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Review Article

Investigating the Causes of Cellular Aging and Uncovering the Novel Vision of Anti-aging Therapeutics

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Abstract

Anti-ageing is an idea that has always been a part of human life. Eternal life and immortality are parts of fantasy which were given a hope to become true with developing technology. Anti-ageing research is being conducted with the hopes of finding ways to improve lifespan, reversing the impacts of certain diseases in humans which leads to cell death and faster progression of aging and to develop regenerative medicine. There are genes in certain species of animals and other organisms which tend to reduce the speed of aging in the organisms, it is also found out that these genes are also present in human beings but are inactive. There are also ways to manipulate these genes and activate them using certain compounds and their activation might prove to be helpful in improving lifespan and curing various diseases which leads to the destruction of cells and their aging. This also helps in regenerative medicine. Gene therapy is one of the developing approaches for almost every disease and their progression which also includes the age-related ones. Gene therapy helps to alter the genome to produce the desired results but when it comes to anti-ageing gene therapy there have been a lot of hindrances like ethical issues, unsatisfactory results of the therapy and side effects so this therapy hasn't been brought into application yet but if the hurdles were overcome, clinical implementation of this technology and with the upcoming CRISPR technology it will give rise to various closed disciplines from the past.

Key words: Anti-ageing; Regenerative medicine; Gene therapy; CRISPR.

Introduction

Aging is characterized by reducing cellular and metabolic functions over a period. As a person gets older, one is generally subjected to various stresses physically, mentally, biologically, and genetically. With the reduced rate in cellular metabolisms and

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immunological responses a person becomes vulnerable to multiple diseases which are associated with ageing such as loss of hearing capacities, loss of proper eyesight, refractive errors, cataracts, heavy pain in the back, joints and neck region, pulmonary and cardiac diseases, diabetes, and some neurodegenerative diseases [1-4]. The common reasons for aging are loss, mutations and changes in genome and its instability, epigenetic changes associating genome, loss of telomere, lack in metabolism and proteostasis, mitochondrial dysfunction, cellular senescence etc. In this review we will be discussing anti-ageing technology and its subsidiaries. This technology is currently under study, and it deals with the age reversing effects of certain drugs and compounds, causes of aging, genes involved in aging, diseases that accelerate and are associated with aging, CRISPR technology in anti-ageing, stem cell based anti-ageing therapy and cellular mechanisms involved in anti-ageing and normal ageing. Anti-ageing medicine is a blooming study with the recent developments in CRISPR technology and next generation sequencing [5]. Senolytics, hormonal drugs and certain chemical compounds are said to reduce the pace of aging and are currently being studied.

Causes of aging

Aging leads to the loss of innate ability of cellular repair and is caused by changes in genome, epigenetic modifications, telomere shortening, and certain diseases. Mechanism of aging is being studied to improve lifespan [6]. Anti-ageing medicine solely relies on the way to manipulate and slow down the mechanisms that are involved in aging.

Genome and epigenetic modifications

The human genome consists of nuclear DNA, mitochondrial DNA, etc. DNA gets damaged over time due to various factors that are endogenous or exogenous to the cell, DNA gets damaged due to loss of genetic material, reduction in the ability of a cell to repair its DNA, random epigenetic modifications and sometimes mutations caused by endogenous or exogenous factors. Mitochondrial DNA can also get mutated or degenerated leading to a lot of degenerative diseases, lack of energy for multiple cellular pathways and reduced cell metabolism which naturally progresses cellular aging. Mitochondrial DNA and chromosomal DNA mutations have been reported in multiple patients with aging associated diseases [7].

DNA of a cell undergoes various stresses across time and usually loses its ability to function properly in aging cells. The DNA repair mechanisms that are innate to the cell get slower in aging cells and results in overall DNA damage, DNA loss and epigenetic modifications.

DNA loss in chromosomes generally occurs in the telomeric region, which is the terminal region of a chromosome containing more repetitive sequences. Telomere serves various functions such as prevention of unwanted recombination between different chromosomes, inter-chromosomal fusion, and DNA repair [8,9]. Reduction in telomere size and loss of telomere is called telomere shortening which progresses aging and cellular mutations. Telomerase is an enzyme that serves to preserve and maintain the telomeric length during cell replication and

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protects the nucleotide sequences. Telomere shortening affects cell division and causes cellular senescence. Epigenetic modifications such as histone modification, DNA methylation and RNA interference can also cause mutations and progress aging.

Proteostasis

Metabolic pathways get disrupted in the cell when subjected to multiple stresses from the environment or from within the cell such as heat shock, endoplasmic reticulum stress, and oxidative stress [10]. This can lead to accumulation of unwanted metabolites such as proteins, minerals, ions, and sugars. These accumulated metabolites can induce aging in cells by interfering in multiple pathways involving cell division and repair and cell metabolism. When cells start to age its ability for proteostasis reduces gradually which leads to improper protein synthesis and reduced protein metabolism, which gives rise to neurodegenerative disorders such as Alzheimer's and Parkinson's.

Cellular senesense

Cellular senescence is basically an irreversible arrest of cell cycle due to certain factors and this is widely seen as a defense mechanism against cancer. Cellular senescence helps to stop proliferation of mutated or damaged cells and remove the cells by apoptosis and with the help of immune system and prevents oncogenesis [11]. Sometimes senescent cells get accumulated over time and they won't get removed properly which leads to secretion of senescence-associated secretory phenotype (SASP) this inhibits cell signaling and communication, immune responses, apoptosis and cell cycle.

Aging study models and mechanisms

Aging involves various mechanisms, but they were extensively studied mainly in 4 organisms,

Aging study models

Saccharomyces cerevisiae

Yeast

Caenorhabditis elegans

Nematode

Drosophila melanogaster

Fruit fly

Mus musculus

Domestic mouse

After the experimental studies conducted on these 4 organisms, a few certain genetic pathways were confirmed to facilitate aging and reduced metabolism.

Aging mechanisms

- Mechanistic target of rapamycin (mTOR)
- Sirtuins
- Adenosine monophosphate-activated protein kinase (AMPK)
- Growth hormone/insulin-like growth factor 1 (IGF-1)
- Mitochondrial stress-signaling pathways

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These pathways are said to influence aging in the selected organisms and were studied for understanding and delaying aging in humans, but the extent of how much the study of aging metabolism in non-human organisms can provide biological information regarding aging in humans is still a question to be answered [12,13]. Some basic mechanisms and pathways are however said to contribute to aging in all organisms.

Yeast

Robert Mortimer and John Johnston described aging in budding yeast. Yeast mother cells are found to divide through a certain number of mitotic divisions before reaching replicative arrest. This lifespan of yeast was termed as replicative life span (RLS). Asymmetric division in yeast cells is considered a feature of RLS as mother yeast cells undergoes molecular damage during mitotic division but the daughter cells that are produced are devoid of such damage and have full replicative lifespan.

Yeast cells are also said to have another kind of lifespan in which the cells tend to be in a non-dividing state [14]. Mitotic cell divisions stop, and the yeast cells are maintained in this state, this is called Chronological lifespan (CLS). CLS is the period of time, the yeast cells can survive in their non-dividing state, but its survival is determined by its ability to enter back to its replicative state by itself or by external factors.

CLS and RLS are extensively studied in yeast as models for aging in eukaryotic organisms be it single-celled or multi-cellular, they are being studied to understand the relationships between cell replication and aging in all eukaryotic cells.

Worms

C. elegans is a facultative hermaphrodite that is used as a model to study aging and the factors associated with it. Generally, hermaphrodites take time and certain physical developments before reaching their reproductive stage [15-17]. After reaching their reproductive stage the hermaphrodite will be actively involved in laying eggs before sperm depletion, which is then followed by the post-reproductive period, Adult *C. elegans* are said to be postmitotic. *C. elegans* studies are designed to study lifespan, maintenance of muscle function, tissue atrophy, protein homeostasis and mitochondrial function in aging.

Flies

D. melanogaster was the first invertebrate organism that was selected and used for the study of aging, their life cycle has three stages embryo, larva, and pupa. They later attain reproductive ability and are used as models for aging studies. Life span of Drosophila is the duration of time between eclosion and death and Drosophila undergoes various genetic changes when grown in laboratories but it shows homology to various mammalian organs such as heart, kidney etc. [18-21]. Drosophila is used to study the relationship between aging and neurodegenerative disorders because of their simple nervous system to relate them with humans. It is also observed that mitochondrial function and protein homeostasis affects aging in Drosophila.

