TURNING BACK TIME: IS IT POSSIBLE TO REVERSE AGEING?

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Abstract

Ageing is a biological process that results in physiological and cognitive decline of an individual. During ageing, the risk for certain diseases such as neurodegenerative disorders, cancer, and cardiovascular diseases increases, thereby impacting the quality of life. Ageing is a natural phenomenon characterised by time-dependent accumulation of cellular damage, which leads to cellular dysfunction. Scientists have proposed nine hallmarks which signify that a cell is ageing: genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Scientists have recently attempted to slow or reverse ageing and further understand potential underlying molecular pathways. Administration of nicotinamide mononucleotide, partial reprogramming of cells, and parabiosis are a few of the experimental methods that are being attempted to increase the lifespan of various model organisms. However, poor translation of findings within animal research to the human situation, the potential conflict of financial interests in anti-ageing supplements, the dearth of funding to develop therapies, and a lack of regulation of new research findings by governing bodies are posing limitations to the implementation of research in this field.

Introduction

Ageing is a natural, biological process that occurs over the lifetime of an individual. It leads to functional and cognitive decline, which in turn results in decreased quality of life, and inevitably, death [1]. It is a complex phenomenon in which a myriad of molecular and cellular changes occur [1]. Currently, there are 900 million people worldwide that are aged 60 and above. By 2050, the proportion of people aged 60 and older would compose 22% of the global population [2]. The consistent rise in the elderly section of the population can be attributed to improved healthcare as well as a decline in fertility rate. However, as we age, the risk factors for certain age-related diseases such as neurodegenerative disorders, cardiovascular diseases, diabetes, and cancers also increase [3]. To improve the quality of life of the elderly, and for the prevention or early diagnosis of age-related diseases, it is vital not only to understand the underlying molecular mechanisms that are responsible for damage accumulation during ageing, but also to take into account the genetic and environmental modulators that affect these molecular mechanisms, and if they can be targeted. These topics are in line with a most controversial question that has persisted throughout centuries, being: Is it possible to reverse ageing?

Causes and Hallmarks of Ageing

Ageing is generally known to be caused by the accumulation of cellular damage over time which leads to a loss in cell function and eventually loss of organ and organism fitness [4]. Similar to Weinberg's hallmarks of cancer, there are certain signs that cells display when they start to age. In 2013, *Lopez et al.* proposed nine hallmarks of ageing [5], which can be divided into three categories; **primary hallmarks**: mechanisms that cause cellular damage - genomic instability, telomere attrition, epigenetic alteration, and loss of proteostasis; **antagonistic hallmarks**: processes that occur in response to cellular damage - deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence; **integrative hallmarks**: processes that eventually lead to a reduction in organ fitness - stem cell exhaustion and altered intercellular communication [6] (Figure 1). These hallmarks ultimately result in the clinical phenotype of ageing, and age-related diseases. Their involvement in the ageing process is briefly described below:

1. Genomic instability: DNA damage and mutations are caused by multiple exogenous threats like chemical and biological agents and endogenous factors such as errors during DNA replication and oxidative stress. These mutations compromise signalling pathways essential to the functioning of cells, which disrupts homeostasis. In addition, mitochondrial DNA (mtDNA) and its dysfunction may also play a role in age-associated diseases [5].
2. Telomere attrition: DNA polymerases that are involved in the replication of DNA cannot completely replicate the terminal ends of the DNA. This can only be achieved by an enzyme called telomerase. However, telomerase is not expressed by most vertebrate cells, and hence during every cell division the telomeres become shorter [4]. Over time, there is a cumulative loss of telomeres from chromosome ends. Telomere shortening has been observed during normal ageing patterns in mice and humans and short telomeres lead to a decreased lifespan in mice [5].
3. Epigenetic alterations: epigenetic marks such as DNA methylation patterns, chromatin remodelling and histone modifications signify the age of the cell. Alterations in any of these marks are associated with normal ageing as well as with age-associated diseases [7].
4. Loss of proteostasis: protein homeostasis or 'proteostasis' is a complex process that involves the regulation of proteins within the cell: from its synthesis to degradation to maintaining the healthy functioning of the cell [4]. With ageing, proteostasis is compromised, which is seen by enhanced misfolding and accumulation of damaged proteins, causing age-associated diseases such as Alzheimer's and Parkinson's disease [7].
5. Deregulated nutrient sensing: nutrient sensing systems such as mTOR, AMP-activated kinase, insulin, and insulin-like growth

factor 1 also regulate ageing [7]. For instance, AMPK activates catabolic pathways like β-oxidation of fatty acids and improves insulin sensitivity by suppressing anabolic pathways like cholesterol and fatty acid biosynthesis [7]. Anabolic processes and mutations in nutrient sensing systems lead to accelerated ageing while calorie restriction extends lifespan in murine models by altering these pathways [3].

