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REVIEW ARTICLE

New Concepts in the Manipulation of the Aging Process

Peter Hollands[1,*] and Todd Ovokaitys[2]

[1]*CTO Qigenix, 6125 Paseo Del Norte, Suite 140, Carlsbad, CA 92008, USA;* [2]*CEO Qigenix, 6125 Paseo Del Norte, Suite 140, Carlsbad, CA 92008, USA*

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Abstract: This review explores the current concepts in aging and then goes on to describe a novel, ground-breaking technology which will change the way we think about and manage aging. The foundation of the review is based on the work carried out on the QiLaser activation of human Very Small Embryonic Like (hVSEL) pluripotent stem cells in autologous Platelet Rich Plasma (PRP), known as the Qigeneration Procedure. The application of this technology in anti-aging technology is discussed with an emphasis on epigenetic changes during aging focusing on DNA methylation.

Keywords: Platelet rich plasma (PRP), DNA, epigenetic, methylation, QiLaser activation, human very small embryonic like (hVSEL) stem cells,.

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"When I was younger, I could remember anything, whether it had happened or not; but my faculties are decaying now and soon I shall be so I cannot remember any but the things that never happened. It is sad to go to pieces like this, but we all have to do it".

1. INTRODUCTION

There are two certainties in life: death and taxes. A third certainty is aging because, from the moment we are born, we age until the moment of our death. This death can of course be at any age. In 1935 it was discovered that a reduction in calorific intake in rodents may increase lifespan [1]. More recently, it has been suggested that aging has many complex and interacting factors associated with it including the decreasing effectiveness of intercellular communications [2], stem cell 'exhaustion' [3], mitochondrial dysfunction [4], epigenetic alterations [5], telomere shortening [6], proteostasis [7] and genomic instability [8]. Aging is clearly a multi-system process in adults and in premature aging in children the focus seems to be on atherosclerosis and stroke but with no cognitive damage [9] suggesting that the protein which causes premature aging in children, progerin, does not interact with the central nervous system. This may be due to the inability of progerin to cross the blood-brain barrier.

A particularly interesting area in the subject of aging is the data which are related to aging in astronauts who experience long periods in space in zero gravity [10]. Many astronauts have shown advanced aging on return to Earth (or following simulated space flight) with many reports of diseases related to the elderly such as a decline in the immune system [11], bone loss with the risk of premature osteoporosis [12], muscle degeneration [13] and problems associated with the cardiovascular system [14].

*Address correspondence to this author at the CTO Qigenix, 6125 Paseo Del Norte, Suite 140, Carlsbad, CA 92008, USA;
E-mail: peterhollands@qigenix.com

Many of these changes associated with aging, either on Earth or in space, may be due to one common factor which is the natural or induced aging of stem cells. This stem cell aging is often attributed to the decreased efficacy of mitochondria within the stem cells and to a disruption of the stem cell niche [15, 16]. The haemopoietic stem cell has often been the focus of stem cell aging since it is known that the incidence of pathologies such as myelodysplastic syndrome and acute myeloid leukaemia increase with age [17]. Damage or mutations to genomic DNA within the stem cell is another mechanism by which the aging process may manifest itself [18].

2. OXIDISED NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD+) AND AGING

NAD^+ is a vital redox co-factor in metabolism and ATP production in human cells [19]. As well as being critical in cellular metabolism in normal health, NAD^+ is a substrate for proteins that catabolize NAD^+ to nicotinamide including ADP ribosyl-cyclases (CD38/CD157) [20], NADase sterile alpha [21], TIR motif-containing 1 (SARM1) [22] and poly (ADP-ribose) polymerases (PARPs) [23]. This group of substrates are known as sirtuins and they are implicated in both health and disease [24]. NAD^+ is also suspected to be a key player in the process of natural brain aging and in the development of neurodegenerative disease [25]. It is also important to note that reduced levels of NAD^+ are seen not only in natural aging of the central nervous system [26] but also in accelerated aging diseases such as ataxia telangiectasia (AT) [27] which can be modulated by NAD^+ supplementation, Cockayne syndrome (CS) [28] and xeroderma pigmentosum group A (XPA) [29]. It is interesting that a high