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Mice

M. musculus is a mammalian organism chosen as a model for aging studies. Mice are generally bred in laboratories to be used as models for research purposes as mice can provide information regarding the effects of the study on humans [22]. Several strains of mice were developed for this sole purpose such as transgenic mice and knockout mice. Mice are used to study aging mechanisms that are common to mammals as mice show the difference between the mechanisms involved in aging and its differences between vertebrates and invertebrates. Mice also showcase the metabolic pathways involved in aging and the stresses that can accelerate or decelerate the process of aging.

Mechanisms involved in aging

After experimental studies on all the model organisms several pathways were identified to be directly involved in the aging of an organism. Here the authors will be discussing certain pathways.

Mechanistic target of rapamycin (mTOR)

mTOR is a protein that is common in eukaryotes which regulates and promotes cell division and growth, and it is also directly involved in promotion and control of cancer as it has both positive and negative regulation on proto-oncogenes and tumor suppressor genes [23]. mTOR is found to be a serine/threonine protein kinase of the phosphoinositide-3-kinase-related family. This protein has two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) each of which has their own features and function. mTORC1 complex is said to be involved directly in mRNA translation, autophagy, mitochondrial metabolism, and cellular longevity. The mTORC2 complex functions are not yet completely classified but it is said to respond to insulin and RAS pathway, it is also involved in cell growth and metabolism.

mTORC1 signaling was studied in various model organisms and reduced mTORC1 signaling was found to have increased the lifespan of yeast but later reduced expression of mTOR gene and mTOR protein components showed increased lifespan in worms and flies. Research was conducted by National Institute on Aging's Interventions Testing Program (ITP) to study the importance of mTORC1 in mammalian aging using mice as model organism and it was found that mice treated with mTORC1 inhibiting rapamycin have increased average lifespan when compared to the normal untreated mice in both male and female. Similar results were obtained from worms, flies and mice when mTOR gene expression was inhibited or mTOR complexes were inhibited using rapamycin [24,25]. After multiple experimentation and research rapamycin was found to extend life span and reduce the risks of age-related health span disorders such as cancer, cardiac functioning and disorders and immune system functioning.

Apart from rapamycin treatment, the genetic inhibition of mTORC1 signaling has proved to be effective in improving lifespan and health span and it is said that reduced mTORC1 signaling can prove helpful in preserving organ functioning and maintaining cell senescence. However, the

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mTOR signaling pathway is too complex to be understood completely even though its inhibition has proved helpful in improving the lifespan and reduced chances of cancers, it remains unclear as which mechanism involved in mTOR is important for improving lifespan as it can have a negative effect by inducing cancers, and mTOR is also involved in autophagy and mitochondrial metabolism.

Sirtuins

Sirtuins are enzymes that are nicotinamide adenine dinucleotide dependent (NAD+), and they are responsible for catalyzing post translational modifications of proteins especially deacetylation. Sirtuins are very much distinguished from other protein deacetylases because of their absolute requirement of NAD+, which is used up during deacetylation. Budding yeast Sir2 protein was the first enzyme of this family to be extensively characterized and the term sirtuin was also derived from budding yeast Sir2 protein [26]. It is found that among the major model organisms, yeast and flies have five sirtuin proteins, *C. elegans* has four sirtuin proteins, and both mice and humans have seven sirtuin proteins (SIRT1-7).

Sirtuins impact on aging was first described in budding yeast as overexpression of Sir2 increased the Replicative Life Span of yeast mother cells and this is done by Sir2 as its overexpression increased the genomic stability within the ribosomal DNA and it also helped in asymmetrical retention of damaged proteins in the yeast mother cell. After this observation in yeast more such studies were done in both *C. elegans*, and *D. melanogaster* and they resulted in similar lifespans extensions in both organisms by overexpression of Sir2 but the mechanism behind the increased lifespan in those organisms by sirtuin is still not definitive [27-31].

Mammalian sirtuins are said to be 7 in number and each of them have their own tissues, biochemical activity, and function. SIRT1 is said to the mammalian ortholog of yeast Sir2 and has both positive and negative effect in age-associated diseases such as heart failure and cardiovascular diseases, cancer, and neurodegenerative diseases. Overexpression of SIRT1 throughout the body of mice only improved certain metabolisms which were associated with health span, but it didn't improve the lifespan of the mice, but recently mice with overexpression of Sirt1 in hypothalamus showed both improved health span and lifespan so it is suggested that SIRT1 activity in the brain of mice may help in improving lifespan and health span. Apart from SIRT1 other members of sirtuin family can also improve lifespan such as SIRT6 in mammals and the mitochondrial sirtuin SIRT3 improves mitochondrial function, thus influencing age-related metabolisms. Activation of sirtuins using pharmacological means have been said to improve lifespan and the first sirtuin activating drug was resveratrol and it was said to have improved lifespan of yeast, mice and worms [32]. The mechanism and target of this drug is still majorly under study and the second generation of Sirtuin activating compounds were later used to understand their way of action and target.

Adenosine monophosphate-activated kinase

The 5′-AMP-activated protein kinase (AMPK) is associated with regulation of metabolic pathways that majorly involve energy metabolism and it regulates pathways for glucose uptake and utilization, oxidation of fats and its function is controlled by the presence and concentration of ATP and AMP, where AMPK gets promoted by AMP and inhibited by ATP. AMPK is activated or inhibited by factors affecting the AMP/ATP ratio which includes lack of glucose, mitochondrial respiration, and hypoxia but sometimes DNA damage can affect AMPK directly without influencing ATP/AMP ratio [33]. Activation of AMPK is said to increase longevity and improve health span. Overexpression of AMPK is said to improve lifespan in model organisms, worms have two orthologs of AMPK-α subunit in which activation of just one of the two orthologs AAK-2 has proven sufficient to extend life span and its deletion prevents life-span extension. AMPK activation in flies and mice has been shown to improve lifespan and slow down aging [34,35]. Mice were given AMPK activators metformin and phenformin which improved its lifespan, Metformin is a widely used diabetic drug which has multiple uses, and it has seen that diabetic patients who take metformin have improved lifespan when compared to nondiabetic patients.

Insulin-like growth factor 1 (IGH-1) Signaling

This insulin/IGF-1-like signaling axis (IIS) is supposedly the longevity pathway that has been studied extensively and has proven effective to improve lifespan in model organisms and humans. IGF-1 is said to promote cell growth, proliferation, and cell-cycle progression. This pathway was first worked on and studied in *C. elegans* and further studies in flies and mammals showed a similar overall structure in mechanism flies. Insulin and its similar peptides have proven to be majorly involved in morphological development in organisms, both vertebrates and invertebrates as it directly involves nutrient metabolism and cell proliferation. Nutrients stimulate the production of Hypothalamic growth hormone releasing hormone (GHRH) and Ghrelin which in turn induces the secretion of growth hormone, Growth hormone stimulates IGF-1 production in the liver [36-39]. All these hormones are directly involved in aging and growth of an organism, they also directly affect multiple metabolisms in an organism. Various stresses in cells may be caused if any of these hormones get secreted more or less such as increased oxidative metabolism, fatty acid oxidation, insulin sensitivity and heavily altered metabolisms. This could seriously affect cells and their aging and lead to neurodegenerative disorders, cardiac diseases and cancer which are associated with old age.

Mitochondrial stress-signaling pathway

Mitochondrial metabolism and its function are one of the major factors that affect the aging in cells and organisms. Proper mitochondrial metabolism is essential for healthy aging in organisms and to reduce the chances of age-related disorders. It was believed from the first molecular theory of aging that age-related damage and decline in

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cellular function was caused by free radicals that were produced during mitochondrial metabolic processes. Recent studies shows that mitochondrial oxidative stress has both negative and positive effects on age-related factors as mitochondrial stress and reactive oxygen species (ROS) can also protect cellular mechanisms apart from damaging them, which is called "mitohormesis". Mitohormesis can be defined as a biological response through which an induced mitochondrial stress can improve the viability of a cell, it also describes mitochondrial performance in response to levels of ROS [40,41]. But however, mutations that improve lifespan are also sometimes said to impair mitochondrial function, so a complex relationship is said to exist between longevity and mitochondria.

Genes responsible for aging

Sirtuin gene

Sirtuin family is useful to treat the age-related illness. Sirtuin belongs to the class 3 histone deacetylases. There are seven genes that come under the Sirtuin family. They have some transcription proteins and cytoplasmic proteins. Nicotinamide (NAD) is a non-competitive inhibitor of sirtuin activity. The sirtuin activity was inhibited by decreasing NAD+ level. In humans the functions of sirtuin have not yet been determined.

