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Scientific White Paper

Epiverse Formula. Epigenetic clock (DNAm, cytosine methylation) reversal, Includes phytotherapeutic extracts of: *Berberis vulgaris, Pinus sylvestris (Pollen), Lepidium meyenii, Taraxacum officinale, Elitaria cardamomum, and Cinnamomum verum*. Biological Actions, Molecular Mechanisms, and Their Effects.

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Epiverse Formula is an Epigenetic clock reversing (DNAm, cytosine methylation) synergistic herbal analog formulation of seven (7) plant extracts including: ***Berberis vulgaris, Pinus sylvestris (Pollen), Lepidium meyenii, Taraxacum officinale, Elitaria cardamomum, and Cinnamomum verum***. This formula is an herbal analog to the synergistically-rich formula containing HGH, DHEA and Metformin (with Vitamin D3 and Zinc), that was elucidated in the TRIIM study whose research was published in September 2019, and wherein the first demonstration of a reversal of the Epigenetic clock was observed.

An Overview of Epigenetics

Epigenetics refers to the modification of our DNA, RNA, or protein, which can change and regulate these molecules without altering the primary sequence. Our genetics, lifestyle, the food we eat, and the environment we live in, affects these modifications and therefore how our genes behave. Epigenetic mechanisms play a crucial role in regulating biological processes as diverse as development, learning, metabolism, and the progression of diseases such as cancer. Epigenetics can explain how external factors cause the modifications of our DNA and its structures, which results in gene regulation. Epigenetics is the study of heritable nonencoded genetic changes that turn genes on or off. Examples include activating changes such as histone acetylation and DNA demethylation, repressive changes like DNA methylation and histone modifications induced by noncoding RNAs, such as microRNA and long noncoding RNA (lncRNA). Epigenetic modifications can modulate gene expression and/or alter cellular signaling pathways, which may affect individual susceptibility to various diseases.

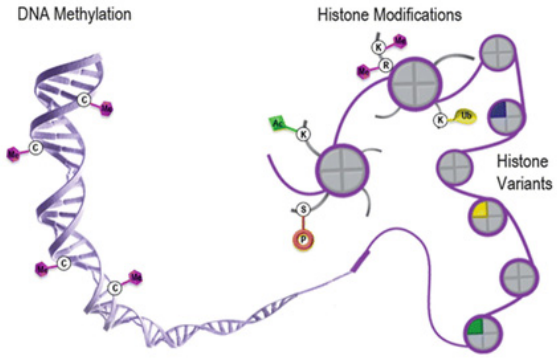
Epigenetic Clock

DNA methylation, the most studied epigenetic modification, is now recognized as a reliable indicator of biological age. Epigenetic aging clocks have been developed that are epigenetic age calculators built using DNA methylation data.

Epigenetic Changes

Epigenetic changes refer to stable and heritable modifications of chromatin, the DNA and its associated histone proteins, that are independent of the underlying DNA sequence and that determine the phenotypic traits of cells during development. The core unit of chromatin is the nucleosome, which consists of 147 bp of DNA folded around histone octamers containing two each of the histone proteins H2A, H2B, H3, and H4. Histone H2A and H3 are known to exist in multiple forms which differ in their primary

amino acid sequence at a limited number of sites in the histone. Changes in chromatin structure allow (or forbid) specific multiprotein transcriptional regulator complexes to access DNA sequences. Such changes in chromatin structure are achieved chiefly by three distinct mechanisms: DNA methylation, histone modifications, and ATP-dependent chromatin remodeling. Epigenetic modifications of chromatin have generally been considered to be both stable and heritable.



Epigenetic mechanisms determine the way genes are organized in the cell nucleus and influence their expression by changing the conformation of the chromatin and therefore the accessibility of the DNA for transcription factors, other factors, and the transcriptional machinery. These epigenetic mechanisms include post-translational histone modifications (PTMs), DNA methylation, and non-coding RNAs, resulting in activation, silencing, or posing of genes and thereby regulating patterns of gene expression. Both the environment and individual lifestyle can interact with the genome to influence epigenetic change. Such as many psychological challenges or stress can induce epigenetic mechanisms.

DNA methylation

DNA methylation, the best-known epigenetic signal, and is associated with condensed and compacted chromatin. It is thought to have the opposite effect, allowing transcriptional activation. DNA methylation typically occurs at cytosines that are followed by a guanine.

Epigenetics and Gene Expression

Epigenetics plays an important role in affecting gene expression. Histone modifications determine the genome's accessibility to transcription factors, while DNA methylation is influenced by post-translational modifications of histone proteins, such as acetylation, methylation, phosphorylation, ubiquitination, and crotonylation. Epigenetic marks can be trans-generationally transmitted through the germline.

