Recent Review on Oxidative stress, Cellular senescence and Age-Associated Diseases

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ABSTRACT
Reactive Oxygen Species (ROS) is considered as the main factor of the Free Radical theory of aging over centuries and it indicates the pathophysiology of aging in mammals. ROS causes oxidative stress, which is a major component in the aging process of higher organisms. ROS also leads to many age-related diseases such as cancer, cardiovascular disease, diabetes, etc. ROS causes damage to most of the biological membranes that cause these chronic diseases. Enhanced ROS levels at the cellular level lead to cellular senescence. It is a stage of cells where growth arrest happens associated with the secretion of Senescence-associated secretory phenotype (SASP) factors. Senescence maintains tissue homeostasis, functions in normal development and restricts tumor development. In this regard, recent experimental evidence has shown that the genetic or pharmacological ablation of senescent cells extends the lifespan and improves the health span. Here, we review the cellular and molecular links between cellular senescence and aging and discuss the novel therapeutic avenues that this connection opens.

INTRODUCTION
The main feature of aging is a steady decline in body functions. It is characterised by increasing damage to various organs and systems that finally ends in improper functioning of the tissues. As a result, aging is a cause of many diseases like cancer, dementia, type 2 diabetes, osteoporosis, cardiovascular problems, osteoarthritis, glaucoma, etc. But our knowledge about aging remains insufficient. General cellular and molecular features related to aging are identified but the biological features are not known at all. Aging hallmarks have helped in gaining more about the studies of aging and to reduce the heterogeneous age-associated diseases. The hallmarks of aging can be classified as,

1. Primary (the causative agents behind age-related damage)
2. Antagonistic (damage responses)
3. Integrative (after-effects of the responses and causes of aging phenotype)
that sometimes can also cause metabolic damage in age (Sun et al., 2016).

**Intrinsic and extrinsic aging features**

Generally, skin aging can be divided into two,

1. **Intrinsic aging** - it usually happens in the sun-protected areas.
2. **Extrinsic aging** - mainly happens due to UV and in areas open to sun (Kammeyer and Luiten, 2015)

As per the Fitzpatrick scale, extrinsic aging is overlaid on intrinsic aging and is based on the depth, time and longevity of UV on a particular skin type (phototype). Dark-skinned people have shown decreased aging due to UV exposure due to the presence of melanin which protects the skin from UV to an extent. Many other factors like smoking, air pollution also causes aging, which can be seen as wrinkles in the faces of large cigarette smokers.

Impaired body functions and skin problems like dry skin, ulcers, fungal infections, itching, dyspigmentation, wrinkles, benign and malignant skin cancers are some characteristics of extrinsic and intrinsic ageing. Both the aging differ at clinical levels and histological levels.

Well, molecular pathways are the same for both intrinsic and extrinsic aging. Damage to the extracellular matrix by matrix metalloproteinases and the development of reactive oxygen species are the characteristics of both the aging ROS build-up also causes the RTKs (receptor tyrosine kinases) to get activated through the deactivating of PTPs (protein tyrosine phosphatases). This, in turn, causes the signaling paths such as mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK to get activated and RTKs get phosphorylated. Expression of procollagen-1 is prevented and activation of transcription factor activator protein-1 (AP-1) and downstream of MAPKs take place. The build-up of senescent cells in the dermis and epidermis takes place both in intrinsic as well as extrinsic aging. The main source of senescent cells is unknown. But it is known that many mechanisms are accountable for their occurrence like the oxidative stress level, or an enhanced inflammation.

**Reactive Oxygen Species, Oxidative Stress and Organismic Life Span**

The part that reactive oxygen species and oxidative stress act in the life span of an organism is contentious. It was found that reports using longevity-related mutants in organisms like Caenorhabditis elegans, Drosophila and mice resulted in a miscellaneous report. Prolonged studies on yeast and rat glomerular cells showed that enhanced oxidative resistance caused enhanced adaptive response due to the reaction from different antioxidants and an increase in the reactive oxygen species production. It was also found on the studies on Caenorhabditis elegans, that there is a substantial increase in the life span due to the inhibition of respiration that led to an enhanced release of mitochondria reactive oxygen species. When there is decreased oxidative stress, through various exercise, caloric control, or some other stimuli, it was proof that shows enhanced life span through the initiation of metabolism in mitochondria. Consider an e.g., when Caenorhabditis elegans are kept under diet control, especially decreasing the glucose level, there is increased resistance to oxidative stress due to enhanced mitochondrial respiration and reactive oxygen species levels. Well, a different study with the glucose control was done with the anti-oxidant N-acetylcysteine (NAC) did not show any proof of an increase in both reactive oxygen species levels and longevity (Yun and Finkel, 2014). Reactive oxygen species levels are also found to enhance during normal exercise and sequentially promotes the anti-aging pathways (Yavari, 2015). But, thorough exercise is injurious and can increase the levels of oxidants and can trigger cell death. Thus, in general, the reactive oxygen species levels below a definite level is an advantage while above a particular level, it is harmful to the cellular components. The theory of mitohormesis is based on the fact that only reactive oxygen species that are harmful to the cellular components are targeted without affecting the oxidants needed in cellular-signaling.