SIRT1

SIRT1 is the most conserved nicotinamide adenine dinucleotide (NAD+) dependent histone deacetylase. It acts as cytoplasmic targets, during the inhibition of insulin signaling [42]. The product of the SIRT1 gene is SIRT1 protein, this protein is homologous to the member of yeast Silent Information Regulator 2(Sir2) protein. It acts as a transcriptional factor for the many different physiological processes. SIRT1 protein is expressed in the brain, kidney, heart, liver, pancreas, spleen, skeletal muscle, endothelial tissue, and white adipose tissue.

The modulation occurs in its downstream pathways by targeting nuclear factor kappa chain enhancer of activated B cells and its co-activator namely, adenosine monophosphate activated protein kinase (AMPK), protein tyrosine phosphate (PTP), fork head transcriptional factors etc., By removal of the acetyl group in this gene modulates the function of these molecules [43,44]. Alterations in this gene expression level were determined the metabolic diseases, cancer, aging, neurodegenerative diseases.

1. In yeast, sirtuin proteins regulate the gene silencing and suppress the recombination of DNA.
2. In mice, SIRT1 showed the better improvement in its lifespan and health span.

SIRT2

SIR2 is eponymous gene in the family of sirtuins located at 19q13.2 which is present in bacteria, plants, and animals. It is also known as senescence marker. It suppresses the formation of extrachromosomal toxic rDNA circles in yeast which increases longevity. SIR2 codes for the protein SIRT2, the only cytoplasmic protein found in the

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sirtuin family. SIRT2 is considered to be microtubule deacetylase as it removes acetyl groups from the α-tubulin at lysine 40, a major component of microtubule. The overexpression of SIRT2 leads to delaying of cell cycle progression. The levels of SIRT2 protein levels vary during the cell cycle phase, specifically it increases during mitosis phase. The SIRT2 transcript is abundantly present in the adipocyte compared to the other members of the mammalian sirtuin family, thus decreasing level of SIRT2 during pre-adipocyte differentiation and deacetylation of FOXO1 directly influence the adipocyte differentiation in a negative manner [45-51]. The ability to deacetylase the FOXO3 by SIRT2 helps to the regulation of pre-apoptotic protein BIM expression. The overexpression of SIRT2 helps in the regulation of cell death in order to the respond the DNA damage and reduce the cell proliferation. The overexpression of SIRT2 in neurons results in the inhibition of neurite elongation which impairs the migration pf primary hippocampal neurons**.**

SIRT3

SIRT3 gene is the only sirtuin which influences the longevity in human directly. It has the ability to promote the extension of lifespan. SIRT3 interacts with mitochondrial acetyl-coA synthase which is required for G1 arrest; thus, it plays a major role in apoptosis. In early embryo, oocytes, granulose cells this gene are the sensors and guardians of the redox state. It plays a major role in female fertility.

It is located in the mitochondrial matrix. It controls the flow of mitochondrial oxidative pathways and rate of the reactive oxygen species production. SIRT3 knockout mice have a chance to develop age related disorders. It improves regenerative capacity in hematopoietic stem cells (HSC) and regulates stress response in it. The main function of it is to protect neurons by the interaction with NAD+ and polymerase 1.

SIRT4

SIRT4 is also a mitochondrial sirtuin which regulates the multiple mitochondrial functions connected to age related diseases [52]. It plays a major role in toxic stress and acts as a bridge protein between mitochondrial metabolism and tumorigenesis. In mice, it inhibits the activity of pancreatic β-cells. By TCA cycle it can be identified as “Guardian of cellular metabolism.

SIRT5

SIRT5 gene encodes the protein, which is responsible for glycolysis, electron transport chain, TCA cycle and thus it plays a vital role in cardiac physiology and stress responses. Mostly prokaryotic sirtuins are SIRT5. In humans it encodes two isoforms namely SIRT5iso1 which contains 310 amino acids and SIRT5iso2 which contains 299 amino acids. SIRT5 gene expression was inhibited by metformin-mediated activation of AMPK.

SIRT6

SIRT6 gene is also known as “longevity gene” because of its role in DNA repair mechanism. The product of the SIRT6 gene is SIRT6 protein which is the determinant factor of extending the lifespan. There are 5 amino

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acids responsible for the stronger SIRT6 protein. It inhibits the aging by four pathways:

1. Promotion of DNA damage repair.
2. Maintenance of the normal telomere structure.
3. Regulation of glucose and NAD+.

In mice, if SIRT6 is less expressed, then the uptake of glucose increased and the insulin signaling also increased.

SIRT7

SIRT7 is a NAD+-dependent H3K18 deacetylase located in the nucleus and also in the nucleolus of mitotically active cells. It removes acetyl groups from histone H2A and H2B in particular. The deacetylation of H3 histone (H3K18) leads to silencing of gene transcription. SIRT2 helps in the regulation of RNA polymerase I which promotes rDNA transcription [53,54]. It catalyzes desuccinylation of H3K122 at double strand break sites which promotes chromatin condensation and DSB repair.

The induced expression of the nuclear receptor TR4/TAK1 by SIRT2 regulates the ubiquitin-proteasome pathway, thus controls the lipid metabolism in liver. SIRT7 inhibits SIRT1 which promotes adipogenesis. The SIRT7 helps to promote wound healing by stabilizing TGF-β type 1 receptor protein which leads to well organized TGF-β signaling. SIRT7 plays a protective role in cardiomyocytes as it protects them from hypoxia-induced damage.

Klotho gene

The klotho gene generally codes for three proteins α-Klotho, β-Klotho and Klotho-related protein (Klrp). β-Klotho is expressed in adipose tissue and liver and α-Klotho is expressed in distal tubule epithelial cells of the kidney while α-Klotho is expressed primarily in the kidneys and brain choroid plexus. Metabolic regulation, glucose uptake, bile acid synthesis, and fatty acid metabolism are a few functions of β-Klotho. The functioning of β-Klotho is independent from the α-Klotho though both the proteins are coded by the same gene. In humans, mice and rats there are 5 exons and 4 introns in the coding region which transcribe 3036, 3042, 3042 nucleotide mRNA [55,56]. Klotho protein is a transmembrane protein. Naturally in the human body the klotho levels decrease when the authors reach the age of 40. This gene regulates the protein Wnt5a which is seen in cancerous cells. The receptor of Fibroblast Growth Factor-23(FGF23) is α-Klotho protein, which regulates phosphate and vitamin D. Deficiency of Klotho causes hypertension, endothelial dysfunction, impaired angiogenesis and vasculogenesis.

FOXO3 gene

FOXO3 is a gene that encodes the transcription factor forkhead box O-3, belonging to the forkhead family. It acts as a transcriptional activator that specifically recognizes and binds to a particular DNA sequence 5'-[AG]TAAA[TC]A-3'. It regulates DNA repair, cell death, cell cycle arrest, autophagy, mitophagy, lipophagy, cellular homeostasis of stem cell and inflammation,

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proteostasis and counteracts the oxidative stress caused by Reactive Oxygen Species (ROS). It also helps in counteracting metabolic stress and growth factor deprivation [57]. This gene is associated with various age-related diseases like neurodegenerative diseases, intervertebral disc degeneration (IVD), cardiovascular diseases (CVD) and cancer. The activity of FOXO3 is regulated by several upstream factors via post transcriptional modifications like phosphorylation, acetylation, methylation, ubiquitination, glycosylation, prenylation, and sulphation. The miRNA, long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) also help in the regulation of FOXO3 activity. The subcellular localization is vital for the activity of FOXO3, most of these subcellular localization and DNA binding are altered by the post transcriptional modifications.

Phosphorylation and dephosphorylation

(Dephosphorylates PIP3 at position D3 to generate PIP2)

The negative regulation by IIS-PI3K-Akt signaling makes the FOXO3 gene inactive under normal conditions. PTEN dephosphorylates PIP3 at position D3 to generate PIP2 to antagonize the effect of P13k and induce the activation of FOXO3. The activity of FOXO3 is majorly suppressed by the phosphorylases that includes extracellular signal-regulated kinase (ERK), IκB kinase isoform β (IKKβ), serum-and glucocorticoid-inducible kinases (SGK), and inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKBKE). In contrast phosphorylation by AMP-activated protein kinase (AMPK), mammalian sterile 20-like kinase 1 (MST1) and c-Jun N-terminal kinase (JNK) activates the activity of FOXO3 [58].