The TRIIM Study

"In a small trial, drugs seemed to rejuvenate the body's 'epigenetic clock', which tracks a person's biological age. For one year, nine healthy volunteers took a cocktail of three common drugs — growth hormone and two diabetes medications — and on average shed 2.5 years of their biological ages, measured by analyzing marks on a person's genomes. The participants' immune systems also showed signs of rejuvenation. The Thymus Regeneration, Immuno-restoration and Insulin Mitigation (TRIIM) trial tested 9 white men between 51 and 65 years of age. It was led by immunologist Gregory Fahy, the chief scientific officer and co-founder of Intervene Immune in Los Angeles, CA and was approved by the US Food and Drug

Administration in May 2015. It began a few months later at Stanford Medical Center in Palo Alto, California. In the TRIIM trial, the scientists took blood samples from participants during the treatment period. Tests showed that blood-cell count was rejuvenated in each of the participants. The researchers also used magnetic resonance imaging (MRI) to determine the composition of the thymus at the start and end of the study. They found that in seven participants, accumulated fat had been replaced with regenerated thymus tissue. Checking the effect of the drugs on the participants' epigenetic clocks was an afterthought. The clinical study had finished when Fahy approached Horvath to conduct an analysis.

Horvath Clock

Dr. Steven Horvath used four different epigenetic clocks to assess each patient's biological age, and he found significant reversal for each trial participant in all of the tests. "This told me that the biological effect of the treatment was robust," he says. "What's more, the effect persisted in the six participants who provided a final blood sample six months after stopping the trial, he says. Because we could follow the changes within each individual, and because the effect was so very strong in each of them, I am optimistic," says Horvath.

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Active Herbal Ingredients

Berberis vulgaris, source of Berberine is an analog to Metformin, an anti-diabetic and component of the TRIIM study formula. Berberine, a major isoquinoline alkaloid present in ***Berberis vulgaris***, is a potent inhibitor of inflammation and has shown anti-diabetic activity. Type 2 diabetes and obesity are rapidly becoming a worldwide epidemic and they are associated with the development of insulin resistance. Insulin resistance is believed to be an underlying feature of type 2 diabetes and metabolic syndrome. Berberine has a wide range of pharmacologic actions, such as antidiarrheic, anticancer, and antiinflammation. It has been used for the treatment of infective and inflammatory disorders. It improves insulin resistance, lowers blood sugar, and treats lipid metabolism disorders by activating the AMP activated protein kinase (AMPK) pathways. Berberine inhibits gene expression of proinflammatory cytokines in adipose tissue of obese mice and suppresses inflammatory response through AMPK activation in macrophages, while demonstrating its anti-inflammatory potency.

Pinus sylvestris (Pollen), a source high in natural Growth Hormones. Pine pollen is a potent source of androgenic substances composed of the bioidentical steroid hormone testosterone, along with lesser amounts of other steroids including androstenedione, dehydroepiandrosterone (DHEA) and androsterone. Pine pollen also contains estrogens including estrone, estriol and estradiol, as well progesterone.

Lepidium meyenii, Maca, is a natural source of DHEA. It contains polysaccharides, polyphenols (flavonolignans), macaenes, macamides, glucosinolates, and alkaloids. Various bioactivities of Maca include enhanced reproductive health, antifatigue, antioxidation, neuroprotection, antimicrobial activity, anticancer, hepatoprotection, immunomodulation, and improving skin health and digestive system's function.

Taraxacum officinale, Dandelion, a source high in Vitamin D. A source of the autophagy inducing flavonoids quercetin, luteolin, apigenin and luteolin-7-glucoside Extracts have anti-influenza, anti-retrovirus activity, antioxidant and hepatoprotective effects. Ethanol extracts reduce inflammation and inhibit angiogenesis. Dandelion contains sesquiterpene lactones (believed to have anti-inflammatory and anticancer effects).

Elitaria cardamomum, is one of the highest sources of plant-based zinc. Extracts of cardamom may be effective against a variety of bacterial strains that contribute to fungal infections.

Cinnamomum verum, Cinnamon health benefits are attributed to its content of a few specific types of antioxidants, including polyphenols, phenolic acid and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease. The effects of cinnamon on life span implicated major longevity pathways. These include the DAF-16 transcription factor in the insulin signaling pathway, which promotes expression of stress resistance and the longevity genes. Cinnamon activates the insulin signaling pathway, anti-oxidative pathway and serotonin signaling for its lifespan prolonging effect.

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ORIGINAL ARTICLE

Aging Cell WILEY

Reversal of epigenetic aging and immunosenescent trends in humans

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Abstract

Epigenetic "clocks" can now surpass chronological age in accuracy for estimating biological age. Here, we use four such age estimators to show that epigenetic aging can be reversed in humans. Using a protocol intended to regenerate the thymus, we observed protective immunological changes, improved risk indices for many age-related diseases, and a mean epigenetic age approximately 1.5 years less than baseline after 1 year of treatment (−2.5-year change compared to no treatment at the end of the study). The rate of epigenetic aging reversal relative to chronological age accelerated from −1.6 year/year from 0–9 month to −6.5 year/year from 9–12 month. The GrimAge predictor of human morbidity and mortality showed a 2-year decrease in epigenetic vs. chronological age that persisted six months after discontinuing treatment. This is to our knowledge the first report of an increase, based on an epigenetic age estimator, in predicted human lifespan by means of a currently accessible aging intervention.

