group Box 1 (HGMB1) protein from the nucleus and subsequent secretion, and the p53-independent senescence-associated secretory phenotype (SASP) composed by numerous inflammatory cytokines, growth factors and proteases that locally modulate tissue microenvironment (● **Figure 1**).

By means of these markers, senescent cells are found in multiple tissues, they accumulate during aging and are present at sites of age related pathology as chronic obstructive pulmonary disorder and Alzheimer’s disease [1, 4, 5]. Interestingly, secreted proteins, both HGMB1 and components of SASPs, can drive inflammation and consequently the accumulation of senescent cells during aging can stimulate chronic inflammation that in turn contributes to age-related pathology.

Two strategies can counteract pathologies fuelled by senescent cells, and thus aging: i) suppressing the secretory phenotypes and ii) the elimination of senescent cells. Three pathways are involved in regulating the SASP: the DNA damage response, p38MAPK-NF-KB signalling and the mTOR pathway, that is also involved in re-

• **Figure 1.** Selected markers of senescent cells



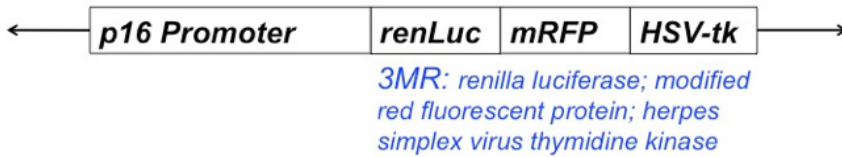
Source: for details, see Dimri et al., 1995 [1]; Rodier, Campisi, 2011 [2]; Davalos et al., 2013 [3].

gulating longevity in several organisms. Until now, some drugs are available against these pathways but unfortunately they are not suitable for long term, continuous therapy. So, a more promising idea is to initially preserve the senescence process, that also protects against cancer, and only after to eliminate senescent cells.

To test this hypothesis, a transgenic mouse model was generated with multiple gene cassettes driven by p16^{INK4a} senescent-sensitive promoter: the luciferase gene, to detect cells by *in vivo* imaging, the red fluorescent protein to sort senescent cells, and herpes simplex virus thymidine kinase (HSV-TK), which is able to convert ganciclovir (GCV) into a toxic product that allows selective elimination of TK-expressing cell (p16-3MR trimodal reporter mice) [6] (• **Figure 2**).

Using this mouse model, it is possible to reveal some beneficial results of eliminating senescent cells. One example concerns the adverse effect of chemotherapy. Whole body non-lethal ionizing radiation (IR) on p16-3MR transgenic mouse results in senescent cells that persist in several organs (skin, lung, kidney, etc.) for many months, and are specifically eliminated by GCV treatment. Senescent cells can be also eliminated from naturally aged mice by GCV administration and effects on longevity are currently under investigation. In parallel, a recent study on chemotherapy revealed that children with lymphoma or leukaemia are cured by chemotherapy but 20 years later they look older and present with several age-associated pathologies such as atherosclerosis, diabetes and osteoporosis [7]. To assess the role of senescence in response to chemotherapy, p16-3MR mice were combined two different mouse cancer models. Both B16 melanoma cells or MMTV bre-

• **Figure 2.** Schematic of the p16-MR transgene



Source: for details see Demaria et al., in press [6].

ast cancer cells injections in p16-3MR mouse give rise to metastasis and, notably, these are reduced by GCV treatment administrated after chemotherapy. As result, elimination of senescent cells after chemotherapy may have potential beneficial effects also in human cancer patients.

A second example is related to Parkinson Disease (PD). Surprisingly, expression of p16^{INK4a} tumor suppressor, a marker of senescence, is increased in brain of PD patients. Exposure to pesticide Paraquat (PQ) induces PD in both human and mice and determines senescence in astrocytes. Notably, GCV treatment eliminates PQ-induced senescent cells and improves motor neuron functions in p16-3MR mice. Again, these results suggest it could be beneficial to remove senescence cells to ameliorate several pathologies, including Parkinson Disease.

The third example identifies a beneficial effect of senescent cells. A question arises about the role of the senescence response, selected as tumor suppressor mechanism, but should be redundant to apoptosis, which avoids inflammation. The answer seems to be that senescence probably evolved to improve tissue healing and stimulate tissue repair. Indeed, senescent cells are present transiently during wound-healing and elimination of senescent cells in p16-3MR mice with GCV treatment retards the wound healing process. Of note, analysis of secreted molecules from senescent fibroblast and endothelial cells revealed the presence of an understudied form of PDGF, PDGF-AA, that is able to reverse slow wound healing in GCV-treated mice [6].

At the same time, senescent cells accumulate in naturally aged skin and wound healing slows during aging. Indeed, in a mouse model of genetic Sod2 deficiency, persistent senescence was observed in the skin together with a delay in wound healing [8]. In summary, the transient presence of senescent cells is required for optimal wound healing and is positively associated with tissue repair, while the persistent presence of senescent cells, as occurs during aging, retards the wound healing process.

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Telomeres and the origin of disease

Christian Bär

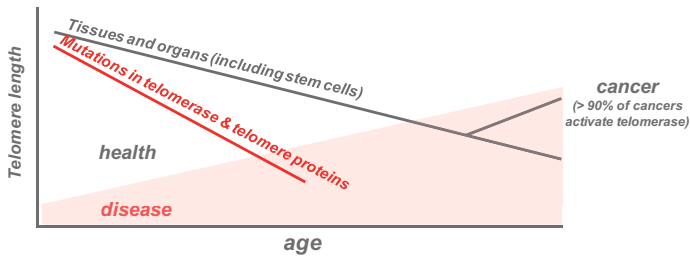
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Several pathologies such as cardiovascular disease are virtually absent in first four decades of life, therefore aging itself can be seen as a leading risk factor. Understanding the molecular basis of the aging process is necessary to reach comprehension of the origin of disease. In this regard, telomere attrition represents one of the recently classified hallmarks of aging [1]. Telomerase, the enzyme that synthesizes telomeres, is silenced at birth in somatic cells and therefore telomere length decreases with age since telomeric sequence is lost upon each cell divisions, a phenomenon described as “end-replication problem”. Cancer cells on the other hand reactivate telomerase thus escaping the mortal fate of adult cells and becoming immortal. In contrast to telomerase reactivation, mutations in telomerase or telomere proteins give rise to telomere syndromes characterized by telomere defects and accelerated telomere attrition especially in high turn-over tissues [2] (• **Figure 1**).