Acetylation and deacetylation

The FOXO3 is basically acetylated by CBP and p300 while deacetylated by SIRT1 and SIRT2. The role of these Sirtuins is not fixed as the expression of target genes that are associated with antioxidant stress is promoted by SIRT1, while at the same time it suppresses the expression of proapoptotic genes.

Ubiquitination involves in the regulation of the FOXO3 activity, multiple ubiquitination leads to the degradation of FOXO3.

Drugs involved in anti-aging

Anti-ageing properties of lithium; association of lithium and length of telomere promoting longevity through GSK3/NRF2 dependent hormesis

Commercial drug name: Eskalith, Lithobid

Composition: Lithium Carbonate.

Molecular formula: Li_2Co_3

Molecular weight: 73.89 Da

New studies from King's college London state that there is a slowdown in the molecular ageing process with the usage of naturally occurring metal, lithium.

Increased amount of lithium in water have been linked to decreased risk of neurodegenerative disease and longer life spans in an individual. This metal has been

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said to treat bipolar disorder and has been prescribed as a psychiatric drug for over 50 years.

The length of telomeres which serves as molecular markers was monitored in 384 people taking lithium as a drug (lithium carbonate and lithium citrate are FDA approved drugs) for bipolar disorder to analyse lithium's potential as an anti-ageing drug which showed that the length of telomere is directly associated with the duration of intake of lithium as a drug.

DNA tails or telomeres tend to shorten each time the cells divide, as an individual gets older the shorter telomeres divide and replaces less comparatively which leads to several age-related issues such as coronary artery disease [59,60]. Dr Timothy Powell, institute of psychiatry, psychology & neuroscience, has stated that lithium slows down the rate at which the telomere shortens and restore telomere over time. Comparison between the polygenic scores among the people who take lithium for bipolarity for long term showed that people who had longer telomere length as an adult benefited the most from lithium's anti-ageing properties. Dr Powell quoted that "few people possess greater capacity to lengthen telomeres in response to higher doses of lithium, depending on a person's genetics. In future a genetic test might come in handy to determine who would benefit from lithium intake".

GSK-3

Initially this enzyme was found to regulate glycogen synthesis in-response to insulin. In recent study, it is found that lithium targets Glycogen synthase kinase-3 beta (GSK3-BETA), an enzyme linked to cellular aging in kidney and responsible for kidney function decline. This enzyme is involved in multiple biological processes such as gene expression, proliferation, cell fate determination, survival, and metabolism. Through series of experiments, they found that knocking out the gene for GSK3-BETA slowed kidney aging and preserved its function in animals. The inhibition of GSK3 gene is done by phosphorylation of serine 21 in the GSK-3α and serine 9 in GSK-3β.

Nrf2

(Nrf2) Nuclear factor E2- related factor 2, is involved in the regulation of cellular defence mechanism against oxidative and xenobiotic stress by the transcription of components for thioredoxin as well as glutathione antioxidant system and enzymes which are involved in phase I and II detoxification, heme-metabolism, and NADPH regeneration. Nrf2 also plays a vital role in stem cell quiescence, autophagy, and intermediate metabolism.

Mechanism

Inhibition of glycogen synthase kinase -3 (GSK-3) by phosphorylation of serine 21 of GSK-3α and serine 9 in GSK-3β is involved, and activation of transcription nuclear erythroid 2- related factor (NRF-2) takes place by binding of GSK3 and NRF-2 to Antioxidant electrophile response element (ARE) in promotor region of several cell defence genes. Nrf2 can initiate the transcription process by itself, providing a

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positive feedback mechanism that amplifies Nrf2 effects. Collaborating genetic loss of NRF-2 repressor kelch-like ECH-associated protein (keap1) treated with lithium showed high levels of NRF-2 activation portrayed stress resistance and low levels of promoted longevity. The identification of GSK-3 as therapeutic target for aging opens ways to effective treatment that can manipulate mammalian aging and health.

Advantages

- Balance of neurotransmitters
- Treatment of bipolar disorders

The inhibition of GSK-3 enzyme by lithium helps in,

- Promoting longevity
- Slow brain aging
- Improve health parameters.

Side effects

- Lithium toxicity
- Muscle weakness
- Fatigue
- Weight gain
- Vision problems
- Severe headaches
- hallucinations
- Hives
- Drowsiness
- Swelling of face, lips, and throat

Metformin in anti-aging: Type 2 diabetes

Commercial name: Glucophage, Fortamet, Glucophage XR, Riomet, Glumetza.

Composition: Metformin Hydrochloride (N,N-dimethylimidodicarbonimidic diamide hypochloride)

Molecular formula: $C_4H_{11}N_5.HCL$

Molecular weight: 165.63 Da

Recent studies shows that metformin, a drug that is in commercial use for type -2 diabetes also known as diabetes mellitus (chronic disorder that affects the blood sugar processing in the body) has the ability to inhibit aging by activating AMPK (Adenosine monophosphate-activated protein kinase) pathway that is said to promote healthy aging (slow aging). High blood sugar levels and insulin resistance are the main characteristics of diabetes which also accelerates aging [61]. In addition to this, the treatment for diabetes has several other benefits such as protection against cancer, cardiovascular diseases namely-coronary artery disease, cardiac arrest.

Likewise, it induces weight loss as well as gives neuroprotection by the regulation of AMPK pathway by modulating a healthy metabolism in the system.

AMPK pathway

Adenosine monophosphate-activated protein kinase is an enzyme which lowers the blood sugar level by energy utilization inside the cell. This plays a crucial role in the regulation of growth and reprogramming metabolism. AMPK is the master metabolic regulator that burns fats and sugars and helps in preventing their accumulation in the body.

mTOR

Mammalian (or) mechanistic target of rapamycin (mTOR) plays a vital role in cellular metabolism which integrates cellular processes like cell proliferation and growth by nutrient sensing [62]. Studies in the past decade have revealed that mTOR has a link to processes involving cellular senescence, aging, autophagy, stem cell function decline and mitochondrial dysfunction.

SIRT1

It is an epigenetic regulator belonging to sir2 protein family (studies have shown that Sir2 protein is a limiting factor which promotes longevity in yeast mother cells), changes in the expression of sirtunin (these are NAD+ dependent histone deacetylases that regulates metabolic pathways in eukaryotes and prokaryotes, also involves in cell senescence, apoptosis, cell metabolism and calorie restriction) are crucial in many diseases such as metabolic syndrome, cancer, cardiovascular disorders and neurodegeneration.

Mechanism of metformin

Blocking and diminishing the fundamental factors that accelerate aging is carried out by Metformin. This drug facilitates DNA repair which aids in cancer prevention, protects from chronic inflammation and fights against age related disorders.

Metformin regulates longevity by promoting cell signaling molecules like AMPK and mTOR as these regulate cell metabolism and survival of neural cells, both enzymes modulate cellular homeostasis at multiple levels [63].

The activation of AMPK triggers higher production of mitochondria. Reduction of mitochondria in a cell leads to faster or accelerated aging. The AMPK induced Mitochondrial Biogenesis slows down the aging process.

SIRT1, an enzyme that is activated along with the activation of AMPK has life extending properties. Metformin is the first ever anti-aging approved study by the FDA. The sketched out 6-year study called TAWM (Targeting Aging with Metformin) and MILES (Metformin in Longevity Study) will be used to evaluate the effects of this drug on longevity.

Metformin as a multi-therapeutic drug

Metformin serves as a multi-target therapeutic drug which deals with treating and controlling several disorders and diseases like cardiovascular disorders, malignancies, tumor treatments, obesity, liver, skin and renal disorders and type-2 diabetes mellitus. Also, metformin has been investigated and produced results in several species for its anti-aging properties.

Clinicals trials and results of metformin as an anti-aging drug

Caenorhabditis elegans commonly known as round worms are used widely as a model for studying pharmacokinetics and pharmacodynamics of drugs capable of delaying aging. Metformin (50Mm dose) supplement was discovered to increase the life span of *Caenorhabditis elegans* by 40%

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through activating LKB1-AMPK-SKN1 pathway which are involved in processes like cell homeostasis maintenance, cellular energy sensing, state and oxidative stress response.

100mg/kg of metformin for 5 consecutive days every month increased the mean (+8%) and a maximum of (+9%) in cancer prone short lived female HER2 mutated oncogene transgenic mice. This drug delayed and slowed down the growth of mammary tumors and prolonged the life span of HER2 transgenic mice [64,65].

Long-term exposure of metformin to female outbred SHR mice increased the mean lifespan (+37.9%) and maximum lifespan (+10.3%) by suppressing spontaneous tumorigenesis and slowing down age related disorders within the estrous cycle without any impact on the food intake or body weight. This proved that metformin could prolong lifespan, and this is independent from its ability to suppress cancer.

