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In search of elixir: Pharmacological agents against stem cell senescence

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ABSTRACT

Stem cell senescence causes different complications. In addition to the aging phenomenon, stem cell senescence has been investigated in various concepts such as cancer, adverse drug effects, and as a limiting factor in cell therapy. This manuscript examines protective medicines and supplements which are capable of hindering stem cell senescence. We searched the databases such as EMBASE, PubMed, and Web of Science with the keywords "stem cell," "progenitor cell," "satellite," "senescence" and excluded the keywords "cancer," "tumor," "malignancy" and "carcinoma" until June 2020. Among these results, we chose 47 relevant studies. Our investigation indicates that most of these studies examined endothelial progenitor cells, hematopoietic stem cells, mesenchymal stem cells, adipose-derived stem cells, and a few others were about less-discussed types of stem cells such as cardiac stem cells, myeloblasts, and induced pluripotent stem cells. From another aspect, 17β-Estradiol, melatonin, metformin, rapamycin, coenzyme Q10, N-acetyl cysteine, and vitamin C were the most studied agents, while the main protective mechanism was through telomerase activity enhancement or oxidative damage ablation.

Although many of these studies are *in vitro*, they are still worthwhile. Stem cell senescence in the *in vitro* expansion stage is an essential concern in clinical procedures of cell therapy. Moreover, *in vitro* studies are the first step for further *in vivo* and clinical studies. It is noteworthy to mention the fact that these protective agents have been used in the clinical setting for various purposes for a long time. Given that, we only need to examine their systemic anti-senescence effects and effective dosages.

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Introduction

Adult stem cells distributed throughout the entire body are responsible for tissue regeneration. However, human beings are not immortal and experience the aging phenomenon, as well as age-related diseases (1). Aging has received much attention in recent years, while stem cells play a crucial role in aging. It is established that the lack of sufficient stem cell regeneration leads to the aging phenomenon. Telomere shortening, DNA damage, harmful epigenetic modification, and depletion of proliferative potential all induce permanent cell cycle arrest, called cellular senescence. Senescent stem cells lose their functions for tissue rejuvenation and perseveration (2). Their high regeneration capacity or serial transplantation increases the rate of DNA damage and leads to telomere erosion, reactive oxygen species (ROS), and ultraviolet (UV) mutagenicity. Studies have demonstrated the accumulation of DNA damage in the stem cell aging process. This damage could affect cellular function or transform cells into cancerous types. Telomeres contain thousands of base pairs of repetitive DNA sequences, TTAGGG repeats, which protect chromosomes from end-to-end fusions. Each round of cell division reduces telomere length unless the cell expresses telomerase. Various checkpoints and mechanisms in the cell cycle monitor DNA integrity and fix most of the mutations. If during this monitoring the genome repair systems fail, apoptotic signals will clear any damaged cells. However, if the involved protein cascades are not strong enough to trigger apoptosis, these cells will permanently stop in the cell cycle; and this state is called cellular senescence. Senescence is a protective barrier against cancerous cell formation (3, 4). P53, p16, p19 (p14 in humans), and retinoblastoma protein (Rb) are tumor suppressor proteins playing a role in the cell cycle checkpoints. They negatively control cell division to reinforce DNA repair, and while genomic damages accumulate by age, their quantity increase in the cell. P19 promotes p53 protein stability by inhibiting Mdm2-mediated p53 degradation (5). P21 and p16 are cyclin-dependent protein kinase (CDKs) inhibitors. CDKs phosphorylate Rb and inactivate it to promote the cell cycle (6). A low concentration of p21 activates CDKs 4 and 6 and promotes the cell cycle; however, a high concentration of p21 suppresses cyclin E-dependent kinase 2 and arrests the cell cycle (7).

Senescent cells secrete pro-inflammatory factors such as IL-2, IL-6, IL-8, and TNF-α to attract the immune system to clear them. The high number of senescent stem cells as a result of the high rate of formation or low

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rate of clearance puts the body in a chronic inflammatory state. This condition causes more senescent cells, aging, and various non-aging pathologies such as metabolic syndrome or adverse drug effects (8).