Over the last years, telomere length has been tested for its potential to represent a novel diagnostic bio-marker for various conditions. A high-throughput assays to measure telomere length was developed to quantify both telomere length and percentage of short telomeres in blood in large human cohorts by quantitative FISH [3].

In this context, shorter telomeres in peripheral blood leucocytes have been identified as predictors of onset of hereditary ovary and breast cancer [4], thus assigning a prognostic value to telomere length and highlighting the potential in personalized and preventive medicine. Furthermore, the question was raised whether telomeres can be predictors of lifespan and degree of cellular aging? To address this point, a longitudinal telomere length study throughout the life of mice was performed showing that mouse telomeres shorten ~100 times faster than human telomeres. Importantly, the increase in percentage of short telomeres over time predicts the individual’s longevity [5].

• **Figure 1.** Lifelong telomere shortening contributes to aging-related diseases



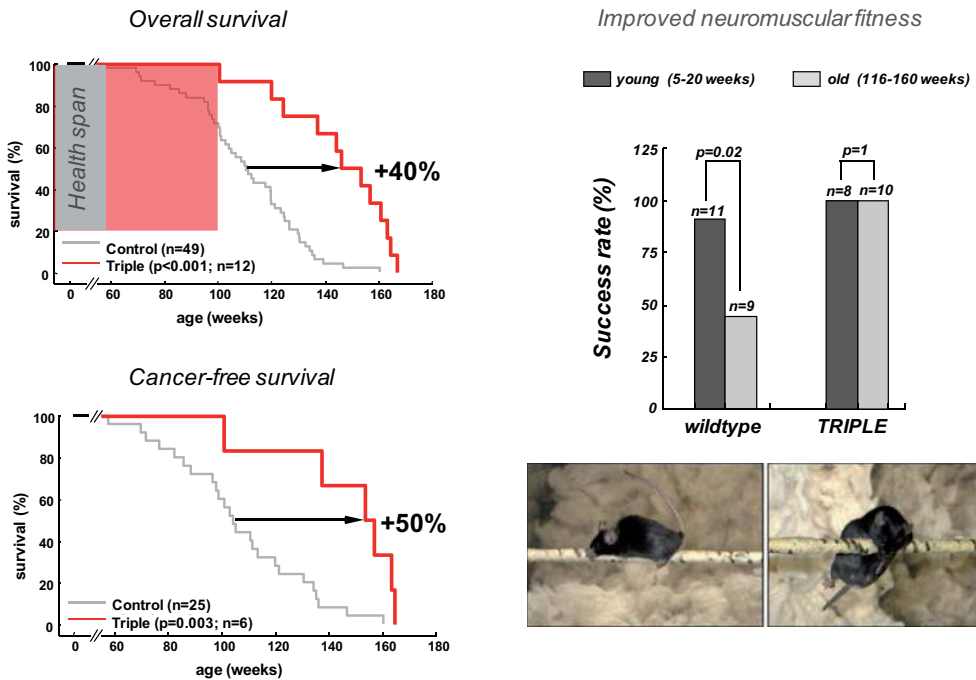
Telomeres shortening is a process that occurs throughout life. Telomeres can eventually become critically short and contribute to the emergence of various diseases. Accelerated telomere attrition due to defects in the telomere maintenance machinery lead to early onset of disease. Cancer cells, characterised by uncontrolled proliferation can activate telomerase and thus escape the mortal fate of normal somatic cells.

Telomere shortening in mice contributes to aging and, accordingly, telomerase-deficient mice ($Terc^{-/-}$) present stem cell dysfunction, premature aging and less cancer in late generation. This has led to the idea that telomerase re-activation may delay physiological aging. Indeed, mice transgenic for the catalytic subunit of telomerase (TERT) showed less aging with a minor increase in survival [6]. However, constitutive expression of telomerase in mice engineered to be cancer resistant by enhanced expression of the tumor suppressors p53, p16, and p19ARF (triple mice) showed less cancer, longer telomeres and less DNA damage with aging. Strikingly, an increase in lifespan of 40% was accompanied by improved signs of aging such as neuromuscular fitness in old animals [7] (• **Figure 2**).

Following this proof of concept study demonstrating that expression of telomerase provides anti-aging activity a TERT based gene therapy of aging with therapeutic potential was tested. Single tail vein injection of adeno associated virus (AAV) expressing TERT (AVV9-TERT) in mice led to longer telomeres and lower DNA damage in a wide range of tissues. Notably, treatment of 1- and 2-year old mice with AVV9-TERT improved health and fitness and extended median lifespan by 24% and 13%, respectively [8].

Given this global anti-aging effect based on AVV9-TERT delivery this approach may be an effective treatment in several diseases associated with short telomeres, such as telomere syndromes (e.g. aplastic anaemia and idiopathic pulmonary fibrosis) and age-associated diseases (e.g. cardiovascular and neurodegenerative disease) in which short telomeres were shown to be a risk factor. In this regard, our laboratory is currently developing different mouse models for human diseases which may help to evaluate the therapeutic value of telomerase gene therapy.