The importance of oxidants in cellular senescence was more emphasised by the studies of an activated Ras gene can also cause senescence in human fibroblasts. Upcoming analysis of activated Ras in diploid fibroblasts reported an enhanced level of oxidants. Moreover, by decreasing the surrounding oxygen or using a cell-permeable antioxidant, the growth arrest caused by activated Ras in human diploid fibroblasts and can be upturned. As a result, it shows that senescence activity is enhanced due to the normal and constant rise in oxidants.

**Sources of Reactive Oxygen Species (ROS)**

Various ROS can be divided into two, Exogenous and Endogenous (Figure 1).

**Exogenous sources**

Various DNA mutations and enhanced reactive oxygen species levels can happen due to various exogenous sources such as UV and ionizing radiations,
smoking, chemotherapeutics, environmental pollutants and other toxins. Since the skin is always exposed to the outside environment, more exogenous reactive oxygen species producing sources are predominant in the skin. The structural integrity of DNA is weakened by the radiation, which reacts with the oxygen to form $O_2$, $OH$ (hydroxide anion) and $OH$ radicals and breakdown the nitrogen base pairing and phospho-di-ester bonds. There is also more risk for cardiac and respiratory problems because of the continuous exposure to cigarette smoke that also increases lipid peroxidation. A few xenobiotics also initiate superoxide release by intervening with the bioenergetics of mitochondria.

**Endogenous Sources**

The main source of reactive oxygen species is a diverse group of enzymes that is present in various cell compartment intracellular. Peroxisomes, from enzymes like xanthine oxidase, show superoxide release and release of hydrogen peroxide from beta-oxidation of fatty acids. On the other hand, reactive oxygen species are formed in the endoplasmic reticulum due to the leakage in the transfer of electrons from NADPH to cytochrome P450. A group of enzymes called NADPH oxidases show a major part in the formation of free radicals due to aging. In the beginning, NADPH oxidases were known to be present in the phagocytes as a natural producer of reactive oxygen species for destroying microbes. Later it was found that other members of the NOX family produced reactive oxygen species not only in the phagosomes but also in many other tissues. Hence these isoenzymes play a major role in the age-associated diseases via redox signaling pathways. Each of the members in the NOX family is associated with a specific regulatory function, and there are a total of seven members of the NOX family such as (NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2) and also downstream targets in the cellular and tissue levels. In response to specific upstream stimuli, NOX proteins act as transmembrane enzymes that transfer electrons from cytoplasmic NADPH to molecular oxygen and form $O_2$ or $H_2O_2$ radicals (Takac et al., 2012).

**Oxidative stress at the macromolecular and cellular level**

Oxidant stress can be defined as the imbalance between the production of free radicals and reactive oxygen species and the antioxidant defense system in order to fight against them. The main cause of impairment to cellular macromolecules is the exposure to reactive oxygen species levels above the homeostatic threshold. The mitochondrial DNA is especially attacked due to reactive oxygen species and it is the chief source of intracellular oxidants. Oxidative stress causes heavy damage to the DNA like breakage of the double helix and more alteration of nitrogen bases. 8-OH-G development is the most studied DNA lesion. Through the excision repair of base or nucleotide, an impaired proliferating young cell can be repaired.

Enhanced reactive oxygen species levels affect the protein’s structure and functions. Among the proteins, cysteine and methionine are more prone to oxidation because they form disulfides among the thiol-groups between the proteins. Reactive oxygen species-mediated oxidation of protein is enhanced by the concentration of carbonyl groups. As the aging progress, there is an increase in protein carbonyl levels, although the exact damage inherited from oxidants appears to be more specific to the particular tissue. As the cell begins to age, proteasomes (agents that breakdown oxidised proteins) activity is decreased that leads to initiation of pro-death pathways. Lipids, especially fatty acid residues of a phospholipid, are also sensitive to oxidants. Hydroperoxides, which are formed by the lipid peroxidation involving free radical species that aim different carbon to carbon bonds is the main indicator of oxidative stress in various tissues. So the there is damage to both membrane and integrity of the cell due to the oxidant radicals. Severe damage to the membrane, change in the fluidity, and several other diseases are the causes of oxidation of lipids.

**Reactive oxygen species, oxidative stress and cellular senescence**

Hayflick and Moorehead really described the concept of cellular senescence in human fibroblasts, but later it was found to be the main cellular phenotype causing cancer and age-related diseases. Also, in recent times it was found that cellular senescence...
also plays a major role in embryogenesis. Hayflick described senescence due to the shortening of the telomere present in the somatic cells that go through mitotic cell division and also not contain telomerase. It is also known as replicative senescence or Hayflick Limit.

Cellular senescence not only occurs in fibroblasts, but it also happens in so many cells like epithelial cells, lymphocytes, endothelial cells, chondrocytes post-mitotic cells such as neurons and glial cells. It is also known non-telomeric signals also induce senescent phenotype, which involves different types of stresses like oxidative stress. Stress initiated senescence is also called stress-induced premature senescence (SIPS) or simply as premature senescence (PS). SIPS/PS also contain other forms of senescence initiated by non-telomeric signals like oncogene-induced senescence (OIS), and telomere attrition-induced senescence (replicative senescence). Some degree of telomere dysfunction/damage is also involved in SIPS/PS and OIS or other forms of senescence (Correa-Melo et al., 2014). Cells that go through cellular senescence are active in their metabolism and depict stable growth arrest, enlarged, vacuolated and flattened morphology. Senescence associated β-galactosidase (SA-β-gal) marker identifies senescent cells using histochemical staining of the cells grown in culture. Senescence-associated heterochromatin foci (SAHF) is another marker of senescence that is not commonly used. A key tumor suppressor, p16 (aka p16INK4a or CDKN2A), is a molecular marker that is upregulated in many senescent cells. Senescent cells also overexpress many secreted molecules, which are known as SASP factors in addition to stable growth arrest phenotype mediated by CDK inhibitors like p16. Under various senescence-inducing signals, few SASP factors differ across multiple cell types though many of them are conserved.