There are different approaches to prevent stem cell senescence, such as using hypoxic conditions (9), exosomes or extracellular vesicles secreted from stem cells (10), supportive matrices or cells (11, 12), microRNAs regulation (13), and gene therapy (14). In addition to these approaches, we can use pharmacotherapy. Pharmacotherapy is a convenient and economical tool that can be applied for systemic aims. For this kind of intervention, there are various natural substances derived from plants and non-natural agents. We have reviewed natural agents previously (15). Therefore, this paper has outlined some problems related to stem cell senescence (16) and all studies about protective medicines and supplements also their protective mechanisms (Tables 1 and 2).

Materials and Methods

We searched databases such as Embase, PubMed, and Web of science with the keywords "stem cells," "progenitor," "satellite," and "senescence." On account of stem cell senescence being considered a therapeutic method in cancer therapy, we excluded the keywords "cancer," "tumor," "malignancy," and "carcinoma." The results were without a time limitation until 2020/06/01. We categorized the protective agents as natural (from plants) and non-natural. Following our review of protective natural agents in our previous paper (15), for the present paper, we selected 47 articles among 2137 results that investigated the protective effects of medicines and supplements which inhibit cellular senescence in various types of adult stem cells.

Results

Protective medicines inhibit adult stem cell senescence

Endothelial progenitor cells (EPCs)

Angiogenic progenitor cells with three surface markers CD133, CD34, and VEGFR-2 are called endothelial progenitor cells, which predominantly locate in bone marrow (BM). When they migrate to the bloodstream and express endothelial markers such as VE-cadherin, eNOS, and von Willebrand factor, their phenotype gradually changes. These cells are involved in re-endothelialization and neovascularization. EPC sources are BM or peripheral blood. Various factors, such as drugs, diseases, and growth factors, influence their number and migratory activity (17). For example, atherosclerotic and obese patients have premature EPC senescence (18, 19). Senescent EPCs have shown a finite migratory and proliferative potential for cell therapy in ischemic diseases (20). Patients suffering from migraines have more senescent EPCs with less migratory capacity leading to the conclusion that there is a possible link between a higher risk of cardiovascular diseases and migraine (21). Angiotensin II (Ang II), which causes hypertension, can accelerate EPC senescence and reduce their differentiation capacity (22). Doxorubicin is an antineoplastic agent that significantly increased the risk of heart failure. This anticancer drug via Nox2 (an NADPH oxidase) could induce EPC senescence (23). Also, studies showed that the rate of senescent EPCs had increased in pre-eclampsia (24).

In vitro studies

According to a study, aspirin (a cyclooxygenase inhibitor that prevents platelet aggregation in cardiovascular disease, 50-100 mM) protected h-EPCs against senescence and improved their migratory and adhesion ability. The authors declared the protective mechanism was not via eNOS (25).

Coenzyme Q10 (a vitamin-like substance that is also made naturally in the body, ten μM) phosphorylated AMPK, thus increased Akt/eNOS phosphorylation, HO-1 expression, which in turn reduced ROS and high glucose-induced senescence in EPCs of healthy volunteers. In this pathway, NO protected EPCs against high glucose (26).

Statins are anti-hyperlipidemia medicines that inhibit HMG-CoA reductase. Atorvastatin (1 μM) increased Akt phosphorylation and telomerase activity, thus reversed homocysteine-accelerated senescence in h-EPCs (27). Atorvastatin (0.1 μM) and mevastatin (0.1 μM) decreased cellular senescence in h-EPCs. The protective mechanism was independent of NO, ROS, and telomerase activity. In this study, atorvastatin up-regulated cell cycle proteins such as cyclin A, cyclin F, and down-regulated p27 in a dose-dependent manner via the PI3K/Akt pathway. Maybe the inactivation of FOXO by Akt could result in p27 down-regulation (28). Atorvastatin at the concentration of 0.1 μM activated the Akt pathway without affecting telomerase activity. However, the higher concentration of atorvastatin (10 fold) increased the Akt phosphorylation and telomerase activity. Telomerase activation through Akt may be dose-dependent in the EPCs.

The physiological range of insulin (0. 1, 1 nM) increased Akt phosphorylation, and eNOS expression in rat bone marrow generated EPCs in high and normal glucose conditions (29).