• **Figure 2.** Telomerase reactivation delays aging in mice



Constitutive transgenic overexpression of telomerase in cancer-resistant mice leads to an 40% in median lifespan and 50% improvement in cancer free survival compared to wild-type mice (left, top and bottom panel). Those mice also show healthier aging compared to wild-type as indicated by improved neuromuscular fitness in old mice. Mice were subjected to a tight-rope-test in which we check whether a mouse is able to hold on to a rope or whether it falls off (right, top and bottom panel).

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Telomere-driven chromosome instability and cell senescence: a dual mechanism in tumorigenesis

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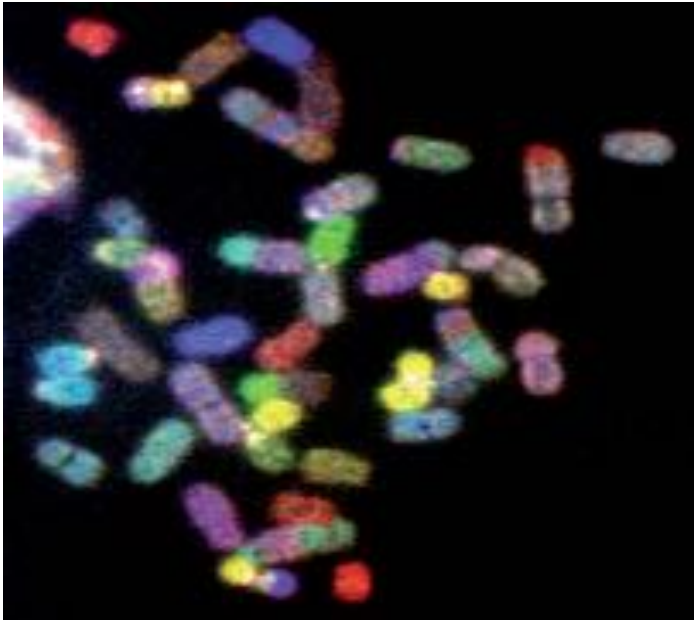
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Age is the major risk factor for cancer development since the frequency of carcinomas increase exponentially with age beyond the 4th decade. Three major non-exclusive hypotheses potentially explain this tight connection: 1) the drop in evolutionary constraints after reproductive life makes genome surveillance mechanisms less efficient with age; 2) both the mutational load per cell and the number of cells with mutations increase with age; 3) the accumulation of senescent stroma cells in proliferative epithelial tissues impinges upon the progression of pre-cancerous lesions, promoting invasion and dissemination. On the other hand, no other chromosome structure has been so tightly linked to aging, genome stability and the transformation process as telomeres. Telomeres, which protect chromosome ends from degradation and fusion, shorten with age. In normal somatic cells, telomere shortening (which occurs progressively with each cell division) leads to senescence (permanent arrest of cell proliferation) thus acting as a barrier to tumor development. However, in cells with dysfunctional p53/Rb signaling pathways, telomere shortening leads to chromosome instability (CIN) -a hallmark of cancer cells-, which fuels the transformation process (• **Figure 1**).

Increasing evidence points to a major role of telomere shortening in carcinogenesis in humans: 1) tumor evolution is characterized by pronounced telomere shortening [1-3]; 2) individuals with shorter telomeres have an increased risk of developing carcinomas [4]; 3) cancer patients with short telomeres carry a higher risk of dying from metastasis [5]. Yet, massive sequencing approaches applied to progressing tumor stages have failed to pin down late gene-specific changes that could underline a metastatic behavior [6], thus lending support to the implication of epigenetic mechanisms. Indeed, growing *in vitro* experimental evidence indicates that metastatic cells often, if not always, follow a transdifferentiation pathway

- **Figure 1.** Whole Genome Multicolor FISH analysis of a HEK cell line after telomere-driven CIN



Changes in color along a single chromosome indicate a translocation. The presence of multiple unbalanced chromosome translocations are commonly detected in many carcinomas, the most prevalent type of cancer in aged individuals.

that is largely ruled by epigenetic mechanisms, including chromatin modifications, large non-coding (nc) RNA induction and microRNA (miR) expression deregulation, particularly those miRs belonging to the miR-200 family [7]. Interestingly, in cancer prostate (which is the most prevalent cancer in aged men), patients who presented a combination of variable telomere lengths in tumor cells and short telomeres in stroma cells had a higher risk of lethal outcome [8]. Thus, the available data strongly suggest interplay between cell transformation-related telomere-driven CIN and age-related telomere-driven senescent microenvironment (SM) in the evolution of aggressive carcinomas.

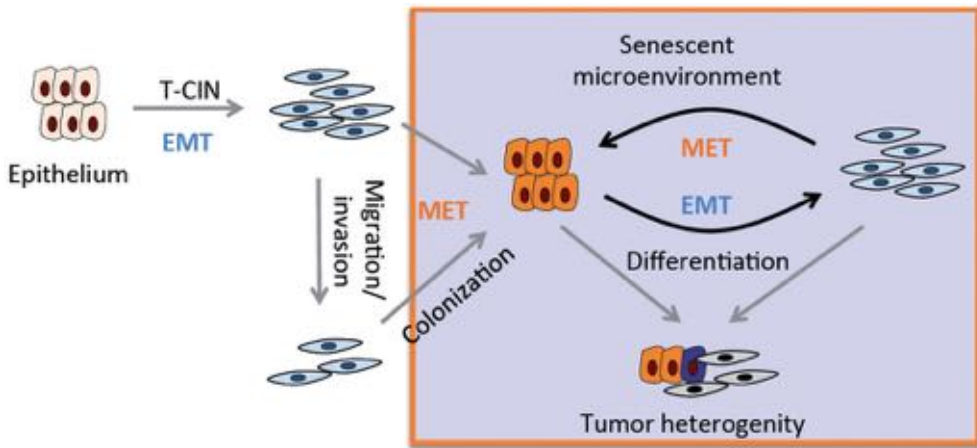
Investigations in the “Telomere and Cancer” lab using an *in vitro* model of human epithelial kidney (HEK) cell transformation established by Professor Silvia Bacchetti (McMasters University, Hamilton, Canada), have shown that recurrent breakage-fusion-bridge formation following telomere shortening leads to widespread CIN and to the selection of spontaneously immortalized post-crisis cells having undergone an Epithelial-to-Mesenchymal transition (EMT) and displaying increa-