KEYWORDS

c-reactive protein, lymphocyte-to-monocyte ratio, naive T cells, PD-1, PSA, thymic regeneration

1 | INTRODUCTION

Population aging is an increasingly important problem in developed countries, bringing with it a host of medical, social, economic, political, and psychological problems (Rae et al., 2010). Over the last several years, many biomedical approaches to ameliorating aging have been investigated in animal models, and some of these seem able to reverse general aspects of aging in adult mammals based on a variety of physiological measurements (Das et al., 2018; Ocampo et al., 2016; Zhang et al., 2017). However,

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Epigenetic clock analyses of cellular senescence and ageing

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ABSTRACT

A confounding aspect of biological ageing is the nature and role of senescent cells. It is unclear whether the three major types of cellular senescence, namely replicative senescence, oncogene-induced senescence and DNA damage-induced senescence are descriptions of the same phenomenon instigated by different sources, or if each of these is distinct, and how they are associated with ageing. Recently, we devised an epigenetic clock with unprecedented accuracy and precision based on very specific DNA methylation changes that occur in function of age. Using primary cells, telomerase-expressing cells and oncogene-expressing cells of the same genetic background, we show that induction of replicative senescence (RS) and oncogene-induced senescence (OIS) are accompanied by ageing of the cell. However, senescence induced by DNA damage is not, even though RS and OIS activate the cellular DNA damage response pathway, highlighting the independence of senescence from cellular ageing. Consistent with this, we observed that telomerase-immortalised cells aged in culture without having been treated with any senescence inducers or DNA-damaging agents, re-affirming the independence of the process of ageing from telomeres and senescence. Collectively, our results reveal that cellular ageing is distinct from cellular senescence and independent of DNA damage response and telomere length.

INTRODUCTION

While ageing at the level of the organism is obvious and easily understood, the biological aspect of ageing is far from clear. Even the definition of ageing is not self-evident. It is reasonable to consider ageing as a natural biological process that in time, leads to the eventual failure of organs, as it is this that gives rise to the phenotypic features of ageing; from the benign, such as thinning of the skin and greying of the hair, to the pathological, such as cataracts and cardiovascular disease. Understanding why tissues and cells function sub-optimally and eventually fail in time, will greatly aid our understanding of ageing.

One model of ageing posits that the failure of tissues to function properly is due to the depletion of stem cells [1]. Stem cells, which are the reservoir cells of tissues, may have finite capacities of proliferation such as being limited by the lengths of their telomeres. Their eventual depletion leads to the deficit of properly functioning cells, causing phenotypic changes that constitute ageing. While this model is plausible and supported by strong circumstantial evidence, it is presently difficult to prove or refute directly, not least because the identification of specific tissue stem cells is difficult. Similarly, the association between telomere length and ageing, although widely reported, is not without inconsistencies [2-4].

There is however, another model of ageing which is based on the observation that the number of senescent cells in the body increases in function of organism age [5-7]. While this could be interpreted to mean that senescent cells cause ageing, it could also equally mean that senescent cells are a consequence of ageing. In this regard, it is noteworthy that there is increasing evidence to demonstrate that senescent cells are not benign. Instead they secrete bio-chemicals that are detrimental to normal functioning of neighbouring cells. The senescence-associated secretory phenotype (SASP) proteins include cytokine, chemokines and proteases [8, 9] and their paracrine activities are very diverse and include oncogenic characteristics that stimulate cellular proliferation and

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Genome Biology

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DNA methylation aging clocks: challenges and recommendations

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Abstract

Epigenetic clocks comprise a set of CpG sites whose DNA methylation levels measure subject age. These clocks are acknowledged as a highly accurate molecular correlate of chronological age in humans and other vertebrates. Also, extensive research is aimed at their potential to quantify biological aging rates and test longevity or rejuvenating interventions. Here, we discuss key challenges to understand clock mechanisms and biomarker utility. This requires dissecting the drivers and regulators of age-related changes in single-cell, tissue- and disease-specific models, as well as exploring other epigenomic marks, longitudinal and diverse population studies, and non-human models. We also highlight important ethical issues in forensic age determination and predicting the trajectory of biological aging in an individual.