Angiotensin-2 (Ang2) decreased telomerase activity, increased peroxynitrite, superoxide, and AT1R mRNA and protein expressions in h-EPCs. Pre-treatment with pioglitazone (PPAR- γ agonist, an anti-diabetic drug, 10 μM) reversed these effects and decreased senescence in h-EPCs (30). Peroxynitrite and superoxide could damage the cellular genome. AT1R increases the action of Ang2 on cells. As mentioned before, PPARγ like Nrf2 controls the expression of HO-1at the transcriptional level. Then, HO-1 reduces the activity of NADPH oxidase and consequently the content of intracellular ROS (31).

Hormone replacement therapy with 17 beta-estradiol has positive effects on cardiovascular diseases in menopausal women (32). In a study, 17 beta-estradiol (100 nM) inhibited Ang2, reduced telomerase activity, increased AT_1R mRNA and protein expressions, and elevated gp91phox (NOX2) mRNA expression, thus inhibited h-EPC senescence. Ang2 induces peroxynitrite formation. Subsequently, peroxynitrite activates redox-sensitive NF-κB and leads to AT1R up-regulation. This study also showed that 17 beta-estradiol inhibited the p38/MAPK pathway (33). MAPK and p38 activation contribute to p53-induced replicative senescence (34).

Table 1. The summary of protective mechanisms of medicines (*In vitro* studies)

Agent	Concentration	Cell source	Mechanisms	Reference
Rapamycin	20 ng/ml	Mice BM-HSCs	↓ mTOR ↑Bmi-1 ↓p16	(66)
1,25-Dihydroxyvitamin D3	100 nm	h-MSC	↓ p16	(99)
Ascorbic acid	200 µM	rat BM-MSCs	↓ROS ↓ Akt/mTOR signaling	(98)
Aspirin	50 -100 mM	h-EPCs	Not studied	(38)
Atorvastatin	1 µM	h-EPCs	↑Akt phosphorylation ↑Telomerase activity	(40)
Atorvastatin Mevastatin	0. 1 µM	h-EPCs	↑PI3K/Akt ↓p27 ↑Cyclin A, cyclin F	(41)
Carbamylated Darbepoetin or Darbepoetin	100 ng/ml	non-dialysis stage 4–5 CKD patient EPCs	↑Telomere	(50)
Coenzyme Q10	10, or 100 μm/l	rat BM-MSCs	↓p21,p16,p53 ↓ Akt/mTOR signaling	(96)
Coenzyme Q10	500 nM	mouse neural progenitor cells	↓ ROS ↑Telomere length ↑SIRT3	(136)
Idebenone	10, 20 or 30 µg/ml	Rat MSCs	Progressed cell cycle	(97)
Insulin	0. 1, 1 nM	Rat BM-EPCs	↑ Akt phosphorylation ↑eNOS expression	(42)
Isosorbide dinitrate	50 µM	Rat MSCs	↑ERK/FOXM1 ↑ mir-130b	(94)
L-Carnitine	0.2 mM	Rat ASCs	Anti-oxidant	(128)
Melatonin	100 µM	CKD-Mice BM-MSCs	↑ PrPC ↑ Mitochondrial function	(103)
Melatonin	10 nm, 1 µM and 100 µM	Mice BM-MSCs	↓ROS level ↓p53, p21,p16 ↓p53/ERK/p38	(104)
Melatonin	10 nm, 1 µM, and 100 µM	BM-MSCs	↑SIRT1 mRNA level ↓p16 ↓Phosphorylated p38	(70)
Melatonin	100µM	h-ASCs	↑ AKT pathway ↑ catalase ↓ ROS AMPK/mTOR↓ SMP30↑	(130)
Melatonin	10 µM	Mice ASCs	↓ NADPH oxidase ↓ ROS	(131)
Melatonin	10 µM	CPCs	↑Long noncoding RNA H19 ↑mir-675 ↓p21, p53	(142)
Metformin	1 mM	Rabbit annulus fibrosus stem cells	↓Inflammatory factors	(140)
N-Acetyl cysteine	1 mM	h-iPSCs	Oxidative stress ↓	(149)
N-acetyl-cysteine	1mM	h-MSCs	↓ROS level ↓p53 ↓p38	(102)
Nicotinamide	5 mM	h-MSCs	↓ROS level ↑NAD+/NADH ratio ↑SIRT1	(105)
Nicotinamide	1 mM	h-iPSCs	↓ROS ↓p21,p53, p27 and p16	(151)
Nicotine	10 nM	h-EPCs	↑ PI3K/Akt ↑Telomerase activity	(51)
Pioglitazone	10 µM	h-EPCs	↓Peroxynitrite ↓ Superoxide ↓AT1R	(43)
Pravastatin	25 µM	h-MSCs	↑Telomerase activity ↑SOD ↓ROS level	(106)
Rapamycin	100-500 nM	BM-MSCs of Systemic lupus erythematosus patients	↓mTOR over-activation	(95)
Rapamycin	10 nM	Muscle stem cells of progeroid mice	↓ mTORC1	(134)
Vitamin C		mice ASCs	↓p21	(127)
Vitamin c		h-iPSCs	↓p21,p53	(150)
Znso4	0. 14 µg/ml	Rat ASCs	↑TERT	(129)