sed migration/invasion capacities [9]. Massive parallel RNA sequencing showed that CIN directly impacts the expression of miRs genome-wide independently of local rearrangements, and in particular represses the miR-200 family [9]. This repression is directly responsible for driving the EMT observed in post-crisis cells [9]. Interestingly, although none of these post-crisis cell lines are able to form tumors in immunosuppressed mice, some of them do form tumors when co-injected together with fibroblasts that have entered senescence due to telomere shortening (Castro-Vega *et al.*, submitted). Strikingly, only the post-crisis cell lines that were able to form tumors when a senescent microenvironment was provided were also able to form “spheres” when put in contact with culture medium that had been conditioned by the presence of senescent fibroblasts. Sphere formation by cultured cells has been interpreted as being dependent on self-renewal capacities [10] and therefore it is considered to be a manifestation of stemness. Further work indeed demonstrated that these cells are endowed of differentiation capacity (Castro-Vega *et al.*, submitted), thus confirming the acquisition of stemness by CIN+ HEK cells. That work has also confirmed that post-crisis cells but not CIN- cells undergo further transdifferentiation when in contact with the senescent microenvironment. In all, our experiments suggest that telomere-driven CIN impacts the genetic program of epithelial cells, thus promoting EMT, a first step towards not only the acquisition of tumor-related properties but also the dedifferentiation pathway. However, as suggested by our observations, these CIN+ cells have not acquired full tumorigenicity yet and require the input of environmental cues, which further promote dedifferentiation and the development of self-autonomous tumorigenic capacity (● Figure 2).

The fact that media conditioned by the presence of senescent fibroblasts is sufficient to trigger stem-like manifestations in pre-malignant cells strongly suggest that the Senescent-Associated Secretory Phenotype (SASP) plays an important role in the acquisition by transformed cells of stem-like properties, thus promoting malignization [11]. These observations provide a mechanistic rationale for the connection between aging (which favors CIN in pre-transformed cells and senescence in stroma cells both through telomere shortening) and the age-related increase in carcinoma frequency and aggressiveness. Understanding the relative contributions of CIN and SASP, and the dynamics of tumor and senescent compartments is currently a major scientific challenge in the study of age-related carcinomas.

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- **Figure 2.** Interplay between telomere-driven CIN (T-CIN) and telomere-driven senescence in the stroma (the senescent microenvironment)



Chromosome instability due to telomere initiated breakage-fusion-bridges cycles trigger an epithelial to mesenchymal transition (EMT) through miR deregulation. T-CIN+, EMT+ epithelial cells become sensitive to secreted factors by the surrounding senescent fibroblasts. These factors promote further transdifferentiation (akin to a mesenchymal-to-epithelial transition), allowing CIN+ cells to acquire stem-like characteristics and aggressive behavior. This transdifferentiation may occur locally allowing the progression of the primary tumor lesion or distally, triggering the colonization process that allows the emergence of metastasis. Cancer stem-like cells remain responsive to the senescent microenvironment, undergoing successive transdifferentiations, which could explain the heterogeneous composition of many carcinomas.

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Emerging concepts in senescence and reprogramming

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Tissue remodelling during normal and pathological conditions involves several mechanisms. Developmental remodelling needs coordinated regulation of cell proliferation and apoptosis. Recently, senescence is emerging as a beneficial processes during wound healing. Both processes, apoptosis and senescence, eliminate damaged cells in tissues after injury operating through selective and regulated cell death (in the case of apoptosis) or “assisted” cell death (in the case of senescence and the elimination of senescent cells by macrophages). Newly, an unexpected role for senescence has been discovered during embryogenesis where it orchestrates tissue remodelling in response to developmental cues [1, 2].

The main tumor suppressor pathways triggering apoptosis and senescence in response to cellular damage involve p53 and Rb signalling, respectively. These tumor suppressors trigger transcriptional programs with the potential to induce apoptosis or senescence, the final result depending on unknown factors but the coexistence of both programs ensures compensatory mechanisms in case one response fails. What about programmed cell death during development? Here, apoptosis is a fundamental process for the elimination of unwanted cells, tissue remodelling and cell balance. To study the role of senescence in embryogenesis, mouse embryos at different stage of development were stained with senescence-associated β -galactosidase (SA- β -Gal). Senescence is widespread during embryonic development at multiple locations, including the mesonephros, neural tube, interdigital webs, vibrissae.

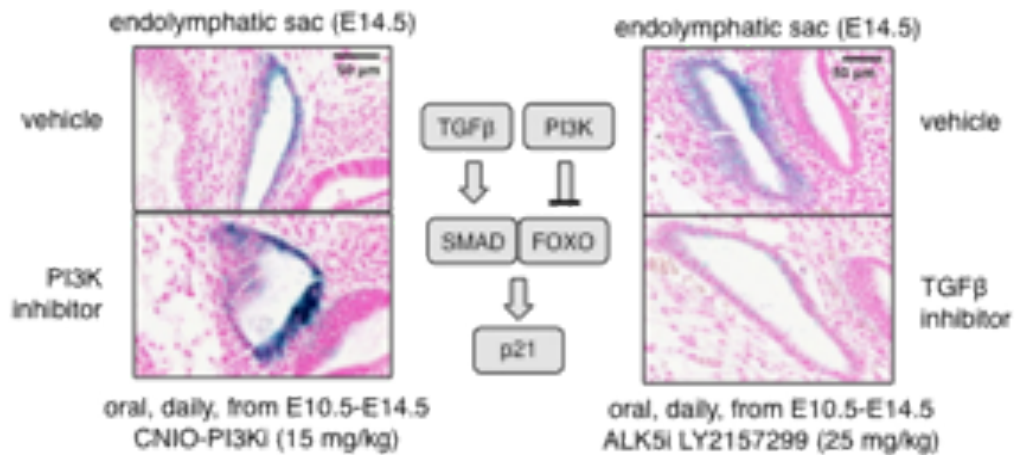
Developmentally-programmed senescence during embryogenesis is present in mouse, human and chicken, a therefore it appears conserved across evolution. To identify the main players of developmental senescence, SA- β -Gal staining of embryos was performed in mouse models lacking p53, p27, p16ARF, mediators of damage-induced senescence and other mediators of DNA damage. Surprisingly, none of them