**Reactive oxygen species, mitochondria, and cellular senescence**

Improper functioning of mitochondria definitely leads to senescence and aging, since mitochondria mainly produce reactive oxygen species. Damage to telomere and non-telomere DNA can activate DDR due to reactive oxygen species and it was also found that DDR can produce reactive oxygen species through a positive feedback mechanism. This mechanism proceeded through a p53-dependent signaling pathway that includes p21, GADD45A, p38, GRB2, TGFBR2, and TGFβ in human diploid fibroblasts. Non-mitochondrial reactive oxygen species are also included in the intracellular reactive oxygen species. So, non-mitochondrial reactive oxygen species and mt reactive oxygen species precipitate and collaborate in the initiation of cellular senescence.

The current study reported that mitochondria and mt reactive oxygen species are vital for initiation of senescent phenotype via common markers such as SA-β-gal, and producing a full spectrum of senescent features. In the above study, Proteasome mediated pathways and autophagy cause the degradation of mitochondria using CCCP (Carbonyl cyanide m-chlorophenyl hydrazone), an uncoupler that induces mitochondrial depolarization, and degradation which leads to cell cycle arrest but SA-β-gal induction and SASP factors were absent. P21 and p16 were not upregulated by the cells as it is usually observed with senescence initiation. The studies also added the importance of mTORC1, which may integrate DDR signals, and PGC-1β in mitochondrial biogenesis and senescence initiation. Hence, the studies indicate that mitochondria play a major part in senescence and the degradation of mitochondria can affect many cellular functions, which could even dominate some of the phenotypes linked with senescence (Ziegler et al., 2015).

**Phenotypes and signaling pathways**

Cellular senescence happens due to replicative and non-replicative stress, as depicted in vitro studies. Studies were further done in senescence (replicative) in cell cultures show low proliferative capacity in human cell culture. Exogenous sources such as to expose to x-ray, DNA damage, oxidative stress, chromatin damage, mitochondria damage and endogenous sources like transcription stress like overexpression of activated oncogenes can cause non-replicative senescence. Senescence due to transcriptional stress is also known to be stress-induced premature senescence.

Senescent cells are not properly identified because many senescence programs do exist and most of the senescence markers are not specific but still, senescence phenotype and its features can be studied in vitro and in vivo. Morphological characteristics of Senescent Cells include huge, flat and aberrant structures containing a big nucleus as well as the nucleolus, vacuoles in the cytoplasm. A usual lysozyme β-gal i.e., senescence-associated beta-galactosidase, constitutes the “senescence phenotype.” SA β-gal effect is mainly considered as a marker of senescent cells both in vitro and in vivo even though, and some scientists say it should be used in combination with other markers, such as p16. Transcriptomic and pharmacologic studies were done to study in detail about the pathways involved in the senescence phenotype. Lack
of proliferation is the main feature of senescent cells. The inhibition of the cell cycle is long known as the method to prevent premalignant expansion in cancer cells. Premalignant tumors are features of senescence, and senescence must be absent for the development of malignancy. The latest studies prove that senescence plays a part in aiding tumor relapse in autonomous and non-cell autonomous activities. The high expression of p53, p161InK4a, p21 (a protein that inhibits) and the proteins that down-regulate the replicating cells such as pCNA, c-Fs and cyclins constitute the repression of the senescent cells. Hence, p16, p21 has highly researched biomarkers associated with senescence.

Senescent cells depict many modifications in mitochondria, DNA, lipid metabolism, oxidation balance, glucose metabolism and inflammation signaling other than of replicative arrest.

Some markers of DNA damage include DNA markers linked to senescence-like SDF Senescence Associated DNA damage foci (SDF) and Senescence Associated Heterochromatin Foci (SAHF) includes p-γH2AX, TAF is commonly noticed in senescent cells. The development of cellular senescence involves improper mitochondrial function and the so caused imbalance in the associated oxidative metabolism. Reactive oxygen species (reactive oxygen species) are evolving as the main cause of spreading senescence from senescent cells to the adjacent cells. Many times, improper mitochondrial functioning can lead to problems in fatty acid metabolism and it can even lead to age and diabetes-related hepatic steatosis. Hence, senescence causes drastic changes to metabolism and bio energy. Senescent cells initiate cell cycle arrest via AMPK activation and depict an enhanced and ineffective glycolysis, with regards to ATP reduction and AMP increase. AMPK enhances ATP release and decreases its uptake, enhances fatty acid oxidation, glycolysis slightly suppresses mTOR and also stops cell initiation, proliferation and biosynthesis. mTOR exists in cells as two different complexes, complex 1 (mTORC1) and complex 2 (mTORC2) complex 1 respond to the signals associated to the nutrient and in the initiation of cell initiation and release of protein, and lessens autophagy, while complex 2 involved in the arrangement of the cytoskeleton. mTOR activity in senescent cells is enhanced and it plays a major part in many cell activities like cell metabolism, discharge of proinflammatory causes, cell cycle inhibition. mTOR network is also evolving as the biological activity that controls the nutrition activity to senescent cells and adjusts the inflammatory response (Weichhart et al., 2015).