Overall, the roundworms exposed to metformin lived longer by 20% than the controls and the mice treated with metformin had prolonged life span of about 6% in non-diabetic mice and almost up to 15% longer in the mice with diabetes in comparison with mice without diabetes.

Advantages

- Cancer prevention
- Prevention of Cardiovascular disease
- Healthy Weight loss
- Neuroprotection
- Anti-aging

Disadvantages

- Vitamin B12 deficiency
- Allergic reactions
- Low blood sugar level
- Loss of appetite
- Diarrhoea

Rapamycin: an antifungal agent to anti-aging drug

Commercial brand name of the drug: Rapamune

Composition: Sirolimus

Molecular formula: $C_{51}H_{79}NO_{13}$

Molecular weight: 914.2 Da.

Rapamycin was widely used as an immunosuppressant to reduce organ rejection in cases of kidney transplantation, it was initially isolated and discovered from Streptomyces hygroscopicus as an antifungal metabolite. This drug targets mammalian mechanistic target of rapamycin (mTOR) which regulates cell growth, proliferation, and metabolism. Rapamycin acts as an inhibitor of mTOR signalling pathway.

Further studies and research showed that this drug was able to inhibit tumour cell lines. Abnormal increase in activation of mTOR signalling pathway is observed in several human cancers by gain-of-functional mutations or loss-of-function mutations in oncogenes and tumour suppressor genes respectively. The abnormally high cell proliferation requires alterations in nutrient uptake and energy metabolism which tends to meddle with the mTOR signaling

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pathway. As the drug of interest deals with the inhibition of this pathway, rapamycin is under consideration as a viable drug for cancer treatments [66].

Rapamycin as an anti-aging drug has paved way to identify the link between histone levels and TOR signaling, as the changes in histone levels plays a major role in the aging process and mTOR regulates cellular metabolism. Change in histone levels before and after switching off the mTOR pathway through rapamycin was tested in flies by Yu-Xuan Lu and his team. Increased histone protein production after turning off the mTOR pathway through rapamycin treatment showed improvised gut health, reduced tumor growth and extended lifespan.

mTOR

Mammalian mechanistic target of rapamycin (mTOR) is a serine-threonine kinase and an evolutionary conserved signaling pathway that regulates nutrient sensing and growth factors from intracellular and environment to coordinate proliferation of cells, apoptosis and cell growth. mTOR pathway comes from (PI3K) phosphatidylinositol-3 kinase family also referred to as phosphoinositide 3- kinase family which controls all the major biological functions through signaling, metabolic processes and membrane trafficking of cells and tissues in the system [67]. On-going research suggests that this pathway is intertwined with longevity and aging. Several experiments proved that inhibition of mTOR complex1 (mTOR C1) along with rapamycin improved the lifespan and slowed down age-related issues in all the model species studied.

The mTOR C1 consists of 3 main components:

a) mTOR
b) Regulatory protein associated with mTOR (raptor)
c) Mammalian lethal with sec13 protein8 (mLST8)

Along with these mTOR C1 subunit also consist of inhibitory components such as:

a) DEP domain containing mTOR interacting protein (DEPTOR)

b) proline rich Akt substrate of 40KDa (PRAS40).

The initial indications that mTOR is involved in the regulation of lifespan were determined from the studies conducted in *Caenorhabditis elegans* (round worms) and *Drosophila melanogaster* (fruit fly). Later, experiments conducted in *Saccharomyces cerevisiae* (Yeast) to inhibit mTOR pathway with Rapamycin doubled its lifespan. Deletion of S6K1 substrate in mTOR C1 subunit increased the lifespan of female mice and not in male mice. The activation of mTOR C2 by phosphorylation of AKt (it is a signal transduction pathway which promotes growth and survival in response to extracellular signals) of mTOR C1 aids in the formation of heterodimeric complex of tuberous sclerosis 1 and 2 (TSC1 and TSC2) which plays a vital role in inhibition of mTOR C1. The mTOR C1 subunit can also be inhibited by (PI[3,4]P2) phosphatidylinositol 3,4-bisphosphate synthesized by class II

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PI3Kβ under growth factor deprived condition.

FK506

These are highly conserved family of proteins in eukaryotes that consists of peptidyl prolyl cis/trans isomerase domains involved in apoptosis, protein folding, cellular signaling and transcription. These binding proteins are subunits of FKBP12 which is a 108 amino acid peptide that is considered to be physiological regulator of cell cycle mechanism by down-regulation of TGF-β receptor signaling. FK506 when combined with rapamycin provides an immunosuppressive reaction and helps with the inhibition of mTOR signaling pathway.

Mechanism of rapamycin

Rapamycin binds to FK506 (enzymes with peptidyl-prolyl cis-trans isomerase activity)-binding protein-12 (FKBP12- receptor for immunosuppressant drug) cytoplasmic receptor to form immunosuppressive complex. FKBP12-rapamycin complex inhibits the mTOR complex 1, which phosphorylates the substrates such as S6 kinase 1 (these are mechanistic target of rapamycin that is involved in cell growth control), eif4E-binding protein (regulation of cell cycle and apoptosis), transcription factor EB (master regulator of energy metabolism, autophagy, lysosomal exocytosis, lysosomal biogenesis, lipid catabolism) and several other factors associated which suppresses the cytokine-driven T-cell proliferation and the progression of cells from G1 to S phase of cell cycle is stopped [68]. Rapamycin is said to form a gain-of-function complex along with (FKBP12) 12-kDa FK506-binding protein which binds and behaves as an allosteric inhibitor of mTOR C1 complex.

Clinical trials and results of rapamycin as an anti-aging drug

Treatment with rapamycin on *Drosophila melanogaster*, fruit fly demonstrated an increased level of histone proteins after switching off the mTOR pathway, this effect was observed specifically in the gut of the flies and not in any other tissues. On further research, Yu-Xuan Lu and his team showed higher levels of histone protein production in certain specific cell types known as enterocytes which reduced tumour growth, improved lifespan and healthy gut. This experiment showed that there is a link between TOR pathway and Histone protein that aids in longevity. In 2009, experiment conducted by Harrison et al demonstrated that treatment with rapamycin extended median and maximal lifespan of female and male heterogeneous mice at the age of 1.5-2 years. In the following years many experiments were conducted which helped to build a strong list of evidence that rapamycin could potentially be an anti-aging drug. These conclusions were attained through the following observation:

- Treatment with rapamycin at an early age slows down aging and increases the lifespan of an organism.
- Rapamycin is also said to reduce neoplastic diseases or cancers and metabolic diseases independent of their ability to delay aging.

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Advantages

- Reduced risk of cytomegalovirus infection in organ transplanted immunosuppressed patients.
- Inhibition of viral replication.
- Blockage of neoplastic cells from growth and replication, serves as an effective anti-cancer drug.
- Promotes longevity by inhibition of mTOR pathway.
- Improvised cardiovascular functions.
- Protection against age related cognitive decline.

Disadvantages

- Increased risk of diabetes.
- High blood pressure on prolonged use.
- Anaemia
- Fatigue
- High cholesterol and elevated lipid levels.
- Sore and inflamed mouth.

Anti-aging of resveratrol

Role: Serves as a multipurpose drug for various age-related disorders and has strong anti-aging properties.

Structure: 3,4′,5-Trihydroxystilbene

Molecular weight: 228.2 g/mol

Resveratrol as a compound was first extracted in 1940 from the roots of Veratrum grandiflorum O. Loes by Takaoka. It has a planar stilbene structure that gave it hydrophobic characteristics. The rate of absorption of resveratrol is quite high, and approximately 75% of administered resveratrol is absorbed in the human body. Resveratrol is a natural antioxidant polyphenol which is found in a lot of foods consumed by people regularly such as grapes, red wine etc. This particular compound has gotten a lot of attention from many scientists as it is found out through various research that it can serve as an effective multipurpose drug for various age-related disorders and even reduce the pace of cellular aging in organisms. Resveratrol has proven effective in treating cancers, diabetes, neurodegenerative disorders, cardiovascular diseases and also as an antioxidant. In aging, resveratrol has proven effective as it reduces mitochondrial stress and improves its metabolism while also maintaining apoptosis.

Anti-aging mechanism of resveratrol

Resveratrol has been administered to the model organisms that are taken for the study of cellular aging and effects of resveratrol on aging [69]. Resveratrol induces deacetylase activity, and it has more potential among other polyphenols. *S. cerevisiae, C. elegans, Drosophila melanogaster,* and mice are the model organisms that were administered with resveratrol and their aging was slowed but only when the gene that codes for Sir2 homolog was present.