played a role in this process. Developmental senescence is conversely completely impaired in mice lacking p21: the senescent cells present in the mesonephros of wild-type mice are absent when p21 is lacking. Mechanistically, developmental senescence is regulated by the TGF- β /SMAD and PI3K/FOXO pathways. In agreement, mouse overexpressing the PI3K-inhibitor PTEN (PTEN-Tg), an enhanced developmental senescence is observed. Moreover, chemical manipulation of TGF- β /SMAD and PI3K/FOXO pathways reveal a central role of p21 in developmental senescence: chemical TGF β inhibitor given to pregnant mothers present same phenotype of p21null mice in embryos i.e. absence of senescence; in contrast, chemical PI3K inhibitor, as PTEN-Tg mice, results in an enhanced senescence (• **Figure 1**).

Interestingly, mice with altered developmental senescence have quite normal phenotype because absence of senescence is compensated by apoptosis. So both apoptosis and senescence are fine-tuning phenomena in development that promote tissue remodelling. Developmental senescence is proposed to be the evolutionary origin of damage-induced senescence. In this view, both developmental and damage-induced senescent cells trigger inflammatory responses, induce self-elimination and clearance thereby allowing tissue regeneration.

Therefore, senescence is just one step in tissue regeneration and a virtuous sequence of senescence-clearance-regeneration can be depicted [3]. Alterations of these sequen-

• **Figure 1.** Senescence during embryonic development



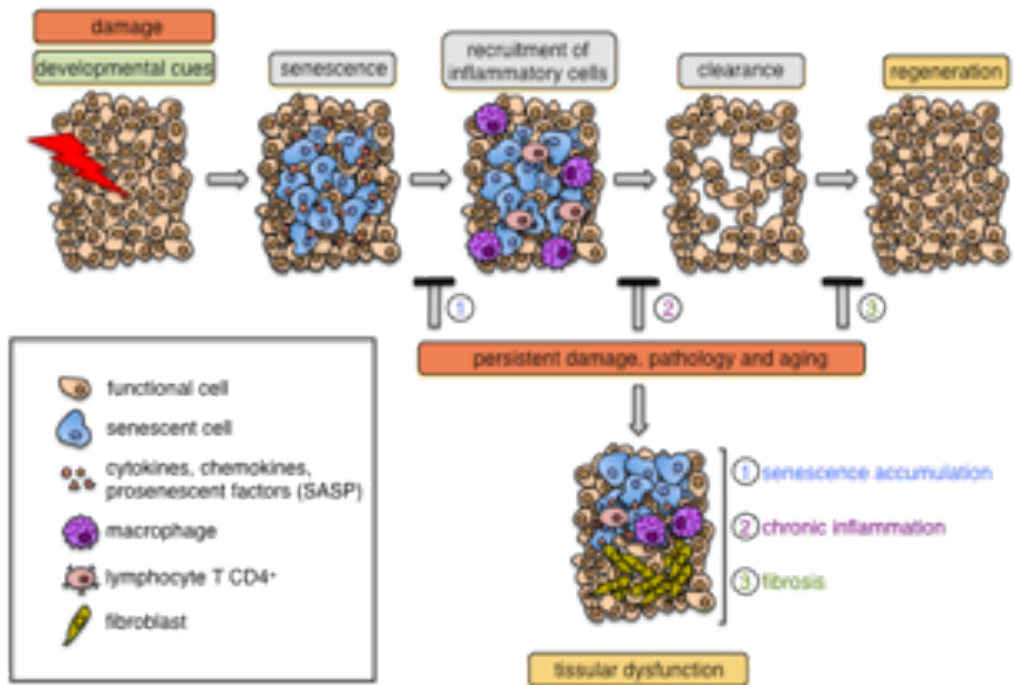
The pictures correspond to the endolymphatic sac of the inner ear at day E14.5 of mouse development. The blue staining corresponds to senescence-associated beta-galactosidase (SAbG). Treatment of the pregnant mothers with a PI3K inhibitor enhances senescence and treatment with a TGF β -Receptor inhibitor decreases senescence. These observations are in agreement with the biochemical pathway depicted in the middle.

tial three steps due to pathology or aging may result in accumulation of senescent cells which may contribute to aggravate tissular dysfunction, chronic inflammation and fibrosis (• **Figure 2**).

Actually, there are many pathologies associated with cellular senescence, in some cases senescence protects from the diseases, as in liver fibrosis, but in other contexts it is detrimental. So, senescence can be categorized as an antagonistic hallmark of aging and both pro-senescent therapies and antisenescent therapies can be beneficial: antisenescent therapies may help to eliminate accumulated senescent cells and to recover tissue function. Conversely, in cancer and during active tissue repair, pro-senescent therapies contribute to minimize the damage by limiting proliferation and fibrosis, respectively [4].