Antioxidants

Superoxide dismutase enzymes have a major role in regulating oxidative stress and redox signaling by catalysing the dismutation of two superoxide anions into oxygen and hydrogen peroxide. Presently, SOD1, SOD2 and SOD3 are the three types of SODs present inside the cell in the cytosol, mitochondrial region and extracellular region, respectively (Kwon et al., 2012). There are so many studies showing the changes in SOD levels with the aging process but still, many studies appear totally different. As aging proceeds, most of the animals and humans depicted less SOD1 activity in many tissues but did not show major changes inside the plasma, muscles and red blood cells in humans. But there was enhanced SOD1 activity in animal tissues like in rat brain and skeletal muscles. Well there are few cases where some areas of the body will first show an increase in SOD2 and gradually reduction is seen in the early sixties. Any removal of enzymes can cause major phenotypic changes in the skin. Still, the actual reason for age-related SOD1 and SOD2 activity is not yet known, but there exist sharp tissue-dependent differences.

Glutathione (GSH) is an important antioxidant that occurs in large quantity and it functions as defending and protecting against oxidative stress. It acts as a cofactor of enzymes such as glutathione peroxidase, or glutathione-S-transferase (GST), and it counteracts hydrogen peroxide, lipid hydroperoxides, or xenobiotic. People around the age of 70 showed a decrease in the activity of GSH concentrations in the brain and erythrocytes than that of younger people of the age of 20 (Zhang et al., 2011). In human plasma, the GSH concentration does not change like that of SODs. The enzyme inductions of GSH synthesis maintains the GSH homeostasis. So the increase in oxidative stress associated with the aging enhances both GSH usage and degradation to maintain the cellular redox balance. There is an increase in the oxidants and probable cellular destruction due to the whole reduction in the adaptive response of GSH synthesis because of the weakened process. Cigarette smoking is also a reason for the decrease in GSH activity in the extracellular lining of fluid in the lungs. Results depicted that smoke-exposed lungs of aged mice showed reduced receptiveness of GSH levels than that of younger mice.

Catalase is an important enzyme localised in the peroxisome and it deactivates hydrogen peroxide to water. Catalase activity depends on tissue activity like the other anti-oxidants with respect to aging. Well, reports of human and rat models depicted absolute difference, indicating that more studies should be done to know the part of catalase in the oxidative stress.
aging process (Doria et al., 2012). Vitamin C, E, A, flavonoids and minerals are few other antioxidants. Some studies have shown that direct intake of supplements did not show any change in the cure of diseases like cardiovascular disease (CVD) or cancer. Hence to keep the redox homeostasis, methods of absorption and sources of these exogenous antioxidants are an important factor.

Forkhead box class (FOXO) family of winged-helix transcription factors regulate the antioxidant enzymes like Mn-SOD (SOD-2) and catalase. The human homologs of DAF-16 in Caenorhabditis elegans constitute these transcription factors like FOXO1a, FOXO3a, FOXO4, and FOXO6. DAF-16 maintains oxidative stress and longevity in C. elegans by the inactivation of DAF-2, which acts upstream of AGE1/ AKT, and it is also an orthologue of the mammalian IGF1-R. Transcriptional Maintenance of Mn-SOD and catalase in mammalian cells mediates the resistance to oxidative stress that was done by FOXO3a like DAF-16 does (Klotz et al., 2015). Insulin and other growth factors activate the FOXO3 acting downstream of the InsR/IGF1-R-P13K-AKT signaling pathway and it is similar to DAF-2-AGE-1-AKT pathway of C. elegans. The antioxidant proteins such as SOD-1, mitochondrial peroxiredoxin-3 (Prx-3), Prx-5, glutathione peroxidase-R1 (GPx-1), mitochondrial thioredoxin 2 (Trx2) and thioredoxin reductase 2 (TrxR2) is regulated by FOXO transcription factors. Through posttranslational mechanism and other similar mechanisms, oxidative stress and reactive oxygen species also cross regulate expression of FOXO Proteins, designating that interaction between reactive oxygen species and FOXO proteins regulate the expression of cellular and antioxidant proteins and the response of different organisms responses to oxidative stress.

Oxidative stress, senescence and age-associated pathologies

As mentioned earlier, enhanced levels of reactive oxygen species can adversely affect the cellular and tissue physiology and wellbeing of an organism. Hence, age-associated diseases and other pathological conditions are either directly or indirectly affected by cellular senescence. Also, aging is also enhanced through therapy-induced senescence through a compromised immune system. Oxidative stress and interactive oxygen species which are related to cellular senescence also shortens the telomere and deactivates it and shortening of telomere is one of the major causes for aging as well as the cause for several age-dependent pathologies. Moving further, we discuss the impact of oxidative stress and cellular senescence on cancer, cardiovascular diseases and neurodegenerative disorders such as Alzheimer’s disease (AD) and Parkinson’s disease (PD).