Introduction

A key question in biology is to understand why and how we age. Alongside this, the unprecedented gain in the average lifespan in humans, since the mid-twentieth century, has dramatically increased both the number of older people and their proportion in the population. This demographic phenomenon is changing our societal make-up, from only ~130 million being 65 years or older (~5% of the world population) in 1950, to a predicted ~1.6 billion people (~17%) by 2050 [1]. However, the success in reducing mortality has not been matched with a reduction in chronic disease [2]. This leads to the undesirable outcome of many years of this prolonged lifespan being spent in ill health, with an associated massive health care burden. Increasing the productivity and reducing the disease affliction in these extended years7

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Research Paper

Epigenetic ageing is distinct from senescence-mediated ageing and is not prevented by telomerase expression

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ABSTRACT

The paramount role of senescent cells in ageing has prompted suggestions that re-expression of telomerase may prevent ageing; a proposition that is predicated on the assumption that senescent cells are the sole cause of ageing. Recently, several DNA methylation-based age estimators (epigenetic clocks) have been developed and they revealed that increased epigenetic age is associated with a host of age-related conditions, and is predictive of lifespan. Employing these clocks to measure epigenetic age *in vitro*, we interrogated the relationship between epigenetic ageing and telomerase activity. Although hTERT did not induce any perceptible change to the rate of epigenetic ageing, hTERT-expressing cells, which bypassed senescence, continued to age epigenetically. Employment of hTERT mutants revealed that neither telomere synthesis nor immortalisation is necessary for the continued increase in epigenetic age by these cells. Instead, the extension of their lifespan is sufficient to support continued epigenetic ageing of the cell. These characteristics, observed in cells from numerous donors and cell types, reveal epigenetic ageing to be distinct from replicative senescence. Hence, while re-activation of hTERT may stave off physical manifestation of ageing through avoidance of replicative senescence, it would have little impact on epigenetic ageing which continues in spite of telomerase activity.

INTRODUCTION

Although ageing is readily observed at the level of the organism, our understanding of why and how this process occurs has remained speculative until normal human cells were successfully cultured outside the body, where they were found to have a finite capacity to proliferate. Hayflick estimated that a population of human cells grown *ex vivo* can double approximately sixty times after which they adopt a permanent state of dormancy termed replicative senescence [1, 2]. The cause of this natural limitation to proliferation was eventually found to lie in the "end-replication problem", which if not addressed by the cell, would lead to telomere attrition at every round of DNA replication [3, 4]. It was eventually demonstrated that this does indeed occur and when telomeres shorten to a critical length they trigger cells to adopt the senescent state [5, 6]. The identification of telomerase, which replicates telomeres [7, 8], and the fact that most adult somatic cells do not produce this enzyme, provided the last major piece of the puzzle that describes the ageing process from events beginning with molecules, proceeding to cells and culminating in the organism. Significantly, this chain of

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Epigenetics & Chromatin

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Age reprogramming and epigenetic rejuvenation

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Abstract

Age reprogramming represents a novel method for generating patient-specific tissues for transplantation. It bypasses the de-differentiation/redifferentiation cycle that is characteristic of the induced pluripotent stem (iPS) and nuclear transfer-embryonic stem (NT-ES) cell technologies that drive current interest in regenerative medicine. Despite the obvious potential of iPS and NT-ES cell-based therapies, there are several problems that must be overcome before these therapies are safe and routine. As an alternative, age reprogramming aims to rejuvenate the specialized functions of an old cell without de-differentiation; age reprogramming does not require developmental reprogramming through an embryonic stage, unlike the iPS and NT-ES cell-based therapies. Tests of age reprogramming have largely focused on one aspect, the epigenome. Epigenetic rejuvenation has been achieved in vitro in the absence of de-differentiation using iPS cell reprogramming factors. Studies on the dynamics of epigenetic age (eAge) reprogramming have demonstrated that the separation of eAge from developmental reprogramming can be explained largely by their different kinetics. Age reprogramming has also been achieved in vivo and shown to increase lifespan in a premature ageing mouse model. We conclude that age and developmental reprogramming can be disentangled and regulated independently in vitro and in vivo.

Keywords: Age reprogramming, Epigenetic rejuvenation, Somatic cell nuclear transfer (SCNT), iPS cells, Reprogramming factors, Epigenetic clock, eAge

Background

Animal cloning experiments using somatic cell nuclear transfer (SCNT) revealed that ageing is reversible. SCNT was initially described in amphibians [1, 2] and later in mammals [3]. These influential experiments showed that nuclear reprogramming of somatic cells was a process by which adult differentiated cells reacquired developmental and ageing potential (Box 1). The result was a newborn clone, which was genetically identical to the somatic cell transferred into the recipient oocyte, a clone that now possessed the potential of a normal lifespan even when the somatic cell was derived from an old donor [4, 5]. Thus, measurable age-associated changes found in old cells can be reversed by SCNT. More recently, the seminal studies of Yamanaka and colleagues have shown that "reprogramming factors", *Oct4*, *Sox2*, *Klf4* and *c-Myc*, can reprogram somatic cells into induced pluripotent stem (iPS) cells even from an elderly 82-year-old donor [6, 7]. Importantly, senescent fibroblasts from elderly donors can be de-differentiated into iPS cells by introduction of reprogramming factors and then redifferentiated back to fibroblasts that have lost the senescent phenotype and acquired the characteristics of young fibroblasts [8]. Putting it short, induction of iPS cells can, like NT-ES cells, reset the ageing clock.