AMPK pathway and Sirtuins

AMPK (Adenosine monophosphate activated protein kinase) is a regulator of energy metabolism in the cells, but it also has been found to directly influence cellular aging in

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organisms. Resveratrol has proved to be able to activate AMPK independently and also with SIRTs. Resveratrol has reduced obesity and risks of type 2 diabetes mellitus in model organisms especially mice as it improved glucose-lipid metabolism and energy metabolisms in association with AMPK and SIRTs. Resveratrol induced phosphorylation in mice which in turn improved AMPK pathway, thus improving the overall glucose-lipid uptake and metabolism with improved insulin sensitivity.

Resveratrol mechanism with AMPK and SIRTs are very complex and intertwined with one another like a complementing pathway [70,71]. It can activate both SIRTs and AMPK directly, but its action varies with the levels of resveratrol, when resveratrol levels are low SIRT1 activates liver kinase B1 by deacetylation and increases AMPK activity as LKB1 is an upstream kinase of AMPK, whereas at high levels of resveratrol it increases AMP/ATP ratio and activates AMPK. AMPK in turn increases cellular NAD+ levels and compliments SIRT1.

Resveratrol gets its anti-inflammation and anti-cancerous ability as AMPK has negative effect on mTOR and induces autophagy while SIRT1 inhibits the nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) and mitochondrial biogenesis.

Resveratrol involves more mechanisms, and it directly affects and slows down aging and reduces risks of age-related disorders by controlling mitochondrial stress, cellular senescence and autophagy.

Resveratrol effects on age-associated disorders

Resveratrol in cancer

Resveratrol reduced the rapid proliferation and metastasis of ovarian cancer cells and induced apoptosis with the help of AMPK. It induced apoptosis to remove damaged cells and improved antioxidant and detoxifying capacity. Resveratrol proved to inhibit cell cycle to a certain extent. It also reduced metastasis and invasion, and sensitizing tumor cells when used in combination with another anti-cancer agent during chemotherapy. Resveratrol induced apoptosis in leukemia, colon, breast, and prostate cancer cell lines.

Resveratrol in neurological disorders

Resveratrol has been proved to prevent neurodegenerative disorders in model organisms by promoting neurogenesis in hippocampus, neuron proliferation, neurotransmitters and reduce inflammation and oxidative stress in neurons. Inhibition of neuroinflammation by resveratrol slows down brain aging and reduces the risk of neurodegenerative diseases. This improved memory and reduced the chances of neurodegenerative disorders such as AD and PD.

Resveratrol in cardiovascular diseases

Resveratrol experiments in mice have provided information that resveratrol can reduce the risk of atherosclerosis and help in improving cardiovascular health. Resveratrol improved the production of NO, reduced and controlled oxidative stress, controlled

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renin-angiotensin system, and enhanced the Sirt1 functions in mice. This proved resveratrol improved cardiovascular health in model organisms and contributed to enhanced lifespan.

Resveratrol in diabetes

Resveratrol with the help of AMPK and SIRT1 has proven very effective in controlling energy metabolisms such as glucose uptake, lipid metabolism, mitochondrial biogenesis and improved insulin sensitivity in the model organisms. Resveratrol can control and reduce the risk of T2DM with its AMPK and SIRT1 regulation.

Clinical trials

Resveratrol was found out to be more effective in certain types of cancer. For instance, it epigenetically reduced the expression of breast cancer-related genes, but at the same time it caused adverse effects in multiple myeloma patients [72]. Treatment of patients with AD and stroke was beneficial in all three clinical trials conducted, suggesting that resveratrol would be an effective treatment for neurological disorders. Future clinical trials should study whether resveratrol is more effective in certain patient types regarding its pharmacokinetics, pharmacodynamics and pharmacogenomics.

Stem cell and aging

There are many kinds of stem cells, but only pluripotent stem cells have the ability to differentiate into different kinds of cells. Every cell undergoes aging and so does the stem cells and they lose their ability to differentiate into different cells and self-renewability. Stem cell aging is being studied in hopes of understanding aging in all tissues and cells, mechanisms involved in age related disorders, therapeutic medicine for aging and stem cell-based therapy for aging.

Aging is an absolute process, and it is an unavoidable physiological change in all living cells and organisms, but the pace of ageing can however vary from cell to cell and organism to organism [73]. Aging is being studied in stem cells to understand the mechanisms that control the pace of aging so that in future aging could be slowed to improve lifespan and health span in all organisms. However, these mechanisms are driven by cellular metabolisms and genetic material, so understanding it might help controlling the pathways involved in cellular aging to improve precision for anti-aging therapy.

Types of stem cells and aging in stem cells

Somatic stem cells

This type of stem cells is more common and are spread out throughout the body of an organism. Somatic stem cells are actively involved in repair mechanisms as they replenish the old dying cells and also regenerate injured or damaged cells with newer cells. As this stem cell is present in almost all tissues and organs their function is highly crucial for an organism to thrive and have a healthy lifespan but however as an organism grows older these cells gradually lose their ability to proliferate and differentiate thus reducing their ability to

replenish and repair tissues and organs in an organism. This is the reason why people at their young age heal and recover sooner from injuries and tissue damage when compared to people at their old age.

Neural stem cells

Neural stem cells (NSCs), as its name suggests this type of stem cell is found particularly in the neural tissues and facilitate neural cells renewability, they are multipotent and self-renewing stem cells. These stem cells basically differentiate into different types of neural cells that are a part of central nervous system such as neurons, astrocytes and oligodendrocytes [74]. These stem cells differentiate in response to various different signals and help to maintain brain homeostasis but as these cells start to age, they gradually lose their ability to replenish the neurons that are lost or lose their ability to differentiate into neurons which in recent studies have been related to lack or loss of function in brain and sensory organs such as eyes, ear etc. This is also said to pave the pathway for multiple neurodegenerative disorders and loss of memory because of their senescence.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are also multipotent stromal cells that differentiate into cells of mesenchyme tissues such as osteoblasts, chondrocytes, myocytes and adipocytes. MSCs also age gradually with time and their renewability reduces along with time. These cells were first isolated from bone marrow of guinea pigs and was later isolated from mice. MSCs are majorly derived from bone marrow and adipocytes and as these cells age bone marrow MSCs depletes then they start favoring adipogenic differentiation over osteoblastic differentiation. This change in MSCs is greatly associated with the change of hematopoietic bone marrow to fatty bone marrow and leads to osteoporosis and more bone related disorder as osteoblastic differentiation reduces.

Adult skeletal muscle stem cells

These cells are also called satellite cells and they have very high renewability and regeneration capabilities. However, like all stem cells these stem cells also lose their ability to differentiate and proliferate gradually as they start to age. This aging in stem cells leads to loss in tissue function and also its structure with gradual aging but the factors that cause this aging is not yet entirely determined if it is extrinsic or intrinsic to the stem cells.

Hematopoietic stem cells

Hematopoietic stem cells (HSCs), as the name suggests these stem cells directly involve in hematopoiesis, a process that involves the formation of blood cells and their elements. These stems cells are located in the blood cell producing bone marrow (red bone marrow) and these cells produce blood elements and immune system elements. This bone marrow function is very crucial for the immune system and blood circulatory system of an organism [75]. These cells constantly produce immune system elements and blood elements that protect and facilitate mechanisms that directly affect

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the lifespan of an organism. The aging of this stem cell affects the HSC cell cycle and its function. HSC functions are controlled by a network of mechanisms involving intrinsic and extrinsic factors of the cell. This cell is responsible for hematopoiesis, bone homeostasis and immune system regulation.

Causes of aging in stem cells

DNA damage in aging stem cells

Stem cells are present in quiescent stage for a prolonged period of time, in most cases throughout its life span. This results in the encounter of genotoxic assaults to the stem cells via various exogenous and endogenous provenance, the accumulation of these DNA damaged stem cells is observed in many studies.

In aged hematopoietic stem cells and muscle cells (satellite cells) there is an increased staining for histone H2A.X (γ H2A.X) variant phosphorylation, which is an evident marker for DNA double strand breaks. These can be detected via single cell gel electrophoresis assays. In addition to this, telomere specialized chromatin structure that resembles a cap structure which protects chromosome ends from gene erosion and chromosome fusion gets shorter and shorter after each cell division in aged hair follicle stem cells.