6. Mitochondrial dysfunction: dysfunctional mitochondria can not only lead to an increase in reactive oxygen species (ROS), but also disrupt cellular signalling and inter-organellar cross talk. This can contribute to ageing. As cells age, ROS levels increase, electron leakage increases, and ATP generation is reduced, which further disrupts cellular homeostasis [4].
7. Cellular senescence: this is a physiological state of proliferative arrest which cells enter upon exposure to stress, DNA damage, telomere shortening, etc. [8]. It is also related to tissue ageing and many of its features correlate to the other hallmarks of ageing such as epigenetic modifications and metabolic changes [8].
8. Stem cell exhaustion: the decline in cell and tissue renewal is a characteristic of ageing. With an increase in cell cycle inhibitory proteins, oxidative stress, DNA damage and telomere shortening, multiple factors drive stem cell depletion [4].
9. Altered intercellular communication: senescent cells express a secretory phenotype, the so-called senescence-associated secretory phenotype (SASP). This phenotype promotes low level chronic inflammation by dispersal of pro-senescent signals to nearby tissues and extracellular matrix [3]. The pro-senescent signals lead to an induction of interleukins and other inflammatory molecules that enable a senescence messaging system and thereby harm the tissues' cells especially at older ages. The progressive accumulation of tissue damage results in organismal ageing wherein one tissue accelerates the ageing of other tissues through gap junctions and ROS signalling [3].

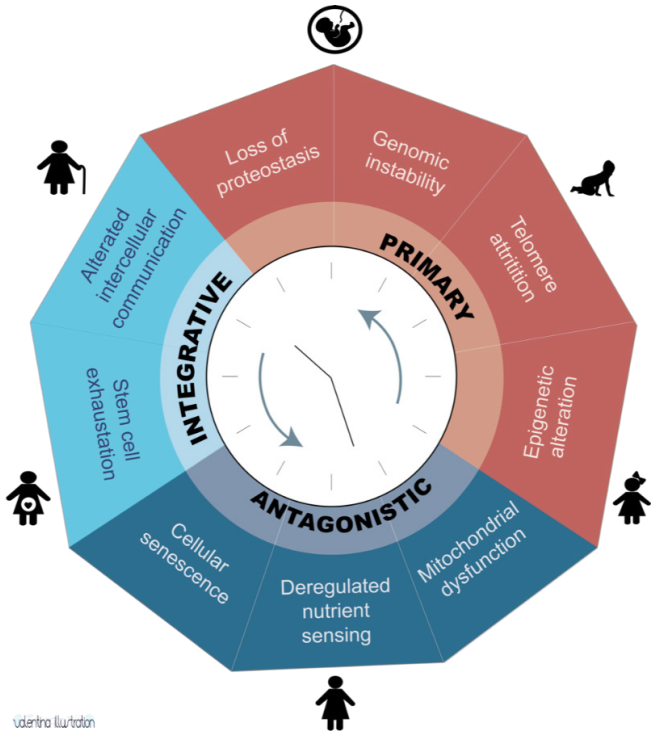

Figure 1: *The hallmarks of ageing. This illustration displays the various hallmarks of ageing signifying a basic set of mechanisms to help understand the process of ageing. The illustration is inspired by a previously published figure [6].*

An approach to measure biological ageing was developed by Steve Horvath, a biomathematician who invented the epigenetic clock or 'Horvath's Clock' [9]. Chemical modifications to the genome such as methyl groups on DNA change during the course of a person's life and can be used to track humans' biological age: the age of an individual based on certain biomarkers and physiological parameters [10]. Horvath developed an algorithm which works on the methylation status of a specific set of positions on DNA [9]. The epigenetic clock's median deviation was 3.6 years, meaning that from a broad range of tissues, it can estimate the chronological age of half the donors with a range of 3.6 years [9]. The clock may therefore be used to detect age-related changes and diseases early and may even be seen as mortality predictors.

Recent insights: can ageing actually be reversed?

The question if ageing can actually be reversed has plagued people and scientists for decades. As the elderly population of society increases, so does the average individual risk for chronic diseases [1]. With more research being done into the molecular mechanisms of ageing, in parallel, longevity research and interventions to improve health-span have emerged as well.

Calorie restriction

Dietary interventions such as calorie restriction without malnourishment can establish an increase in lifespan and slow the ageing process [11]. A study performed in rats whose food intake was restricted showed an extension of the median and maximum lifespan and delayed the onset of chronic diseases [11]. In human studies, calorie restriction was shown to protect against development of cardiovascular disease, hypertension, obesity, and cancer, as it may directly or indirectly target pathways responsible for cell growth, mitochondrial function, and autophagy. However, the effect of calorie restriction on lifespan is not known yet.