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NAD^+/NADH ratio has been shown in pluripotent human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) [30] which are candidate stem cells for the treatment of neurodegenerative diseases. ESC and iPSC are noted for their complexity, cost, and safety concerns which up to now were the reason that kept them out of the clinic. It is likely, but as yet unproven, that easy-to-obtain, cheap, and safe autologous pluripotent hVSEL stem cells may have similar increased NAD^+/NADH ratios and can easily cross the blood-brain barrier to have a beneficial effect on neurodegenerative disease.

3. SENOLYTICS AND AGING

Recent publications suggest that the use of senolytics (molecules that induce apoptosis of senescent cells such as Dasatinib [31], Quercetin [32], and Fisetin [33]) may be able to extend health, quality of life, and possibly even lifespan. Obesity-induced cellular senescence has been associated with anxiety and impaired neurogenesis [34]. In renal aging and disease, it is thought that cellular senescence plays a considerable aetiological role and that senotherapy (using senolytics) to reduce or remove senescent cells may lead to both renal anti-aging and therapeutic benefits in renal disease [35]. Type 2 diabetes is another disease in which senolytics may be useful in either the prevention or moderation of the disease [36]. Similar reports have been made on the use of senolytics for anti-aging and in the treatment of chronic disease [37]. More recently, Quercetin has been associated with possible therapeutic effects in patients suffering from Covid19 by providing anti-inflammatory action and thrombin inhibition [38]. Overall, senolytics seem to have some potential in terms of removing senescent cells with the associated benefits to disease progression but their role (either alone or in combination with other therapies) is still to be determined in anti-aging.

4. PYRUVATE AND AGING

Perhaps the most recent and interesting publication about the role of pyruvate and the related molecules in aging is that of the Hydride Transfer Complex (HTC) [39]. The HTC is repressed in senescent cells and induced by p53 inactivation. This raises the possibility that exogenous expression of HTC may be able to inhibit senescence which may represent a multi-enzymatic complex to inhibit cellular senescence and consequently minimise aging. Animal studies have shown that pyruvate can prevent oocyte aging [40], suggesting that pyruvate is involved in anti-aging processes from the point of female gametogenesis. This is, of course, a negative observation because if oocytes do not mature then they cannot be fertilised. In terms of male gametogenesis it has been reported that pyruvate is an essential component in the capacitation of spermatozoa [41] and that in the absence of pyruvate and related metabolites, human spermatozoa cannot become capable of fertilisation. These observations are not classical anti-aging processes, but they illustrate the critical importance of pyruvate from the point of gametogenesis. Pyruvate, along with nucleotides, is also essential for the early metabolism of human embryos [42]. Pyruvate-enriched fluids may in the future prove to be valuable in disease intervention and also in aspects of anti-aging [43]. The role of pyruvate in the diseased or aging brain, in relation to glycolysis dysfunction, is also an area of considerable research [44] in the field of schizophrenia. In addition, pyruvate appears to be protective in the senescence of skin cells through a mechanism of control of mitochondrial and lysosomal function [45], emphasising the importance of the role of the lysosomal-mitochondrial axis in cellular senescence [46].

5. THE MAMMALIAN TARGET OF RAPAMYCIN (MTOR PATHWAY) AND PLASMA EXCHANGE IN AGING

5.1. The mTOR Pathway

The mTOR pathway is a protein kinase that was first identified in the 1990s and has been shown to be important in the growth factor signals to direct eukaryotic cell growth to maintain homeostasis [47-49]. The mTOR pathway is now recognised as making a major contribution to the aging process [50]. Inhibition of the mTORC1 (mTOR Complex 1) [51] pathway by depletion of the mTOR or raptor [52] has been shown to extend the life span in mammals [53]. The 'exhaustion' of stem cells has been implicated in mTORC1 which is thought to hinder tissue repair by stem cells and therefore enhance the aging process [54, 55]. It has also been shown that low doses of mTOR inhibitors may improve immune function in elderly patients [56]. The modulation of the mTOR pathway, possibly by the administration of Rapamycin, may in the future become a useful tool in the overall management of the aging process [57].