Both techniques can reverse molecular hallmarks of ageing [9]. For example, telomere attrition can be reversed by induction of iPS cells whereupon telomerase lengthens the telomeres [10]. Telomeres are also extended in nuclei of reconstructed embryos [11] although the mechanism(s) involved is likely to be more complicated, using both telomerase and telomere sister chromatid exchange [12]. iPS cells also have reduced DNA damage [13] and enhanced mitochondrial function [14]. Cells differentiated from iPS cells lose expression of markers of

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Aging, Rejuvenation, and Epigenetic Reprogramming: Resetting the Aging Clock

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Abstract

The underlying cause of aging remains one of the central mysteries of biology. Recent studies in several different systems suggest that not only may the rate of aging be modified by environmental and genetic factors, but also that the aging clock can be reversed, restoring characteristics of youthfulness to aged cells and tissues. This Review focuses on the emerging biology of rejuvenation through the lens of epigenetic reprogramming. By defining youthfulness and senescence as epigenetic states, a framework for asking new questions about the aging process emerges.

Introduction

The inexorable tolls of aging are evident in almost all living beings. From the onset of reproductive maturity, organismal aging is generally characterized by a decline in fecundity, an increased susceptibility to disease and tissue dysfunction, and increased risk of mortality (Kirkwood, 2005; Hayflick, 2007; Kirkwood and Shanley, 2010). Aging is associated with a gradual loss of homeostatic mechanisms that maintain the structure and function of adult tissues. A major challenge of aging research has been to distinguish the causes of cell and tissue aging from the myriad of changes that accompany it. One of the hallmarks of cellular aging is an accumulation of damaged macromolecules such as DNA, proteins, and lipids. These become chemically modified by reactive molecules, such as free radicals, that are generated during normal cellular metabolism and whose production increases with age (Haigis and Yankner, 2010). DNA damage may lead to cellular dysfunction directly by altering the expression of specific genes or indirectly as result of cellular responses to damage that can alter gene expression more globally (Seviour and Lin, 2010; Campisi and Vijg, 2009). Damage to proteins may independently contribute to cellular aging if mis-folded or damaged proteins are replaced more slowly than they are generated, especially when they form stable aggregates that are not degraded by the cell (Koga et al., 2011). Such "proteotoxicity" has been postulated to underlie many age-related diseases and may also be an important part of normal cellular aging (Douglas and Dillin, 2010).

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Stem Cell Research & Therapy

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Rejuvenation by cell reprogramming: a new horizon in gerontology

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Abstract

The discovery of animal cloning and subsequent development of cell reprogramming technology were quantum leaps as they led to the achievement of rejuvenation by cell reprogramming and the emerging view that aging is a reversible epigenetic process. Here, we will first summarize the experimental achievements over the last 7 years in cell and animal rejuvenation. Then, a comparison will be made between the principles of the cumulative DNA damage theory of aging and the basic facts underlying the epigenetic model of aging, including Horvath's epigenetic clock. The third part will apply both models to two natural processes, namely, the setting of the aging clock in the mammalian zygote and the changes in the aging clock along successive generations in mammals. The first study demonstrating that skin fibroblasts from healthy centenarians can be rejuvenated by cell reprogramming was published in 2011 and will be discussed in some detail. Other cell rejuvenation studies in old humans and rodents published afterwards will be very briefly mentioned. The only in vivo study reporting that a number of organs of old progeric mice can be rejuvenated by cyclic partial reprogramming will also be described in some detail. The cumulative DNA damage theory of aging postulates that as an animal ages, toxic reactive oxygen species generated as byproducts of the mitochondria during respiration induce a random and progressive damage in genes thus leading cells to a progressive functional decline. The epigenetic model of aging postulates that there are epigenetic marks of aging that increase with age, leading to a progressive derepression of DNA which in turn causes deregulated expression of genes that disrupt cell function. The cumulative DNA damage model of aging fails to explain the resetting of the aging clock at the time of conception as well as the continued vitality of species as millenia go by. In contrast, the epigenetic model of aging straightforwardly explains both biologic phenomena. A plausible initial application of rejuvenation in vivo would be preventing adult individuals from aging thus eliminating a major risk factor for end of life pathologies. Further, it may allow the gradual achievement of whole body rejuvenation.

Keywords: Aging, Epigenetics, Rejuvenation, Cell reprogramming, Therapeutic potential

Rejuvenation: a perennial dream

The longing of man for eternal youth is universal and time immemorial. Initially sought in religions, during the Middle Ages, alchemists, a blend of mystics and proto-chemists, tried to synthesize a mysterious potion, the elixir of eternal youth, able to confer indefinite youth to those that dare to drink it. Now, it seems that science has found the biological fountain of rejuvenation—the cytoplasm of the oocyte.