A second relevant topic for tissue regeneration is “cellular reprogramming”. Reprogramming of adult cells *in vivo* may be a strategy of tissue rejuvenation, i.e. de-differentiation of aged tissue and induction of re-differentiation into a rejuvenated tissue. *In vitro*, Yamanaka’s laboratory generated embryonic pluripotent cells known as induced

• **Figure 2.** Model of the role of senescence during the repair/regeneration of damaged tissues

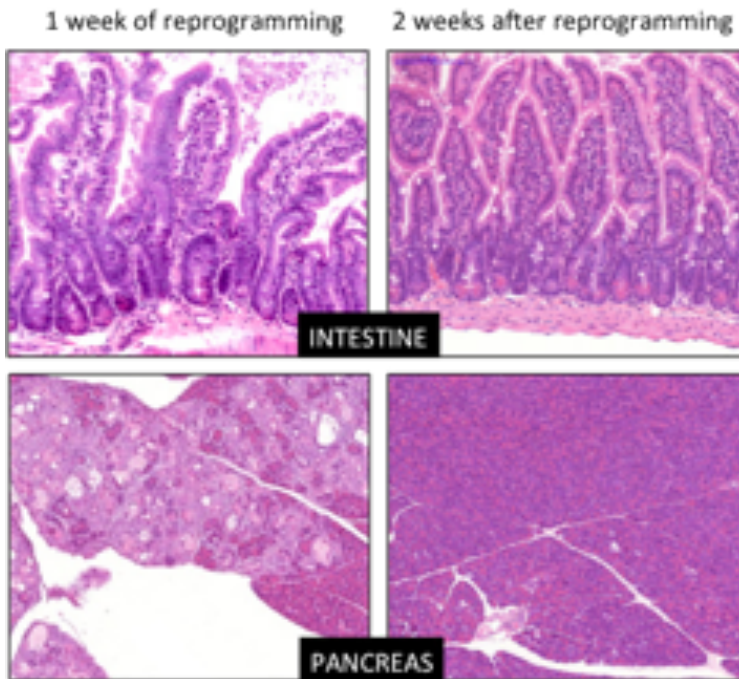


If the full process is not completed, cellular senescence may contribute to aggravate tissue damage.

pluripotent stem cells (iPSCs) from adult human dermal fibroblasts by the introduction of four factors: Oct3/4, Sox2, Klf4, and c-Myc [5]. To test the hypothesis that this reprogramming can occur also *in vivo* within tissues, a “reprogrammable mouse” was generated which has ubiquitous and inducible expression (driven by ROSA26::rtTA) of the four Yamanaka factors [6]. As short term effects (1-3 weeks), multiple cells within tissues become dysplastic and de-differentiate, also circulating iPSCs were recovered from the blood. Notably, two weeks after the doxycycline removal, a reversion of phenotype is observed and correct re-differentiation with complete recovery of tissue homeostasis takes place in all organs, like stomach, liver, pancreas, kidney, and intestine (• **Figure 3**).

Only a minority of cells present an imperfect re-programming giving rise to teratomas and to non-teratoma tumors. The majority of cells, however, undergo correct re-differentiation probably because cells retain some memory, like epigenetic memory, or the environment guide them towards correct differentiation. In conclusion, *in*

• **Figure 3.** The activation of the Yamanaka reprogramming factors



The activation of the Yamanaka reprogramming factors induces dysplasias and loss of differentiation in the intestine and pancreas (left panels). Switching off the reprogramming factors for 2 weeks is sufficient for the tissues to re-acquire a normal architecture.

in vivo conditions are permissive for reprogramming, although it is still necessary to find the right conditions to avoid tumors [6, 7]. In agreement with these concepts, some examples of *in vivo* reprogramming have been recently published [8].

Lastly, both senescence and reprogramming can be considered as processes favouring tissue regeneration and tissue remodelling.

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Tumor-suppressor and aging pathways

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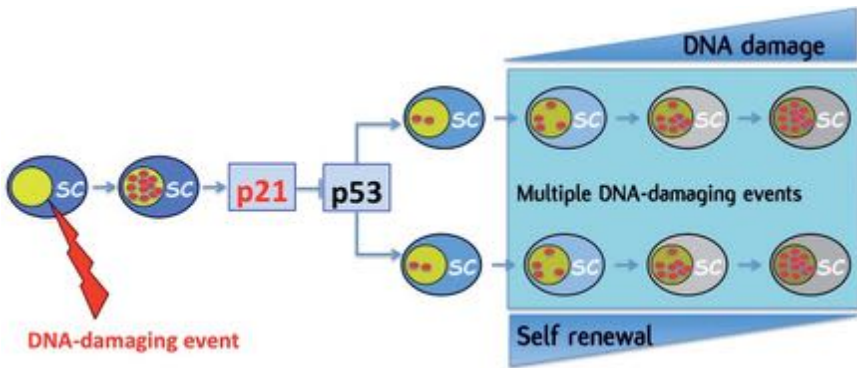
Studies on DNA damage checkpoint and self-renewal properties of normal stem cells can uncover molecular mechanisms implicated in tumor development and physiological aging. As experimental model, aging B1/6 mice were subjected to whole body irradiation, and analyses on both DNA damage checkpoints and self-renewal were performed on highly purified stem cells derived from them. Cancer stem cells (CSCs) from leukemia and mammary tumor cancer models were also studied.

Normal stem cells (SCs) can undergo both asymmetric and symmetric divisions. In steady-state conditions, SCs mainly divide asymmetrically (70% of divisions) through a pathway regulated by p53. In more mature cells, DNA damage induces p53 activation and p53-dependent apoptosis/senescence. In SCs, instead, DNA damage caused by X-ray treatment does not activate p53 but induces a stem-cell specific checkpoint which involves a strong up-regulation of the cell cycle inhibitor p21, inhibition of p53 activation and apoptosis/senescence, induction of one cycle of symmetric division, which leads to immediate expansion and doubling of functional SCs, activation of p21-dependent DNA damage repair and maintenance of self-renewal [1]. DNA damage is, however, never completely repaired and, with time, that results in progressive accumulation of persistent DNA damage and consequent loss of self-renewal potential, suggesting that a flawed p21 checkpoint may act as a tumor suppressor mechanism by limiting the lifespan of normal SCs. Interestingly, also during aging the frequency of SC symmetric divisions decreases, likely due to accumulation of persistent DNA damage over time resulting in a reduction in self-renewal potential (• **Figure 1**).