Continued table 1

Coenzyme Q 10	10 μM	h-EPCs	↑AMPK phosphorylation ↑eNOS/Akt activity ↑HO-1 ↓ROS	(39)
17β-Estradiol	100 nM	h-EPCs	↑Telomerase activity ↓AT1R ↓NOX2 ↓Phosphorylation of p38/ MAPK	(46)
17β-Estradiol	100 nM	rat BM-MNC EPCs	↑Akt phosphorylation ↑Telomerase activity	(48)
17β-Estradiol	10^{-12} M	Mini-pigs BM-MSCs	Not studied	(100)
17β-Estradiol	10^{-7} and 10^{-9}	h-MSCs	↓ROS ↑Telomere	(101)

Akt: Cellular homolog of murine thymoma virus Akt8 oncoprotein, Ang2: Angiotensin-2, ASC: Adipose-drived stem cell, BM: Bone marrow, BP: Blood pressure, CAT: Catalase, CDKs: Cyclin-dependent protein kinases, CKD: Chronic kidney disease, DNA: Deoxyribonucleic acid, EGF: Epidermal growth factor, EPC: Endothelial progenitor cells, FBS: Fast blood sugar, FGF-2: Fibroblast growth factor-2, FOXO: Fork head transcription factor, GH: Growth hormone, GPX: Glutathione peroxidase, h-: Human-, HbA1c: Hemoglobin A1c, HO-1: Heme oxygenase-1, HPC: Hematopoietic progenitor cells, HSC: Hematopoietic stem cells, h TERT: Human telomerase reverse transcriptase, IGF-1: Insulin-like growth factor 1, IL: Interleukin, iPSC: Induced pluripotent stem cells, KSC: Keratinocyte stem cells, MAPK: p38 mitogen-activated protein kinase, MSC: Mesenchymal stem cells, Nox: NADPH oxidases, Nrf2: Nuclear factor erythroid 2–related factor 2, ox-LDL: Oxidized Low-Density Lipoprotein, PI3K: Phosphoinositide 3-kinase, PPAR γ: Peroxisome proliferator-activated receptor gamma, Rb: Retinoblastoma protein, ROS: Reactive oxygen species, SOD: Superoxide dismutase, t-BHP: Tert-butyl hydroperoxide, TERT: Human telomerase reverse transcriptase, TGF: Tumor growyh factor, UV: Ultraviolet

Table 2. The summary of protective mechanisms of medicines (*In vivo* and clinical studies)