Cardiovascular diseases

Shortening of the telomere can lead to cardiovascular risks such as atherosclerosis, heart failure and hypertension. Chronic oxidative stress can also occur due to the improper functioning of the telomere, and its shortening is a distinct feature of senescent cells that undergo replicative senescence. Cardiovascular diseases are initiated via the SASP factors produced by senescent cells through autocrine and paracrine activates, which triggers the degenerative and proliferative activities in various tissues and other organs. Improper function of the epithelial cells and the senescence of human umbilical venous endothelial cells (HUVECs) can cause atherosclerosis with aging. Many studies reported the increased sections of pro-inflammation cytokines such as TNF-α and IL-6 levels amongst older individuals. Reactive oxygen species are also produced in association with the damage to the arterial walls and it also initiates the oxidation changes to the low-density lipoproteins (LDL). The most critical step that causes atherosclerosis is the migration of LDL from the bloodstream to the subendothelial space. TNF-α also activates NADPH via the enhanced production of reactive oxygen species, which leads to oxidative stress. Activation of different inflammatory activities inside the arterial walls is involved with a redox-sensitive transcription factor NF-kB which leads to the translocation of immune cells and cytokines to the region. NFκB expression is maintained by TNF-α. In comparison, with a young and aged healthy person, it was found that both NFκB and NADPH levels were increased. The major regulator of the SASP phenotype in senescent cells is NFKB. It was found that the lifespan of mice was enhanced by a senescence suppressor protein called Klotho and showed negative results in atherosclerosis and endothelia dysfunction. It was also found to deactivate TNF-alpha-induced expression of adhesion molecules and NFκB activation.

Alzheimer’s disease

Alzheimer’s disease is a major disease associated with aging, and it is the most predominant form of dementia and affects a major group of people above. Neuronal death in selective parts of the hippocampus and other brain areas is the main cause of this disease. Formation of senile plaque due to the accumulation of amyloid B peptide (AB) and neurofibrillary tangles are some other factors. The brain always consumes a large amount of oxygen
genes are known to play a critical part in protecting syn, PINK1, and Parkin also constitute the improper oxygen species. Mutation of genes such as alpha-syn, PINK1, and Parkin also constitute the improper oxygen species after that oxidized dopamine is converted to neuromelanin and it gets accumulated in the SnpC. Patients with irregular PD observed are infrequent, and around 15% of it is familiar and is associated with some diseased gene.

Cell death pathways are initiated by oxidative stress in both of the forms. The first sign of PD development is because of the dopamine molecule itself. Mitochondrial permeability in the brain is changed due to the immediate auto-oxidation of dopamine. Ferritin catalysed reactions and depletion of NADPH cause the production of reactive oxygen species after that oxidized dopamine is converted to neuromelanin and it gets accumulated in the SnpC. Patients with irregular PD usually depict a reduction in Complex I activity of the respiration chain. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, is one of the biomarkers of PD that inhibits complex I activity in the form of 1-methyl-4-phenylpyridinium (MPP+), disturbing the electron flow and producing reactive oxygen species. Mutation of genes such as alpha-syn, PINK1, and Parkin also constitute the improper functioning of mitochondria. As a whole, all these genes are known to play a critical part in protecting against oxidation and functions of mitochondria. α-synuclein is a protein that gets accumulated and it is a feature of PD like as in AD. Presynaptic signaling and neuroplasticity are the main functions of the protein. But, α-synuclein is found to regulate mitochondrial membrane permeability and decrease the complex I activity, thus enhance the reactive oxygen species levels. Parkin and PINK1 genes found in the mitochondria is having specific roles in an organelle’s proper functioning. PINK1 usually acts as a neuroprotective agent by getting into the outer membrane and employs Parkin to initiate mitophagy in the impaired mitochondria. Autosomal recessive PD is a characteristic of PINK1 mutations. While deactivated complex I activity constitutes Parkin mutations and it is much prone to oxidative stress because of the accumulation of damaged mitochondria, thereby causing cell damage in the neurons. In recent times, it was found that environmental stressors linked to PD also provoke senescence in glial cells and the glial cell SASP in the non-neuronal glial cells while the brain is getting old.

Type 2 diabetes (T2D)

Senescent cells are the causative agent for many age-related diseases like T2D. Senescent cells in peripheral adipose tissue and senescent pancreatic beta cells induce the SASP factors and it amplifies the T2D pathogenesis, and the senescent cells are again initiated by metabolic and signaling changes induced by high circulating glucose, growth hormone and lipid metabolites. It was found that elevated glucose levels can initiate premature senescence in pre-adipocytes, fat cells and fibroblasts through the enhanced reactive oxygen species and mitochondrial dysfunction. Senescent cells in pre-adipocytes can cause improper adipose tissue functioning causing obesity, which is probably caused by SASP factors, is the main reason for developing resistance to insulin. Well, some anti-diabetic drugs like metformin inhibit SASP factors (secreted through NFkB) by senescent cells and cure diabetes. Metformin treatment resulted in extending the lifespan in diabetic patients and mice model, and less chances of cancer were by inhibiting of SASP factors by metformin (Palmer et al., 2015).

Cancer

Based on the cellular context, various stages of cancer progress, and other therapeutic involvements, oxidative stress and cellular senescence act as both pro-oncogenic and anti-oncogenic. Since the intracellular and mitochondrial reactive oxygen species are produced in large amounts, cancer cells have an oxidative stress environment continuously and it...
also causes mitochondrial and nuclear mutations in DNA and genetic instability. All the factors that are necessary for tumorigenesis like activated hypoxia-inducible factors (HIFs) and PI3K pathways, and metabolic that results in enhanced proliferation due to adaptation, increased cell survival, migration and invasion, and inhibited cell death pathways are activated with the enhanced mitochondria and NOX-generated reactive oxygen species (Glasauer and Chandel, 2014).