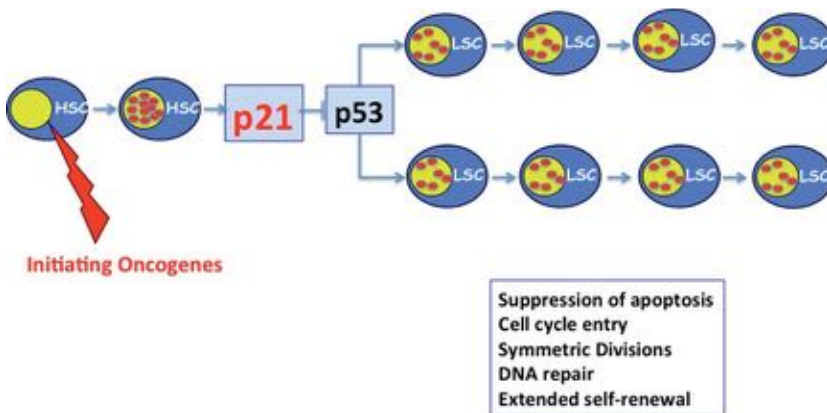
Like normal SCs, CSCs are capable of dividing both asymmetrically and symmetrically but due to loss or attenuation of p53 signaling, which is also indirectly responsible for reprogramming of progenitors into stem cells, divide mainly symmetrically (70%), leading to *in vivo* expansion of CSC numbers. [2]. Moreover, in CSCs, DNA dam-

age accumulation due to oncogene expression constitutively activates the p21 checkpoint leading to active DNA repair, with continuous “waves” of DNA damage-DNA repair (mutator phenotype) (• **Figure 2**). Thus in CSCs, on the one hand p53 loss and p21-checkpoint activation increase symmetric division, favor progenitors’ reprogramming, and extend replicative potential, ultimately resulting in the maintenance of a pool of expanding CSCs responsible for tumor growth, on the other, through alternation of symmetric and asymmetric divisions and the acquisition of a “mutator phenotype”, determine tumor-cell heterogeneity.

• **Figure 1.** Unique DNA-damage checkpoint in stem cells (SCs). SCs have evolved a p21-dependent response to DNA damage that leads to their immediate expansion and limits their long-term survival



• **Figure 2.** Oncogene-expression into pre-leukemic HSCs induces DNA-damage, constitutive activation of the p21 response and extended self-renewal

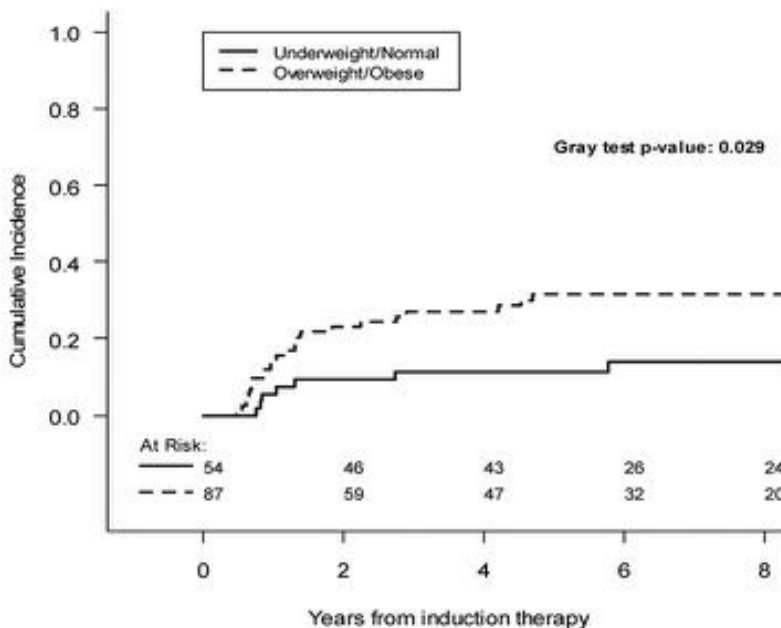


The self-renewal properties of normal and cancer SCs can be also influenced by the environment. In particular, this second part describes the effects of diet, focusing on both obesity and caloric restriction.

Obesity is associated with increased leukemia incidence [3] and with worse outcome in acute promyelocytic leukemia (APL) patients [4]. Thus, obesity appears to affect both APL tumor initiation and APL tumor progression (● Figure 3).

Obesity-associated APL has been modeled in the mouse using PML-RAR transgenic mice. However, PML-RAR mice develop leukemia with low penetrance (~ 55%), suggesting that other genetic abnormalities are required in order to develop cancer. Indeed, this hypothesis was tested by insertional mutagenesis through infection with the Moloney Murine Leukemia Virus (MLV) in a PML-RAR genetic background. The infection resulted in increased penetrance of the disease (from 55 to 75%), confirming a likely cooperation between MLV and PML-RAR in leukemia induction. Single targeted genes isolated from MLV insertion sites were also identified to cooperate with PML-RAR to induce leukemia, the well-known oncogene cMyc among them (unpublished).

● Figure 3. Cumulative incidence of relapse according to BMI



This research was originally published in *Blood*. Breccia et al. Increased BMI correlates with higher risk of disease relapse and differentiation syndrome in patients with acute promyelocytic leukemia treated with the AIDA protocols. *Blood*. 2012;119:49-54. © the American Society of Hematology."