There are also drugs that partially mimic the effect of calorie restriction by activating or repressing the pathways involved. For example, rapamycin, an immunosuppressant at high doses but immunostimulatory at low doses, is an antagonist of the mTOR signalling pathway that is responsible for nutrient sensing, protein translation, autophagy and ultimately leads to a lifespan extending effect in mice [11]. When mTOR signalling is reduced, a lifespan extension effect is observed.

Administration of NAD^+-boosting molecules

In recent years, David Sinclair, a molecular biologist from Harvard Medical School studying ageing, is controversial since he consumes nicotinamide mononucleotide (NMN), a precursor of NAD^+, to slow or even reverse ageing [12]. NAD^+, an abundant metabolite in the body, plays a role in cellular metabolism, cell survival and stress, and genomic stability [13]. NAD^+ levels decline with age and is related to age-associated physiological dysfunction [14]. Hence, increasing NAD^+ production through precursors such as NMN and nicotinamide riboside (NR) is hypothesised to reduce age-associated decline [15]. This hypothesis was found to be true for various species. Increased NAD^+ production in yeast, worms and flies led to prolongation of their lifespan. Similarly, mice administered with NMN during 12 months showed an improvement in insulin sensitivity, plasma lipid profile, eye function, bone density and age-associated body weight gain [15].

Sinclair's group administered NMN in 20-month-old mice (human equivalent ~70 years) and observed higher blood flow to muscles

compared to mice not given NMN. Moreover, they saw a restoration of the normal blood capillary density in NMN-administered mice, which is also observed in younger mice[15]. In addition, the NMN treated mice were able to run 430m compared to the 230m run by the untreated mice, hence displaying an increased exercise capacity [15].

In human trials, NR has shown to increase NAD^+ levels and is considered to be safe in short term [16, 17]. NR supplementation also improved symptoms of participants with ALS such as pulmonary function, muscular strength and slowed its progression [18]. However, administration of NR did not seem to improve glucose and insulin sensitivity [19, 20]. Clinical trials regarding NMN are in the initial stages and multiple studies testing its bioavailability, dosage, safety and efficacy are currently underway [21]. In 2021, a clinical trial in which prediabetic women who were overweight were administered 250mg/day of NMN for 10 weeks showed an increase in insulin sensitivity and insulin signalling in muscles as well as upregulated the expression of certain factors responsible for muscle remodelling [22].

Another interesting protein within this context is Sirtuin1 or SIRT1. SIRT1 belongs to a family of NAD dependent deacetylases. Sirtuins have multiple roles, including tissue regeneration, cell survival, protection from DNA damage and oxidative stress, cellular metabolism, and neuronal signalling [23]. Sirtuins are also responsible for maintaining genomic and epigenomic stability and control of gene expression [24]. For instance, SIRT6 is associated with chromatin and is involved in the transcriptional regulation of metabolic and DNA repair pathways by maintaining various genetic elements such as centromeres, transposable elements, and telomeres [24]. Overexpression of SIRT6 promoted healthspan and extended lifespan of mammals by improving the metabolic ability of the liver, promoting the generation of new neurons, and preventing endothelial cell dysfunction. In male mice where SIRT6 was overexpressed, a 27% increase in median lifespan was observed compared to the littermates [25]. Moreover, with age, blood capillaries die, which gradually reduces blood flow to muscles and organs, and eventually leads to cardiovascular and neurological disorders. Reduced blood flow also causes muscle weakness. Since NAD and SIRT1 levels decline with age, SIRT1 levels reduce in endothelial cells, and reduced SIRT1 signalling prevents capillary regeneration in muscle tissue. Furthermore, mice with endothelial cells deficient in SIRT1 showed a decreased number of capillaries and a diminished ability to form new blood vessels [26].

Partial Reprogramming

In 2006, a set of transcription factors known as the Yamanka factors were discovered. These factors Oct4, Sox2, Klf4, and C-Myc allowed the 'reprogramming' of somatic cells to stem cells [27, 28]. The transformed cells, also called induced pluripotent stem cells (iPSCs), have found multiple applications in therapy. A study performed in 2020 used three of the Yamanaka factors, Oct-4, Sox2 and KL4 (OSK) to partially reprogram retinal ganglion cells of mice suffering from age or injury-induced blindness into a functional state [29]. Retinal ganglion cells form the optic nerve, and if damaged, can regenerate axons only during the neonatal and embryonal stage. In the study, there was a restoration of axon density and half of the visual acuity in mice suffering from glaucoma compared to mice not suffering from glaucoma [29]. On a similar note, 12-month-old mice with injury-induced blindness, showed axon regeneration and retinal ganglion cell survival similar to the observation in 1-month and 3-month-old mice within this study [29].