5.2. Plasma Exchange

Extracorporeal plasma exchange by apheresis is a procedure that can reduce circulating auto-antibodies and has been shown to be effective in Guillain-Barré syndrome and myasthenia gravis [58]. The potential of plasma exchange as part of an anti-aging regime resulting in a decrease in age-related disease has been supported through studies using plasma exchange in the treatment of Alzheimer's disease [59].

Double filtration plasmapheresis has been shown to be potentially effective in promoting anti-aging and longevity [60] although the effects are expected to be transient and may need repeat treatments to maintain any benefit. It is also possible that plasma exchange may dilute the inflammatory molecules produced by senescent cells [61]. This novel application of plasma exchange as an anti-aging protocol needs much further research before it can be brought into mainstream use to ensure safety and efficacy.

5.3. Calorific Intake Reduction and Aging

Intermittent fasting and reduced calorific intake have originated to be interesting areas of research in anti-aging technology with hypotheses about stem cell regeneration being one of the benefits [62]. Other workers have shown that alternative day fasting results in a change in the physiological and molecular markers of aging [63] suggesting that diet and fasting may have an impact on the aging process.

5.4. Epigenetic Clocks

Perhaps the best current way of studying aging is by the use of what has become known as 'epigenetic clocks'. There

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are 4 such epigenetic clocks that assess the extent of DNA methylation at Cytosine phosphate Guanosine (CpG) sites in the DNA. These epigenetic clocks are:

- The Horvath epigenetic clock [64] assesses DNA methylation at 353 CpG sites which predict chronological age with a mean absolute deviance of 3.6 years. This epigenetic analysis can be applied to a range of body tissues, including blood, whereas the Hannum and Weidner epigenetic clocks are only valid when using blood.
- The Hannum epigenetic clock [65] based on 71 CpG sites with a mean absolute deviance of 4.9 years
- The Weidner epigenetic clock [66] is based on 3 CpG sites with a mean absolute deviance of 3.3 years.
- The DNAm PhenoAge epigenetic clock [67] based on the measurement of DNA methylation (DNAm) is claimed to be 'a highly robust predictor of both morbidity and mortality outcomes and represents a promising biomarker of aging'.

These epigenetic clocks all have pros and cons, but the overall message is that the measurement of epigenetic changes is an accurate method to assess biological age and potentially future-related morbidity and mortality [68]. This in turn could initiate preventative measures before disease or problems occur thus reducing morbidity and mortality and optimising the quality of life for the global population.

5.5. The Role of Stem Cells and Exosomes in the Anti-Aging Process

5.5.1. Stem Cells

The basic function of stem cells in normal health is to repair or regenerate diseased or physically 'worn-out' tissue/cells and at the same time to self-replicate [69]. In this way, the stem cell pool is preserved and the tissue becomes repaired or replaced *e.g.* the production of blood by the haemopoietic stem cells [70]. Since the discovery of haemopoietic stem cells by Steensma *et al.* [71] stem cells have been identified in almost all tissues of the body with perhaps the most notable being Mesenchymal Stem Cells (MSC) [72]. These MSC have been shown to be capable of differentiating into osteoblasts, chondrocytes, and adipocytes (multipotent) making them an obvious choice for the development of regenerative medicine procedures [73]. Nevertheless, because of technical problems with MSC process optimisation the collection and use of MSC in clinical trials have been slow and inconclusive [74].