Increased levels of DNA damage in aged stem cells results from accumulated damage over time, as there is increased damage in stem cells there is a decrease in the repair mechanism of these DNA damage. On continuous accumulation of these DNA damage in stem cells it surpasses the threshold that affects and impairs the stem cell function in aged animals.

Depletion of stem cells and senescence in older tissues

The depletion of stem cells is caused by the shortening of telomere to some extent, the length of the telomere shortens every single time the cells divide so as the individual ages telomere length shortens which is also a contributing factor in many age-related disorders.

These multipotent stem cells cannot divide endlessly, and these cells lose their self-renewal mechanism by terminally differentiating or by apoptosis or entering into senescence as a result of telomeric attrition and cellular stress [76,77]. Senescent cells remain alive but are irreversibly arrested from being able to divide, after this these cells aides in the increased production of cell cycle inhibitors like p53, p21 and p16INK4a which are also known as Cdkn2a. Moreover, the senescent cells secrete several bioactive mediators such as inflammatory cytokines, degradative enzymes and growth factors which leads to stem cell dysfunction with aging.

A huge part of multipotent stem cell aging occurs by epigenetic changes which either activate or silence a specific gene preventing them from performing their function. Genomic instability as well as epigenetic changes are attributes in producing a defective stem cell. Age-dependent depletion in the number of stem cells has been found in the following stem cells: neural stem cells,

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skeletal muscle stem cells and germ line stem cells.

For stem cell maintenance and quiescence, low levels of ROS (reactive oxygen species) are required. With aging ROS increases causing the loss of quiescence and depletion of stem cells. Continuous and repeated need or demand of stem cells to regenerate replacement cells over a lifetime causes stem cell exhaustion and depletion by eventual replication exhaustion, telomere erosion, epigenetic dysregulation and mutations.

Clinical trials and results of stem cell therapy in anti-aging

Reversal of aging using cellular rejuvenation in mice

The cells that are isolated from older people and animals possess different types and patterns of chemicals along with their DNAs in comparison to young people and animals, these are called Epigenetic markers (the epigenome is composed of several epigenetic markers such as histone tail modifications, DNA methylation, chromosome remodeling that has the ability to transmit epigenetic information).

The combination of four reprogramming molecules which includes: Oct4, Sox2, Klf4 and c-Myc known as the 'Yamanaka factors', they are a group of protein transcription factors that plays a crucial role in the creation of induced pluripotent stem cells, also known as the iPSCs. These factors help in the control and regulation of how the DNA is copied for translation in other proteins [78]. The Yamanaka factors have become an important and irreplaceable tool in longevity research as they are used in the transformation of adult cells into pluripotent stem cells.

Recently, it was discovered that the Yamanaka factors were able to accelerate muscle regeneration in mice. After this the researchers used the same procedure to enhance the functions of other tissues like optic nerve, heart and brain. Continuous treatment with the Yamanaka factors for a period of 7-10 months in the middle-aged and older mice resembled the epigenetics of younger mice, one such instance is that the skin cells had higher ability to proliferate and less likely to leave a permanent scar in-case of an injury. The age-related changes in the metabolic molecules in the blood did not begin. However, the mice treated only for a month or mid-way in the trial did not show any evident changes.

Anti-aging stem cell treatment in humans using mesenchymal stem cells (MSCs)

The MSC based treatments are based on reducing the age-related frailty (it is a clinical syndrome that is common among older individuals which increases the risk of falling sick often and increased occurrence of age-related disorders) in senior citizens. This is the first stem cell based anti-aging treatment which targets specifically on the frailty and is close to receiving FDA approval.

This treatment requires retrieval of mesenchymal stem cells from a healthy adult donor's bone marrow, and these are then infused as a single dose to patients aged approximately 76. The phase1 and phase 2

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clinical trials in humans does not show any adverse side effects [79]. Although these trials were conducted to demonstrate safety and its results were remarkable.

The first trial included 15 frailty prone patients; they were subjected to single MSC infusion derived from adult bone marrow donors aged anywhere between 20-40 years. 6 months later, these subjects showed improvement in physical fitness, tumor necrosis factor levels as well as overall quality of life. The second trial was a double-blind study with placebo group and randomized, these subjects were reported to have no adverse side effects along with enhanced quality of life and found to have remarkable results. These two trials produced favorable outcomes paving way for mass phase 3 clinical trial which is yet to be started.

Calorie restriction

Calorie restriction (CR) is the reduction of the dietary intake below the energy requirements. CR has a potential to increase the life span by 1-5 years with an improvement in health. It moderates the aging process by metabolic and cellular adaptations. The extension of CR will increase stress resistance, insulin signaling and immune function. Metabolic changes, improvement in insulin sensitivity and alterations in neuroendocrine functions in animals were induced by CR. It has been proved that CR produces changes in the hormone level in fat tissue like adiponectin, leptin, TNFα and resistin [80 - 83]. CR lowers glucose and insulin levels in mice, thus it has an advantageous influence over glucose metabolism. In animal models of Huntington's disease, Alzheimer's disease, Parkinson's disease and stroke, the CR gives protection against neurodegeneration. The improvement in Generation of oxidative damage by reducing reactive oxygen species (ROS) due to increased levels of uncoupling protein by CR helps to decelerate aging.

Mechanisms

CR works as a key nutrient and stress response metabolic signaling pathways including IIS/FOXO, TOR-AMPK, Sirtuins. These pathways regulate CR to extend the lifespan.

In AMPK-TOR signaling

Target of Rapamycin (TOR) pathways plays a central role in energy sensing and nutrient to control cellular growth. It regulates growth and metabolism by promoting protein synthesis. In recent research proves that suppression of the TOR pathway will extend the lifespan. The suppression of TOR was achieved by CR. CR activates the AMPK while suppressing the TOR cascade. When the AMPK activated it extended the lifespan of the model organisms. Scientists have studied that acute starvation activates the AMPK, and it depends on the duration of the CR. Thus, they concluded the possibility of the AMPK-TOR dependent lifespan extension.

IIS-FOXO signaling

Growth hormone (GH) was secreted by pituitary gland which promotes the growth by activating the Insulin/Insulin like growth factor-1 signaling (IIS). This activated IIS

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translocating the Forkhead box protein o (FOXO) transcription factor from nucleus to cytoplasm. In absence of the GH, the FOXO transcription factor translocate into nucleus and induces the expression of the genes involved in cell death, DNA repair, cell cycle arrest and detoxification [84]. In mice and rat models, there are more than 40 mutations are seen out of these approximately one third are involved in GH and IIS. These are reduced by CR and the foxo transcription factors are activating.

Sirtuins

Silent information regulator 2 (sir2) protein that require NAD+ as a cofactor for the deacetylation reaction. NAD+ and NADH are involved in many cellular metabolic pathways. NAD+ accumulates under nutritional stress and activates sirtuins. The activation of sirtuins may extend the lifespan, possibly through the mechanisms that extend lifespan by CR. The over expression of the sirtuins extend lifespan in many model organisms including yeast, worms, flies, and mice. The severe form of CR extended lifespan independently of sir2.

Role of CR in mitochondrial oxidative stress

The production of mitochondrial free radicals during the normal metabolic process causes oxidative stress. These free radicals might have the potential to cause damage to mitochondrial DNA which results in the alteration in the functions of mitochondria. CR seems to reduce the production of mitochondrial free radicles in rodents; thus, it reduces the mutation in mtDNA. In rats, the levels of protein oxidative stress were reduced after implementing CR, especially the levels of protein carbonyls (26-28) and Di tyrosine cross-links which leads to the formation of AOPP (Advanced Oxidative Protein Products). During the research on specific protein markers like advanced Maillard products and specific carbonyl modifications (glutamic and aminoadipic semialdehydes) in liver and heart of rat mitochondria, the authors came to know that the protein oxidative damage after CR depends on the implementation time and highly tissue specific [85]. It is observed that the enhancement of the repair/degradation rate of damaged macromolecules results in the reduction of protein oxidative stress in CR animals. Investigation on the cells of heart and soleus muscle in CR animal reported in increased activity of peptidylglutamylpeptide hydrolase activity of the 20S proteasome after short time CR and chymotrypsin-like activity after long time CR. Based on the investigation the capacity to remove the damaged protein increased significantly. Thus, by controlling these factors CR directly plays an important role in the aging process.