Another study in 2022 developed a method called 'maturation phase transient reprogramming or MPTR which refers to reprogramming of cells by induction of Yamanaka factors until their epigenome is rejuvenated, which is then followed by the removal of the factors and allowing regular cell growth and proliferation [30]. In this study, fibroblasts of 50-year-old donors were exposed to Yamanaka factors for 13 days. During this period, the age-related changes are removed and the cells temporarily lose their identity and then reacquire it [30]. These partially reprogrammed cells are then allowed to proliferate under normal conditions. Genome analysis in the study showed rejuvenation of the epigenome and transcriptome of the cells by 30 years. In addition, the cells produced higher levels of collagen and had partially regained their migration speed; attributes characteristic of young fibroblasts [30].

Parabiosis and blood transfusions

Parabiosis is a procedure wherein old mice and young mice are sutured together so that they share a circulatory system. This method was usually used to study the effect of conjoined twins on each other [31]. However, a study first in 2005 exhibited the rejuvenating effect of parabiosis in old mice [32], and in 2014, another study showed that giving young blood to old mice stimulated the growth of brain stem cells and new neurons in the hippocampus as well as improved cognitive function [33]. Parabiosis allows the identification of a study of rejuvenating factors in blood and hence to further investigate the underlying factors responsible for this effect, scientists found an enzyme Tet2, that alters genetic expression by chemically tagging genes with a hydroxymethyl group [34]. Tet2 levels increased in mice that had undergone parabiosis and decreased with age in the hippocampi of mice. The blocking of Tet2 activity in young adult mice reduced neuronal growth and worsened memory and its overexpression led to an improvement in learning and memory [31, 34]. By identifying such molecular factors widens the possibility to target age-specific diseases such as Alzheimer's disease and macular degeneration.

Limitations

One of the most common issues with studies being done in ageing, is the use of animal models and the poor translation of animal research to humans, owing to differences in physiology. This is a major limitation hindering the feasibility of many anti-ageing therapies [3]. Even though preclinical studies show promising results for health and lifespan boosting interventions, data on the efficacy in humans is lacking.

With the increased interest in longevity- boosting molecules, there are several companies developing longevity pills and compounds [35]. Multiple NAD^+ boosting supplements are readily available in the market with claims of slowing ageing and boosting health, however, the current studies completed have mostly been short term and long term studies have not been performed yet; hence the clinical proof to substantiate these claims is either unknown or not yet published [12, 36]. The issue also lies in the fact that such nutraceuticals undergo fewer regulations and hence their safety and efficacy can be compromised [36]. In addition, ageing is not considered a disease and hence biotechnological and pharmaceutical companies are hesitant to fund clinical studies. These supplements are also sold as food products and not as therapy, thereby the lack of safety standards and regulations [21].

An example of how 'anti-ageing' has over time acquired a bad reputation in society is the case of Ambrosia, a company which in 2017 began selling plasma transfusions for $8000/litre in an attempt

to counter ageing and to rejuvenate the body [31, 37]. At present, there is a lack of substantial clinical proof for such procedures which can be harmful and even lethal. Plasma transfusions can overwhelm the circulatory system and present a high risk for allergic reactions, infections, cardiovascular and respiratory distress [38].

On a similar note, multiple limitations exist for the technique of partial reprogramming which is still being developed. How partially reprogrammed cells behave in the body is unknown [30]. Completely reprogrammed cells become cancerous when implanted in the body and this risk remains for the partially reprogrammed cells as well. Moreover, some of the questions that still need to be explored are if these cells retain their 'youth' once inside the body and whether the rejuvenation is age- dependent [39].

In conclusion, further research is needed on the long-term effects of these therapeutic interventions to understand how they affect the inter-related pathways involved in ageing and their systemic consequences. Moreover, epigenetic clocks can be used to pre-emptively test the effects of the various anti-ageing interventions to determine their efficacy at a molecular level. In an age of ever advancing scientific discoveries, perhaps future generations will uncover how to put a stopper in death, and perhaps this will lead to a new utopia, or rather, dystopia?

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EXAM QUESTION

Question 1
In the HPV test for cervical cancer, about 4% of the tests are false positives. What is the consequence of this 4% of false positive tests?

A. 4% of the colposcopies performed are unnecessary.
B. 96% of women tested have cervical cancer.
C. 96% of women tested are infected with HPV.

(Topic from Q3 MGZ Epidemiology, 2021)

Question 2
After an intensive afternoon of sports, a student has lost a lot of fluid due to sweating. He did not drink enough during and after exercise. His blood will be examined and the concentration of electrolytes will be looked at. What is the student most likely to have? The student has a…

A. hyperkalemia.
B. hypokalemia.
C. normokalemia.

(Topic from Q3 MGZ Epidemiology, 2021)

The answer to these questions can be found on page 32 in this journal.