The PML-RAR transgenic mice were fed either a standard or a high fat diet. Interestingly, the high fat diet enhanced leukemia penetrance and reduced its latency, suggesting that obesity contributes to leukemogenesis in the mouse. It is known that metabolic pathways can generate products which cause DNA damage. For instance, the mitochondrial fatty acid β -oxidation pathway is considered critical for hematopoietic stem cells (HSCs) [5] and can generate reactive aldehydes that induce DNA damage exclusively in the pool of HSCs [6]. Obesity might induce DNA damage and mutations by increasing β -oxidation. Indeed, in Lin⁻ cells, a high fat diet increases levels of the M1dG adduct, a DNA adduct generated by reactive aldehydes derived from the β -oxidation pathway. In addition, several peroxisome-specific β -oxidation enzymes (ACAA, ALOX1, HADHB, HSD17B4) are found up-regulated in PML-RAR mice and can, in turn, increase levels of reactive aldehydes which altogether cause DNA damage.

Obesity also affects leukemia prognosis. In fact, besides obesity being associated with a worse outcome in APL patients [4], a higher incidence of oncogenic FLT3-ITD mutations is observed in the obese APL-patients. It appears that obesity confers a competitive advantage to these mutations and increases the aggressiveness of tumors. In the current model, obesity is a systematic disease and might select FLT3-ITD mutations through adipokines, secreted by fat tissues in the obese mice. Of note, the oncogenic potential of the FLT3-ITD mutation seems to depend on Insulin/IGF1 signalling.

Interestingly, the effects of a calorie restricted diet on leukemia initiation and tumor growth are also very potent: i) caloric restriction (CR) delays tumorigenesis by transplanted p53^{-/-} bone marrow cells or by transplanted PML-RAR pre-leukemic cells; ii) CR reduces the growth of bulk PML-RAR leukemic cells (as observed in peripheral blood, spleen and bone marrow) after transplant of APL cells. Surprisingly, however, CR increases the frequency and number of leukemic initiating cells (LICs). This apparent contradiction was partly explained by observing the effect of CR on normal hematopoietic stem cells: CR, in fact, increases their self-renewal, although decreasing proliferation rate and numbers of HSCs and progenitors. Importantly, CR decreases basal level of DNA damage in HSCs.

In conclusion, CR might delay the initiation of leukemogenesis by reducing DNA damage, while obesity, through the induction of DNA damage due to reactive metabolites, increases the incidence of leukemia.

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Conclusions

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During this meeting, all of us had the opportunity to deepen the topic “Aging” from several points of view and to put it into perspective. The interesting question “Is aging a disease?” leads to additional considerations: aging itself is independent from diseases; aging prevents some diseases; aging facilitates the development of some pathological conditions.

The principal hallmarks of aging have been explored starting with the role of stem cells and their exhaustion during aging, moving to the effects of thyroid hormones on these cells as well as the impact of obesity on genomic instability and the resulting effect on stem cells. The possibility to realize “cell-based therapy” using adult stem cells is really fascinating but stem cells derived from older individuals are less effective than those from young subjects. This aspect represents a major hurdle for the treatment of elderly patients with some pathologic conditions such as heart failure. In such cases, one possibility is to try “rejuvenation” of elder stem cells but this approach may increase the likelihood for cancer development.

Today, several aspects of aging were reported revealing the interplay between telomere and cancer, epigenetics and aging as well as the role of diet on mitochondrial dysfunction and epigenetics. Moreover, Edward Lakatta gave a talk about vascular aging and the role of the secretory senescence-associated phenotype (SASPs) in the age-dependent stiffness of the arterial wall, highlighting its implications in developing cardiovascular accidents and heart failure. Further, Pier Giuseppe Pelicci talked about obesity as cause of DNA mutations in hematopoietic stem cells. It is possible that such DNA mutations may occur also in other cell types, for instance, in cells of the vascular wall, and in that case they may provide a link between obesity and cardiovascular diseases.


Of particular interest, Angiotensin II, a key component of SASP produced by vascular cells in the elderly, could be blocked with Angiotensin II receptor antagonists

and Angiotensin-converting enzyme inhibitors which are effective also in the absence of arterial hypertension.

MicroRNAs and other non-coding RNA are emerging in aging field. This is an issue of great complexity, for instance miRNA-34a increases during aging in both humans, primates and rodents, and this is something that it was well established, but today it has been shown that in *N. furzeri*, a very interesting animal model in aging research, levels of miR-34a decrease during aging. Therefore, these animal diversities should be kept in mind when using different animal models to study aging. Overall, circulating non-coding RNA may be a part of novel SASPs and novel biomarkers. In this way, they may play a role in age-dependent changes in cell-to-cell signalling.

Finally, Judith Campisi showed that removing senescent cells is one way to regenerate tissues and to treat some pathological conditions. During wound-healing, High Mobility Group Box 1 (HGMB1) protein, a component of SASPs, plays a key role. Of interest, we found that HGMB1 is decreased in diabetic skin, both in humans and mice, and it has a strong regenerative action as exogenous supplement on skin of diabetic mice. In addition, in a mouse model of myocardial infarction, HGMB1 has a key role in activating stem cells to induce myocardial regeneration.

As a final conclusion, the several components and hallmarks of aging are strictly interconnected among themselves with interplay at the molecular level. Moreover, at higher level, it is possible to delineate interconnections between aging, life style, diseases and the environment itself.



The V Forum IBSA, titled “Aging: is it a disease?” organized by Fondazione IBSA and the Goethe University (Frankfurt am Main), explored in great depth, all the more recent and significant advances in the field of health aging, as well as those from studies of basic research and clinical research, focused on the pathophysiology of the different apparatuses, from cardiovascular to the endocrine system, without neglecting the cultural aspects in the sense most wide, with important implications for corporations or the economy, and not only in the Western world, directly from the voice of internationally recognized top leaders, carefully selected on the basis of their scientific excellence. The border between aging and diseases associated with it, becomes very thin, and research in this area is more than ever, lively and incisive.