Besides HIFs and PI3K pathways, network of transcription factors such as NFκB, STATs, NRF2, p53, AP-1 and PPAR is also activated by reactive oxygen species which additionally cause changes in tumour cells, neighbouring cells and the tumour environment due to oxidative stress causing invasive tumour, metastasis, angiogenesis, cancer stem cell survival and therapy resistance. The enhanced reactive oxygen species makes the cancer cells more prone to the initiation of cell death pathways and therapeutic involvement leading to cellular senescence. Cancer cells are more prone to exogenous reactive oxygen species generating drugs or agents as they are dependent on the enhanced antioxidant capacity. Well, novel drug therapeutic systems are developed to target the adaptive redox response in cancer cells as they develop drug resistance due to this.

On the other hand, it has been debated that cellular senescence has also got tumour suppressing activity because the phenotype that stops growth probably inhibits the tumour cell proliferation thereby tumour growth along with other prominent features are also stopped. Development of cancer and replicative immortality happens in the absence of senescence and re-expression of telomerase is essential for the cancer cells. Senescence is also known to have the ability to suppress tumour since the damage to DNA and oncogenic signals greatly incite senescence-like phenotype because of the response initially given in vitro and in vivo. The pro-oncogenic part of senescence is explained through different SASP factors, which also assist the oncogenic phenotypes in different types of cancer. The SASP factors can be classified into 3 groups:

1. Inflammatory chemokines and cytokines including IL-1, IL-6 and IL-8
2. Growth factors like CSFs (colony-stimulating factors) and VEGF (vascular growth factor)
3. Secreted Matrix remodeling proteases like MMPs (matrix metalloproteases). Pre neoplastic and neoplastic cells show various effects like proliferation, invasion, migration due to the action of SASP factors, which has got countless effects on these cells.

In opposite to that, therapy-initiated senescence can cause progress in the tumour, resistance to treatment as well as reoccurrence through SASP factors. In recent times, it was found that senescence can increase cancer stem cell phenotype the pro-oncogenic effects of senescence is facilitated by the SASP factors through cell non-autonomous approaches, including paracrine signaling. However, some SASP factors also initiate senescence in adjacent cells and maintain senescent phenotype, including initiation of reactive oxygen species through a positive feedback loop. As a whole, senescent phenotype intensifies and assist cancer development and probably act as a part in disease reoccurrence.

**Senescence compounds**

The anti-aging compounds or compounds with anti-SASP functions can be classified into,

1. Fully synthesised, since they do not exist in nature.
2. Semi-synthesised, by incorporating chemical changes to the substances derived from microbial and flora.
3. Entirely natural

Synthetic substances are made to cure the majority of the conditions, like SASP acquisition, which can be modulated by chemotherapeutics, but they possess many adverse effects. So they are always restricted to certain conditions where they possess positive advantages and disadvantages. This happens in the case of a few drugs with anti-senescence activity. Since anti-senescence studies were started recently, preclinical data can reach the human trials only via measurable markers, outcomes and other human parameters.

**Synthetic substances having anti-senescence features**

**Dasatinib**

It belongs to an anticancer agent that stops the cells from multiplying, migrate as well as cause cell death. It is an oral tyrosine kinase inhibitor. Patients with chronic myelogenous leukemia were approved by the FDA to be treated with this drug. But hematological toxicity, gastrointestinal and respiratory side effects are the common adverse effect of this drug. Current studies reported that dasatinib removed senescent cells involving efficiency as much as cell type-dependent.
Navitoclax and other BCL family inhibitors

This is an anticancer drug that was previously known as ABT-263, with senolytic properties researched mainly in animal models. It activates the cell death pathway of mitochondria, and stops Bcl-2, Bcl-xL, Bcl-w (Bcl family members) protein and initiates the discharge of pro-apoptotic factors like Bim. Gastrointestinal problem is usual adverse effect and fatigue is also occurs but not limited to dose. Bcl-xl inhibition can cause thrombocytopenia, which lessens the platelet lifespan. It shows an anti-senescence effect (cell type mediated), as seen in vitro studies. Navitoclax does not affect human senescent primary preadipocytes but harmfully acts on the feasibility of different senescent cell types such as human umbilical vein endothelial cells (HUVECs), MEFs, human lung fibroblasts (IMR-90).

Panobinostat

This drug is an anticancer agent that is used to treat multiple myeloma after it got approval from the FDA in 2015. It is usually used in combination with anticancer drugs like corticoid dexamethasone, bortezomib and it is finely abided in adults. Hematological and gastrointestinal problems are the common adverse effects of this drug. Its anti-senescence activity was proved on lung cancer (small senescent cell) and squamous cell carcinoma (head and neck). Cisplatin/taxol used for cytotoxic treatment was given a continuous prosenescence response in the cell population. This drug when taken alone, particularly destroyed SCs, thus evidencing an active post-chemotherapy senolytic (Samaraweera et al., 1990).

HSP90 inhibitors

HSP90 (Heat shock protein 90) is one of the most effective drugs with senolytic activity in recent times. Geldanamycin, and 17- dimethylaminooethylamino-17-demethoxygeldanamycin (17-DMAG) its derivative, were the two HSP90 inhibitors found to possess anti-senescence function among the 97 drugs tested in vitro. Repeated alternating courses of 17DMAG significantly hindered the starting of symptoms due to aging in mice, which caused an improvement in the overall healthspan along with half the decrease in p16INK4a effect on the kidney (Zhu et al., 2016).