In terms of anti-aging adipose-derived MSC have been investigated as a possible anti-aging treatment for facial skin [75], as a modulator of the immune system (termed inflammaging) during aging [76] and studies of the molecular mechanisms controlling MSC aging may also add to the further potential of MSC in anti-aging procedures [77]. MSC may have a future in anti-aging technology if the current difficulties surrounding collection, processing, safety, standardisation, and mechanism of action can be resolved. There is also the fact that MSC ages with the patient [78] which means that any future anti-aging technology based on MSC may require donated 'young' allogeneic MSC.

5.5.2. Exosomes

Exosomes are vesicles secreted by possibly all cells which carry lipids, RNA, and proteins and are thought to be important in intercellular communications [79]. Exosome-based therapeutics for the disease are a possibility but at present much more work needs to be carried out to fully understand the importance and possible clinical applications of exosomes [80]. Exosomes are being investigated for their potential anti-aging properties especially in skin regeneration [81] and possibly extending the lifespan in animal models [82]. Once again, much research and future clinical trials are needed to fully exploit exosome technology in the context of anti-aging. A particular concern is the current batch-to-batch consistency of exosome preparations [83] which can be variable resulting in both clinical and regulatory challenges.

5.5.3. Human Very Small Embryonic Like (hVSEL) Stem Cells

A very different stem cell has been discovered from those mentioned above which could revolutionise Regenerative Medicine. The presence of pluripotent hVSEL stem cells has been shown in Platelet Rich Plasma (PRP) and the process of collecting, concentrating, and enumerating hVSEL stem cells has been well described [84]. These hVSEL stem cells, circulating in the peripheral blood, are thought to be quiescent [85] in normal physiology but there are reports of increased numbers of circulating hVSEL stem cells in some disease states such as Chronic Obstructive Pulmonary Disease (COPD) and Pulmonary Hypertension (PH) [86]. Similar reports indicate an increase in circulating hVSEL stem cells in Crohn's disease [87]. These observations suggest that pluripotent hVSEL stem cells are being mobilised (probably from the bone marrow) in disease states to assist in the repair of damaged tissue in Chronic Obstructive Pulmonary Disease (COPD), Pulmonary Hypertension (PH), Crohn's disease and cardiac disease [88].

5.5.4. QiLaser Activation of hVSEL Stem Cells in Autologous PRP (The Qigeneration Procedure)

The fact that hVSEL stem cells appear to be quiescent in normal physiology and increase in numbers in some diseases is interesting. Nevertheless, the increased number of hVSEL stem cells in the disease does not seem sufficient on their own to inhibit the progress of the disease or to repair damaged tissue. This raises the question of whether or not these increased numbers of mobilised hVSEL stem cells are actually still quiescent which may explain the lack of tissue repair despite high hVSEL stem cell numbers. These facts need an assessment of whether or not an activation process for hVSEL stem cells would increase the efficacy of hVSEL stem cells in disease states.

Our data has shown very clearly that the activation of hVSEL stem cells in autologous PRP using a QiLaser (The Qigenix Procedure) does produce hVSEL stem with increased expression of $CXCR4^{+}$, Oct $3/4^{+}$, $SSEA4^{+}$ and are $CD45^{-}$, $CD34^{-}$ and Lin^{-} [84]. We have also been able to propose a theoretical mechanism of action of the QiLaser on hVSEL stem cells using principles from Quantum Mechanics [89]. It has also been shown that the QiLaser can activate expanded allogeneic Mesenchymal Stem Cells (MSC). The-

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se MSC were derived from umbilical cord blood and given to patients intravenously to restore the Left Ventricular Ejection Fraction (LVEF) in patients suffering from end-stage heart failure. The patients received an application of the Qi-Laser to their heart region following intravenous administration of QiLaser-activated MSC. These patients all showed an increase in LVEF but two subsequently died from heart failure resulting in an 80% success rate [90]. The application of the QiLaser to the patient is thought to enhance the migration of activated stem cells to the area where repair is needed. In the same study, we have shown that end-stage heart failure patients benefit in the same way in terms of an increase in LVEF when they receive autologous QiLaser-activated hVSEL stem cells in PRP.