The story of biological rejuvenation began in the early 1960s, with the discovery of animal cloning in frogs by John Gurdon and collaborators [1]. Mammalian cloning was achieved 30 years later, in 1996, with the birth of Dolly, the sheep [2]. Cloning of other mammalian species followed soon. It was clear that the cytoplasm of a mature oocyte contained molecules able to turn a somatic nucleus into an embryonic one that could direct the development of a new individual. At the time, it was assumed that in the oocyte's cytoplasm, there should be a complex constellation of reprogramming factors,

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ORIGINAL ARTICLE

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Reversal of epigenetic aging and immunosenescent trends in humans

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Abstract

Epigenetic "clocks" can now surpass chronological age in accuracy for estimating biological age. Here, we use four such age estimators to show that epigenetic aging can be reversed in humans. Using a protocol intended to regenerate the thymus, we observed protective immunological changes, improved risk indices for many age-related diseases, and a mean epigenetic age approximately 1.5 years less than baseline after 1 year of treatment (−2.5-year change compared to no treatment at the end of the study). The rate of epigenetic aging reversal relative to chronological age accelerated from −1.6 year/year from 0–9 month to −6.5 year/year from 9–12 month. The GrimAge predictor of human morbidity and mortality showed a 2-year decrease in epigenetic vs. chronological age that persisted six months after discontinuing treatment. This is to our knowledge the first report of an increase, based on an epigenetic age estimator, in predicted human lifespan by means of a currently accessible aging intervention.

KEYWORDS
c-reactive protein, lymphocyte-to-monocyte ratio, naive T cells, PD-1, PSA, thymic regeneration

1 | INTRODUCTION

Population aging is an increasingly important problem in developed countries, bringing with it a host of medical, social, economic, political, and psychological problems (Rae et al., 2010). Over the last several years, many biomedical approaches to ameliorating aging have been investigated in animal models, and some of these seem able to reverse general aspects of aging in adult mammals based on a variety of physiological measurements (Das et al., 2018; Ocampo et al., 2016; Zhang et al., 2017). However,

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Letter to the Editor

The epigenetic clock: a molecular crystal ball for human aging?

Simone Ecker and Stephan Beck

A hat trick of new epigenetic clocks have recently been published by Horvath et al.: The Skin & Blood clock [1] provides a more precise estimation of chronological age in tissues and cell types frequently used in research and forensics, while PhenoAge [2] and GrimAge [3] aim to capture biological aging and derive an improved prediction of mortality and morbidity risks. Together, these new epigenetic clocks present valuable tools to investigate human aging, shed light on the question of why we all age differently, and develop strategies to extend human life- and healthspan.

In 2013, the first epigenetic age estimation method that works with high accuracy across almost all human tissues and cell types was published by Steve Horvath [4]. The publication of this multi-tissue clock marked a milestone in epigenetics and aging research, and since then, numerous studies have confirmed not only its ability to accurately estimate an individual's age but also the clock's great value for studying the human aging process.

Horvath's multi-tissue clock is based on DNA methylation data. DNA methylation, the addition of methyl groups to cytosine bases of the DNA, is the most widely studied epigenetic modification so far. It plays an important role in the regulation of gene expression, altering the phenotype without changing the genotype. A particular locus in the genome can either be methylated or unmethylated. But as DNA methylation measurements are usually obtained from a pool of tens of thousands of cells, what is measured, is the proportion of the cells in which a locus is methylated. These proportions are given in β-values between 0 (unmethylated in all cells) and 1 (methylated in all cells). Thus, methylation β-values effectively measure cell-to-cell variability within a sample.

In many positions of the human genome, this methylation heterogeneity changes with age. These usually small but consistent age-associated changes in DNA methylation are what make the epigenetic clock work. And it works very precisely, with a median absolute error (MAE) of only 3.6 years, clearly outperforming previously used molecular biomarkers of age such as telomere length [4,5].

However, deviations of the age estimation derived by DNA methylation compared to chronological age do also provide valuable information. There is significant interindividual variability present in the human aging process [6–8]. Biological aging occurs at different rates across individuals who can exhibit considerably distinct physical fitness and age-related disease susceptibilities despite being the same chronological age. The epigenetic clock has intriguingly demonstrated to be able to quantify these differences and give a biologically relevant prediction of age, that is, a measurement of biological or physiological age. DNA methylation age predicts all-cause mortality better than chronological age, and it has also been associated with physical and mental fitness, vegetable and fish intake, obesity, smoking, alcohol use, lifetime stress, social class and multiple other factors [5,9].

Recently, three further improved epigenetic clocks were published: The Skin & Blood clock [1], DNA methylation PhenoAge [2], and DNA methylation GrimAge [3]. While the latter two aim to provide an improved prediction of mortality and are more closely related to physiological dysregulation, the Skin & Blood clock gives an even more accurate prediction of chronological age of easily accessible human tissues – for example, whole blood, saliva and skin – and cell types often used in research such as fibroblasts and lymphoblastoid cell lines.