FOXO4-p53 targeting peptide

This belongs to a section of transcription-factors, it is active along the downstream of IGF-1, related to the aging process. After knowing that FOXO4 does a major part in senescent cells survivance, an altered form of FOXO4 i.e., D retro Inverso was made. The study showed that the therapy particularly made intrinsic cell death and p53 nuclear-exclusion in senescent cells; also, therapy of mice that are fastly aged and normally aged was found to redeem fitness, the density of the fur, and renal activity prolonging healthy life span and improving chemotoxicity initiated by doxorubicin. Even if FOXO4 peptide was tolerable in amounts that show anti-senescence activity, safety issues in human beings are still in concern.

2-Deoxy-D-glucose

When glucose intake is more by SCs, it survives more, which is the same as seen in cancer cells. Substituting a 2-hydroxyl group in 2-Deoxy-D-glucose with H moiety changes into toxic. The safety, efficacy, pharmacokinetics, and maximum tolerable dose of 2-DG combined with docetaxel in patients were assessed by conducting a phase I trial with advanced solid tumours. In recent times, 2-DG has been used for the effective elimination of senescent muscle cells (vascular and smooth) selectively in vitro, and to remove the senescent cells in a tumour model of treatment-incorporated senescence in vivo.

Natural senolytic compounds

Many molecules in nature have the ability to inter-relate with biological activities. They are called as bioactive molecules, and food having these compounds are named "nutraceuticals." Many studies have been done to recognize these compounds that are able to cure pathological conditions, age-related diseases, and/or impersonate anti-aging properties like metformin and rapamycin exhibit, excluding the side effects. Allantoin, ginsenoside, and epigallocatechin gallate are novel metformin and rapamycin impersonate, is currently considered as an effective compound for experimental validation (Aliper et al., 2017). It was also suggested that bioactive compounds present in the food could act on SASP to prolong the lifespan and to lessen the age-related diseases and its progress.

Even though we know that food impacts our health, the molecular approaches through which it acts is not certain yet. It’s been studied that many bioactive molecules can work in controlling gene-expression, methylation arrangement in DNA, the position of the chromatin assembly, and miRNA, siRNA, piRNA non-coding. Foods that are rich in PP have the ability to control the action of DNA methyltransferase, a histone acetyltransferase, histone deacetylases, etc., which are DNA readers and writers indicating that this also helps in healthy life span. Some compounds are cell type and dose-dependent and it controls the progress and management of cellular senescence. Cells acquire an epigenetic profile, which is followed...
by the future cell generations in the form of epigenetic memory.

**Natural compounds having senolytic features**

The studies regarding the senolytic property and anti-SASP function of the natural compounds have done very recently, and there is no much proof of the antiaging activity of the natural compounds. But there are many studies that showed the anti-SASP property of the natural compounds, but that showing the senolytic property is less. This is expected as senolytic activity is new in the research zone. There are many study reports on quercetin, tocopherol, and piperlongumine (PL), while more data regarding the synthetic and natural compounds can be obtained in the upcoming years.

**Tocotrienols**

It has got effective anti-oxidant properties, and also play a major part in cell signaling, immune response, and cell death. It belongs to the vitamin E family but not much known. In recent times, they were considered as compounds with senolytic property. They show two opposite effects like they decrease the malignancy in cancer cells by enhancing senescence in the cells (Malavolta et al., 2014) and reduce the formation of senescent cells in normal tissue, thereby delaying the aging process.

**Quercetin**

Quercetin is known to exhibit two complementary effects like they slow down senescence, initiate apoptosis in aging cells, and endorse the removal of senescent cells in normal healthy tissue. It is used along with flavanol contained in many vegetables/fruits as it shows an enhanced effect with it. (Hwang et al., 2018). In several mouse models, it was seen that quercetin and dasatinib exerted notable differences in the healthspan.

**Piperlongumine**

It is an anticancer agent who is a product of Piper longum. It exhibits its anticancer activity by concealing the cancer stemness and hindering the malignant phenotypes. Senescence due to exposure to ionizing radiations, oncogene (Ras) ectopic expression is prevented by piperlongumine by mainly destroying human WI-38 fibroblasts (Wang, 2016).

**Curcumin**

Curcumin is obtained from Curcuma longa (turmeric) plant as a yellow pigment. It has got many pharmacological effects like anti-oxidant, anti-inflammatory effects. Curcumin decreases the oxidative stress and enhances the feasibility of retinal pigment epithelial cells, it increases the SKN-1/NRF2 (antioxidant response transcription factor), hence contradicting peroxidation in lipids and stress due to oxidation in C. elegans and Drosophila (animal models). HDACs like HDAC1, HDAC3, HDAC8, sirtuins (epigenetic enzymes) have been controlled by curcumin, and p300 histone acetyltransferase (transcriptional co-activator proteins), and to enhance life span in few animal-models. But hydrophobic property and limited oral bioavailability is a disadvantage of this compound. Well, novel strategies have been developed like micelles with curcumin to enhance the delivery.