5.5.5. The Qigeneration Procedure and Aging

It is clear that the Qigeneration Procedure is beneficial to heart failure patients, and we have considered very encouraging as yet unpublished data on the use of the Qigeneration Procedure in patients suffering from neurological disease, neurological trauma, and type 2 diabetes.

The fact that hVSEL stem cells appear to be quiescent in normal physiology suggests that they would not be aging at the same chronological rate as the cells in the rest of the body [91]. This may represent an epigenetically 'young' source of pluripotent stem cells which once activated could have a powerful effect on the aging process. We hypothesise that pluripotent hVSEL stem cells, once activated by the QiLaser and administered by the intravenous route, are capable of regeneration of the stem cell niche in any tissue [92, 93]. The QiLaser-activated pluripotent hVSEL stem cells are also capable of repopulating the aging stem cell pool (which contains stem cells with increased DNA methylation) in all tissues, therefore, producing daughter cells with much reduced epigenetic aging. The biological age and the chronological age may become two very different parameters with the overall benefit to health and the reduction in chronological age-related disease.

We have preliminary unpublished data which shows that the Qigenix Procedure results in a reduction in DNA methylation using the Horvath epigenetic clock. This reduction in DNA methylation means that the epigenetic age of treated individuals is less than their actual chronological or biological age. This Qigeneration Procedure, therefore, represents a possible anti-aging or 'rejuvenative' process [94] that could be used to delay the diseases of old age and decrease human morbidity and mortality [95]. The cost related to the treatment of the diseases of old age such as dementia and Alzheimer's disease alone has a global impact of $600 billion [96]. The Qigeneration Procedure may also improve the biological age and healthspan of many people which alone would be a massive benefit to society [97].

5.5.6. Lifespan and Healthspan Extension

There have been studies on the extension of the overall lifespan, but these have often used animal models such as alpha-ketogluterate to extend murine lifespan [98] and the use of Resveratrol to extend the lifespan of worms, flies, and yeast[99]. In terms of extending the human lifespan, there have been various interesting publications including the use of nutritional and pharmacological life-extending interventions [100] and the use of Metformin to extend the human healthspan [101]. At the time of writing this article, there were no reports on the fully validated use of cell therapy to extend the human lifespan but there are interesting ideas about the genetics of lifespan [102] which when fully understood may lead to cell therapy interventions to extend the human lifespan in the future. This will not only reduce morbidity and mortality but also increase the useful working lives of individuals to create new concepts in healthspan and lifespan.

CONCLUSION

There are many approaches to possible future anti-aging procedures including medication, therapeutics, and cell therapy. The reasons for this need for anti-aging may be purely cosmetic, but the true benefit will be in the delay or prevention of age-related disease with the subsequent reduction of morbidity and mortality and lowering of the associated costs of treating age-related disease. The Qigeneration Procedure is a safe, cost-effective method of introducing QiLaser-activated autologous hVSEL stem cells as an 'anti-aging' intervention. Much more basic research and clinical trials are needed to fully understand the technology, but the preliminary data seem to be very promising. If the Qigeneration Procedure can truly have an anti-aging effect due to the activation of 'young' quiescent pluripotent hVSEL stem cells, then this will represent a new era in how we understand and manage the aging process.

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AUTHORS' CONTRIBUTIONS

Peter Hollands: Composing and revising the manuscript and joint final approval of the manuscript for publication. Todd Ovokaitys: Composing and revising the manuscript and joint final approval of the manuscript for publication.

LIST OF ABBREVIATIONS

COPD	=	Chronic Obstructive Pulmonary Disease
ESC	=	Embryonic Stem Cells
hVSEL Stem Cells	=	Human Very Small Embryonic Like Stem Cells
LVEF	=	Left Ventricular Ejection Fraction
PH	=	Pulmonary Hypertension
PRP	=	Platelet Rich Plasma

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

Professor P. Hollands is the CTO of Qigenix. Dr T. Ovokaitys is CEO of Qigenix.

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