In a set of whole blood samples, the application of the Skin & Blood clock resulted in age estimations with a MAE of 2.5 [1]. The correlation between chronological age and estimated age also improved correspondingly, from a Pearson's r of 0.96 for the multi-tissue clock to 0.98 in the Skin & Blood clock [1].

We observed similar results when we applied the Skin & Blood clock, the multi-tissue clock, and the PhenoAge clock to a dataset of 656 samples (age range 19 to 101 years) with which another highly accurate epigenetic age estimator for whole blood samples was developed by Hannum et al. [10] [of note, we did not include GrimAge in these comparisons, as GrimAge is supposed to be a predictor of mortality rather than an age predictor and takes (chronological) age itself as input, together with sex and DNA methylation measurements]. The Skin & Blood clock exhibited the least error and the best correlation with chronological age (see Table 1, and Figure 1), confirming its improved performance in chronological age prediction.

The high sensitivity of the Skin & Blood clock in the above-mentioned cell types makes it particularly interesting for *ex vivo* experiments, as it provides a critical

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Protective effect of berberine on renal fibrosis caused by diabetic nephropathy

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Abstract. Berberine (BBR) is a material extracted from Chinese herbs, which has been used in the treatment of diabetes in Chinese medicine for thousands of years. However, the importance of BBR in renal fibrosis remains to be elucidated. In the present study, streptozotocin-induced diabetic nephropathy (DN) rats were used to determine the effect of BBR on renal fibrosis. The pathology of the kidneys was examined using periodic acid-Schiff (PAS) and Masson staining. The expression levels of transforming growth factor-β (TGF-β) and α smooth muscle actin (α-SMA) in kidneys were observed using immunohistochemical staining. The mRNA and protein expression levels of TGF-β, α-SMA, vimentin, nuclear factor-κB were examined using reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. PAS and Masson staining revealed that there was notable glomerular hypertrophy and mesangial matrix expansion in DN rats. Immunohistochemistry revealed that there was a significant increase in TGF-β and α-SMA expression levels in the renal tubulointerstitium and the extracellular matrix. However, treatment with BBR may significantly reduce kidney injury. The protein and mRNA expression levels of TGF-β, vimentin and α-SMA were significantly increased in DN rats compared with the control group; however, this increase was reduced following treatment with BBR. The present study revealed that BBR may inhibit fibrosis and ameliorate the symptoms of DN. The current findings indicated that BBR may be used as a potential treatment for patients with DN.

Introduction

Diabetes and its associated complications, such as diabetic nephropathy (DN) have become a serious health problem. Approximately one-third of all diabetic patients suffer from DN (1), which has significant social and economic burdens (2) and may be the leading cause of end-stage renal disease (ESRD). In the United States ~200,000 patients receive ESRD care due to diabetic kidney disease, with 50,000 new patients starting dialysis yearly (3,4). The primary indicators of DN are continuous albuminuria, high blood pressure and progressive renal damage. However, the specific pathogenic mechanisms remain to be fully elucidated. Hyperglycemia has an important part in the development of DN; however, additional factors, such as inflammation due to fibrosis, are considered to be important for the initiation and progression of diabetic nephropathy.

The major pathological alterations of DN include mesangial expansion, extracellular matrix (ECM) alterations, tubulointerstitial fibrosis and glomerular sclerosis. Transforming growth factor-β (TGF-β) has been identified to be a key regulator of fibrosis in DN (5). Overexpression of TGF-β may promote epithelial-mesenchymal transition (EMT) and renal sclerosis, ultimately leading to organ failure (6). Chen *et al* (7) demonstrated that suppression of the TGF-β/mothers against decapentaplegic signaling pathway may greatly ameliorate streptozotocin (STZ)-induced fibrosis and albumin levels in the urine of rats (7). Therefore, therapeutic agents that may inhibit TGF-β and its signaling pathways may also reduce the progress of DN.

At present, despite the wide use of therapeutic approaches focused on managing hyperglycemia and high blood pressure, numerous patients continue to suffer from progressive and severe renal injury. Therefore, it is important to develop novel renal protective drugs for the treatment of DN. Berberine (BBR) is a type of isoquinolone alkaloid that is extracted from the widely used Chinese herb, *Rhizoma coptidis*. Recent studies have indicated that BBR has multiple pharmacological activities, including hypolipidemic, antioxidant and glucose-lowering, suggesting that it may have clinical potential as an alternative therapeutic drug for diabetic complications (8,9). Liu *et al* (10) reported that BBR effectively lowered blood glucose and lipid levels through suppression of oxidative stress. However, it remains to be elucidated if BBR exerts its beneficial effects in DN via regulation of TGF-β. The present study used a STZ-induced DN rat model to investigate the effect of BBR on the activation of TGF-β and its associated pathways in DN.