Role of CR in metabolic changes

Calorie restriction and starvation seem to be similar, but there is a significant difference between them. The CR animals show increased levels of corticosterone and decreased levels of growth hormone, luteinizing hormone, plasma leptin and insulin. During starvation the RQ is observed to be low, lipids replace the glucose as the energy source. White adipose tissue fats are directed toward mitochondrial metabolism,

which produces ketones as a source of fuel for the brain. While in CR animals the RQ is observed to be low before food intake and gradually increases after food intake. Due to the depletion of their glycogen reserves, CR rats primarily used fat metabolism prior to eating and only occasionally used glucose [86,87]. The fuel for mitochondrial beta-oxidation is reduced before food intake in CR animals. Increased levels of ketones and fatty acids are observed. In turn there is a significant decrease in the levels of triglycerides, total cholesterol and free cholesterol, total fats, free fatty acids, and phospholipids in CR animals after food intake. PPAR- alpha and other gene expressions that are related to the mitochondrial beta-oxidation were boosted. While the activity of acyl-coenzyme A carboxylase in liver, synthesis of fatty acids, and the gene expression of ADD1/SREBP1 were boosted after food intake in CR animals. Overall CR increases lipid metabolism's effectiveness and prevents fat buildup in non-adipose tissue.

Effects of CR on the Neuroendocrine System

In CR animals reduced amount of thyroid, gonadal, GH-insulin-like growth factor 1 (GH- IGF-1) axis is noticed. At the same time glucocorticoid levels are high due to long term CR. During CR orexigenic peptide and neuropeptide Y is stimulated and anorexigenic peptide and proopiomelanocortin were suppressed.

Compared to ad libitum rats the levels of plasma IGF-1 level, and plasma leptin are lowered in CR rats. This effect of CR on hormonal pathway plays crucial role in aging by modulating energy homeostasis.

Effects of CR on Rodents

Growth and maturation

After maturation, CR inhibits development and body size while lowering body weight. It is believed that hormones like IGF and GH play significant parts in these reactions. CR also prevents both men and females from reaching sexual maturity. Inhibition by CR is more potent in females than in men. The ability to reproduce is maintained although the levels of Follicle stimulating hormone (FSH) are low due to the reduction of LH. The levels of plasma prolactin and testosterone levels were significantly reduced by 50 % restriction in food intake [88]. At the same time the basal LH and FSH concentrations and testicular weight in male rats were unaffected. In comparison to a control group that did not receive CR, the female rats which undergone CR displays a greater frequency of estrous cycle and high number of offspring.

Hypercorticosteronemia

The hypothalamic-adenohypophyseal-adrenal cortex glucocorticoid system aids in effective stress management in mammalian species. plasma free corticosterone levels are elevated in CR rats. Compared to ad libitum rats the levels of free corticosterone are increased several folds. Long duration of CR is responsible for light stress hormesis in which the corticosterone plays as a stress hormone. Thus, light stress hormesis directly influences aging and is significant in extension of lifespan by CR.

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Anti-aging of senolytics, senomorphics

Role: It's a type of compounds that can remove the senescent cells, it helps to maintain optimal functions and youthful health.in preclinical studies, Senolytics are very slow or reverse aging [89].

Nutrients used in senolytics: quercetin, fisetin and theaflavins.

Class of drug: Senolytics

p16-reporter mice: short-term administration of a Senolytics must yield a sustained reduction in senescence signal.

Human tissue explant (p16, p21 and SAAP): drug treatment yields a reduction in a senescence marker and increase in apoptosis markers.

Transplantation of labelled senescent cells into a host organism: short-term or intermittent administration of a Senolytics must yield a long-term attenuation of labelled cells, as well as expression of senescent and points.

Measure therapeutics efficacy in aged or diseased organisms: short-term intervention with a Senolytics must yield a sustained health benefit in old.

Senomorphic

p16- reporter mice: chronic treatment with a senomorphic is required for a sustained reduction in senescence signal.

p16-INK-ATTAC: chronic treatment with a senomorphic is required to get the same outcome as genetic ablation of senescent cells.

Human tissue explant (p16, p21 and SAAP): drug treatment yields a suppression in a senescence marker with no evidence of cell death.

Transplantation of labelled senescent cells into a host organism: chronic administration of a senomorphic will suppress senescence end point but not attenuate the signal from the labelled cells.

Measure therapeutics efficacy in aged or diseased organisms: chronic administration of a senomorphic is required to yield a sustained health benefit in old.

Advantages

General advantages of anti-aging

Targeting the master regulators like FOXO, mTOR, AMPK, IGH-1 which plays an essential role in autophagy, oxidative stress and inflammation can potentially increase one's lifespan using drugs like resveratrol, metformin, rapamycin, eskalith [90,91].

Therapeutics used for anti-aging administered in humans demonstrated reduced inflammation, prevention of cardiovascular disease, and can slow down functional decline of organs.

These drugs have proved to be effective for treating carcinogenesis by interfering with proliferation, programmed cell death, metabolism of the cell, Angio-neogenesis.

Drug specific advantages

Eskalith-This lithium containing drug which is administered for balancing neurotransmitters and bipolar disorders inhibits GSK-3 enzyme to promote longevity, slowing down of brain cell aging and generally improving health parameters [92-97].

Metformin-Widely used therapeutic drug for diabetes, this is a multi-therapeutic drug which has numerous other benefits such as prevention of carcinogenesis, cardiovascular disease specifically coronary artery disease and cardiac arrest, offers neuroprotection by regulating AMPK pathway.

Rapamycin-Immunosuppressant, which is administered in organ transplantation patients, is also used as an anti-cancer drug that promotes longevity by inhibiting the mTOR pathway, providing protection against age-specific cognitive decline. Resveratrol-It is a multi-therapeutic drug which is involved in the regulation of AMPK pathway, plays a vital role in inhibition of cancer cell proliferation, protection from neurological disorders, reduce risks of cardiovascular diseases and diabetes.

Disadvantages

All these drugs are prone to Harsh side effects when administered in long run including lithium toxicity, hallucinations, hives in eskalith using patients; Vitamin B12 deficiency, low blood sugar level, anaemia with prolonged metformin usage; High cholesterol and elevated lipid levels in case of rapamycin [98].

Other general issues with these drugs are weight gain, muscle weakness, loss of appetite and fatigue. There are six ethical arguments against anti-aging medicines which are as follows: Inequity, denial of aging's immutability, dominating nature, overpopulation, no natural deadline, ageism.

Conclusion

Aging is the onset of several diseases and disorders like cardiovascular diseases, neurodegenerative disorders, cognitive decline and functional decline of the organs, anti-aging therapeutics focuses on overcoming these possible complications and into leading an increased healthier lifespan [99]. Although this concept has been into consideration for over a long period of time, the last decade has been the initial limelight for research on anti-aging drugs. There is no such specific and exclusive drug for anti-aging but drugs that have already been into play for ages for various other complications such as diabetes mellitus-Metformin, immunosuppressant for organ rejection-Rapamycin, bipolar disorders-Eskalith, carcinogenesis and cardiovascular disorders-Resveratrol has proved scientifically to slow down aging by targeting the master regulators like AMPK pathway, mTOR, IGH-1, FOXO which plays an essential role in autophagy, inflammation, oxidative stress by regulation and inhibition [100,101].

Regenerative medicine is solely based on the property of regeneration in stem cells. There are several types of stem cells such as somatic stem cells, neural stem cells, mesenchymal stem cells, adult skeletal muscle stem cells, haematopoietic stem cells;

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in these few are pluripotent (partial regenerative capacity), totipotent (total regenerative capacity) and multipotent (specific regenerative capacity).

Clinically proven results of anti-aging treatments using stem cells like reversal of aging using cellular rejuvenation in mice and anti-aging stem cell treatment for reducing age related frailty in older citizens has opened doors for further stem cell oriented regenerative medicines. The anti-aging therapeutics targets improvising the quality of life and increased health span.

Although they are faced by challenges starting from ethical issues like overpopulation, ageism to clinical trials lasting many years, and heterogeneity in different individuals during drug administration, there are several strategies, studies and discoveries that are made to lead a fruitful healthy prolonged lifespan, later retirement and ultimately a positive societal and economic impact.

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Competing Interests

The authors declare that they do not have any significant competing interests, including financial or non-financial conflicts, that may influence or be perceived to influence the research findings or interpretation of results presented in this work.

Author Contributions

The study was conceptualized and designed with the collaborative efforts of all the authors. Data collection and analysis were performed by Ashwin Prabahar A, Sandra Nixon, Sandhiya P, Gnana Sowndariyan G and Vidhya Shagar S. Following the initial draft written by Ashwin Prabahar A, all authors provided feedback on earlier versions of the thesis. The final manuscript was reviewed and approved by all authors.

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