**Vitamin B3 complex and NAD+**

White meat, peanuts, and mushrooms are very rich in vitamin B3. The family consists of nicotinic acid, niacinamide and nicotinamide riboside that constitutes the precursor of NAD. NAD+ get reduced to form NADH, a decrease in this can initiate senescence onset, impairs DNA repairing and affects SIRT activity (Imai and Guarente, 2014). SIRT2 reduction suppresses the deacetylation of BUBR1 (mitotic checkpoint kinase budding uninhibited by benzimidazole-related 1), which, in turn, inhibits senescence. Mitochondrial dysfunction-associated senescence (MiDAS), a secretory phenotype associated with senescence of mitochondrial SIRT3 or SIRT5. In addition to that, these senescent cells are constituted by a low NAD+/NADH ratio. Senescence slowdown in the presence of nicotinamide mononucleotide supplement and NADH oxidation. Treatment with NAD+ helps to cure age-related metabolic reduction and to initiate longevity in C. elegans. Reduction with NAD+ levels with aging increases the risk of age-related diseases and providing NAD+ actives can reduce the risk.

**Targeting senescent cells for a healthy life span**

Whether senescent cells developed because of stress factors like reactive oxygen species or improper functioning of the telomere, it is doubtfully shown that senescent cells get added in aged and pathological tissues. To assist the in vivo study of senescent cells, SA-β-gal staining, together with CKI-driven senescent cell reporters such as p16-luc, p16-3MR (trimodality reporter) can be used. Fluorescent markers are used to identify the senescent cells by the p16-3MR and INKA-ATTC reporters and destroy the senescent cells in vivo with the help of these drugs like ganciclovir and AP20187, respectively. As mentioned above cellular senescence can cause many age-related diseases such as CVD, T2D AD, PD, Huntington's disease (HD), cataracts, macular degeneration, glaucoma and osteoarthritis, and physiological traits such as skin wrinkling, hair graying, reduced hearing, poor vision, diminished wound healing, sarcopenia and
immune system dysfunction. All the pathological traits mentioned are either related to the activity or expression of p16 and p53 tumour suppressors and they provide a negative influence on the stem cell pool or SASP factors. Though it is tough to prove experimentally, the ability of senescent cells initiate aging and age-related diseases, but it is testable. Few recent studies have proved that the removal of senescent cells indeed enhance a healthier life span and reduces age-related diseases. Baker et al. showed that the p16-positive senescent cells reduce the healthy lifespan in the wild and a BubR1 progeroid mouse model, and their absence slows down most of the age-related diseases and impairs rejuvenation to the animals. The researchers developed transgenic mouse models using INK-ATTAC transgene, which expresses GFP and FK506-binding protein-caspase 8 (FKBP-Casp8) under the influence of a minimal Ink4a promoter which is supposed to be active in the senescent cells only. The authors then administered AP20187, which activates FKBP-Casp8 to initiate cell death, particularly in senescent cells. It was also found that the removal of senescent cells AP20187 in the transgenic animals weakened age-associated pathological traits in most of the organs like kidney and heart with no side effects at all. Independence to the background and sexes of the animals, the average lifespans were enhanced by 24-27%. p16 is an active tumour suppressor; the removal of p16-expressing senescent cells reduced tumorigenesis, indicating that in senescent cells; indeed, the SASP function can dominate the tumour suppressing function. Also, in a mouse model, it was found that pointing senescent cells increases adipogenesis, decreases lipotoxicity, increases insulin sensitivity, and metabolic function in the aging animals. In recent times it was reported to show that, in general, the senolytic drugs that remove senescent cells through inhibition of pro-survival molecules such as p21, BCL-2, BCL-XL, PI3K and PAI2, can enhance cardiac function and healthy lifespan, and slow down age-related pathologies. These drugs were also shown to revitalise organ-specific stem cells especially hematopoietic, muscle and hair follicle stem cells (Yosef, 2016).

Enhanced oxidative stress has been considered as one of the most common etiology in the aging process. Literature has proved that free radicals (ROS/RNS) in the pathophysiology of the health diseases associated with this. Generally, for normal processing of cells, there must be a balance in the amount of ROS and antioxidants present in our body. The formation of ROS takes place in the powerhouse of our cell i.e., in the mitochondria. Antioxidants work by inhibiting or neutralizing the free radicals and prevent the origin of chain reactions hence reduces oxidative stress. In normal circumstances, antioxidants act as scavengers that scavenge the ROS and preserve the cellular atmosphere. Oxidative stress releases ROS, which can harm the biomolecules that initiate lipid peroxidation, mis-folding of protein, DNA damage and DNA mutations, and aggregation. ROS can also harm to neurons and can get collected in the brain, which can also cause neurodegenerative diseases. Improper functioning of mitochondria can cause unevenness in ROS and antioxidant systems in the cellular atmosphere (Singh et al., 2019).

CONCLUSION

Senolytic agents that aim at the aging processes can be changed into clinical practice. These agents have the ability to increase the lifespan, healthspan, deferring, alleviating so many chronic diseases like cardiovascular diseases, type 2 diabetes, Parkinson’s, cancer, etc. which cause an increase in mortality, illness, and cost of health in the nation. Also, they can defer or cure the aged symptoms such as infirmity, sarcopenia, cognitive impairment and immovability and other age-associated problems like loss of physiological pliability to another level of imagination. Senolytic compounds could convert geriatric medicine from being tertiary or quaternary prevention into an important primary option, which is equal to or enhanced than that of other medical specialisms.

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