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Key words: diabetic nephropathy, fibrosis, berberine, kidney

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REVIEW Open Access

Central intracrine DHEA synthesis in ageing-related neuroinflammation and neurodegeneration: therapeutic potential?

Y S L Powrie and C Smith*

Abstract

It is a well-known fact that DHEA declines on ageing and that it is linked to ageing-related neurodegeneration, which is characterised by gradual cognitive decline. Although DHEA is also associated with inflammation in the periphery, the link between DHEA and neuroinflammation in this context is less clear. This review drew from different bodies of literature to provide a more comprehensive picture of peripheral vs central endocrine shifts with advanced age—specifically in terms of DHEA. From this, we have formulated the hypothesis that DHEA decline is also linked to neuroinflammation and that increased localised availability of DHEA may have both therapeutic and preventative benefit to limit neurodegeneration. We provide a comprehensive discussion of literature on the potential for extragonadal DHEA synthesis by neuroglial cells and reflect on the feasibility of therapeutic manipulation of localised, central DHEA synthesis.

Keywords: Steroidogenesis, Extragonadal, Accelerated ageing, Alzheimer's, Immunosenescence, Sulphotransferase, Translocator protein, Species-specific, Neuroprotection, Antioxidant

Background

From the recent literature, it is evident that the processes of neuroinflammation and neurodegeneration are inextricably linked. Given the sequestered nature of the brain, which complicates research sample collection for obvious reasons, many investigators seem to extrapolate data generated from peripheral samples in attempts to explain central events. However, as also illustrated in the pages to follow, there is often a disconnection between adaptation in the periphery versus those occurring centrally. In our opinion, there are multiple reasons for this. Firstly, the neuroimmune system is structurally distinct from the peripheral system in that most immune functions are mediated by cells specific to the nervous system, such as microglia and astrocytes [1]. Incidentally, although recent commentaries and research letters pertaining to the identification of a lymphatic system in the dural spaces are suggesting that the brain may be subject to surveillance by immune cells circulating from the periphery [2], not enough data exist with which to evaluate the relative importance of these immune cells relative to those residents in the brain. Secondly, the brain has a preference for glucose as a substrate, as opposed to most peripheral organs, such as the heart, which mainly derive energy through fatty acid β-oxidation, which may affect the outcome of adaptive—or maladaptive—metabolic responses differently in the brain to peripheral compartments. Lastly, although the brain itself is subject to glucocorticoid-mediated metabolic modulation, as in the peripheral compartment, it is also directly affected by—and thus adapts as result of—psychological input.

From this, it is clear that different factors come into play centrally vs. peripherally in terms of adaptation to stimuli. This questions the validity of treating neuroinflammation—especially in the context of chronic disease aetiology—using the same strategies by which peripheral chronic low-grade inflammation is addressed. It further highlights the need for specific, central investigations for the purpose of answering questions pertaining to central physiological adaptation or maladaptation.

We have identified the hormone (also aptly referred to as a neurosteroid) dehydroepiandrosterone (DHEA) as a relatively under-researched hormone in the context of

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The role of zinc in pre- and postnatal mammalian thymic immunohistogenesis.

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Abstract

Mammalian thymic histogenesis can be morphologically divided into three consecutive stages: a) epithelial, b) lymphopoietic or lympho-epithelial, and 3) differentiated cellular microenvironmental, with formation of Hassall's bodies (HBs). Immunomorphological changes characteristic of human thymic involution begin during or soon after the first year after birth, and continue progressively throughout the entire life span. The 3% to 5% annual reduction in the number of cells of the human thymic microenvironment continues until middle age, when it slows down to less than 1% per year. According to the extrapolation of these results, total loss of thymic reticulo-epithelial (RE) tissue and the associated thymocytes should occur at the age of 120 years in humans. The marked reduction of the thymic cellular microenvironment is a well-controlled physiological process and is presumably under both local and global regulation by the cells of the RE meshwork and by the neuroendocrine axis, respectively. In humans, the age related decline of facteur thymique serique (FTS) levels in blood begins after 20 years of age and FTS completely disappears between the 5th and 6th decade of life. In contrast, serum levels of thymosin-alpha 1 and thymopoietin seem to decline earlier, starting as early as 10 years of age. The influences of a variety of other hormones on the involution of the thymus have also been characterized: testosterone, estrogen, and hydrocortisone treatment results in marked involution, cortisone and progesterone administration have a slight to moderate effect while use of desoxycorticosterone has no effect. The experimental administration of thyroxin yielded dose dependent results: low doses resulted in thymic hypertrophy, higher doses produced a slight hypertrophy, while the highest employed doses caused thymic atrophy. The atrophy was of apicnotic type, very different from that detected after treatment with corticoid hormones. Thymus transplantation experiments indicate that age-related, physiological thymic involution has been genetically preprogrammed. Grafting of the thymus from one week old C3H leukemic strain mice into 6 month old hosts resulted in changes in thymic weight and involution patterns that were synchronous in all recipients, in direct correlation with the glands in the donor, but not in the

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