Agent	Concentration	Cell source	Mechanisms	Reference
Atorvastatin	10 mg/kg for 3 days	AKI mice EPCs	↑ phosphorylated eNOS ↓ ROS	(55)
Celiprolol	(50 mg/kg/day) For two weeks	Spontaneously hypertensive rats EPC	↑ Anti-oxidant ↓NADPH oxidase subunits	(54)
Famotidine	30 mg/kg/d	Spontaneously hypertensive rat CPCs	↓ROS	(143)
Metformin	250 mg/kg/day	Mice HSCs	NOX4↓ ↑SOD1, SOD2, CAT, GPX1	(70)
Metformin	2.8 mg/day oral for eight weeks	Mice ASCs	↑SOD activity ↓ ROS, NO	(132)
Metoprolol	50 mg/kg/d	Spontaneously hypertensive rat CPCs	↓ROS	(144)
Nicotine	Oral 100 ng/ml	Mice EPCs	↑ Sirt 1 ↑ Telomerase activity	(52)
Rivaroxaban	1 or 3 mg/kg/day	STZ-induced diabetic mice EPCs	↑ eNOS ↑ Akt	(56)
Dasatinib and Quercetin	(100 mg) and (1000 mg) Oral administration for three days	Clinical study: diabetic kidney disease patient Fat tissue biopsy	↓ Inflammatory factors	(133)
Recombinant GH	0. 4 mg/d	Clinical study: Healthy middle-aged male EPCs	↑PI3K/Akt/eNOS ↑Telomerase activity	(57)

Akt: Cellular homolog of murine thymoma virus Akt8 oncoprotein, Ang2: Angiotensin-2, ASC: Adipose-drived stem cell, BM: Bone marrow, BP: Blood pressure, CAT: Catalase, CDKs: Cyclin-dependent protein kinases, CKD: Chronic kidney disease, DNA: Deoxyribonucleic acid, EGF: Epidermal growth factor, EPC: Endothelial progenitor cells, FBS: Fast blood sugar, FGF-2: Fibroblast growth factor-2, FOXO: Fork head transcription factor, GH: Growth hormone, GPX: Glutathione peroxidase, h-: Human-, HbA1c: Hemoglobin A1c, HO-1: Heme oxygenase-1, HPC: Hematopoietic progenitor cells, HSC: Hematopoietic stem cells, h TERT: Human telomerase reverse transcriptase, IGF-1: Insulin-like growth factor 1, IL: Interleukin, iPSC: Induced pluripotent stem cells, KSC: Keratinocyte stem cells, MAPK: p38 mitogen-activated protein kinase, MSC: Mesenchymal stem cells, Nox: NADPH oxidases, Nrf2: Nuclear factor erythroid 2–related factor 2, ox-LDL: Oxidized Low-Density Lipoprotein, PI3K: Phosphoinositide 3-kinase, PPAR γ: Peroxisome proliferator-activated receptor gamma, Rb: Retinoblastoma protein, ROS: Reactive oxygen species, SOD: Superoxide dismutase, t-BHP: Tert-butyl hydroperoxide, TERT: Human telomerase reverse transcriptase, TGF: Tumor growyh factor, UV: Ultraviolet

In another study 17, beta-estradiol (100 nM) increased Akt phosphorylation and telomerase activity and led to lower EPC senescence. These EPCs were collected from hypertensive rats (35).

Chronic renal failure is associated with uremia. Uremia accelerated EPC senescence by increasing

ROS generation (36). Carbamylated darbepoetin (recombinant human erythropoietin) or darbepoetin (100 ng/ml) could decrease telomere shortening and senescence induced by TNF-α and uremic serum in EPCs from non-dialysis chronic kidney disease patients. The molecular mechanism was not declared (37).

In another *in vitro* study, nicotine (10 nM) increased telomerase activity through the PI3K/Akt pathway and reduced senescence in h-EPCs (38).

In vivo studies

Oral nicotine (100 ng/ml) intake after one month increased telomerase activity via Sirt1 up-regulation and decreased EPC senescence in mice. However, long-term treatment (3 and 6 months) had opposite results. The effect of nicotine was dependent on the signaling via nAChR-α7. Therefore, the expression of nAChR-α7 was increased in short-term exposure and decreased in long-term exposure due to a negative feedback mechanism (39). Sirt1 regulates the expression of TERT and anti-oxidant enzymes such as MnSOD through FoxO1 (40).

Beta-blockers, also known as beta-adrenergic blocking agents, such as celiprolol and atenolol, are anti-hypertensive drugs. Treating with celiprolol (50 mg/kg/day) for two weeks reduced EPC senescence by an anti-oxidant mechanism. It decreased mRNA expression of NADPH oxidase subunits in spontaneously hypertensive rats. In contrast, atenolol did not have such effects (41). In chronic diseases such as cardiovascular disease, it is preferable to select medicine with multiple therapeutic effects than other drugs in the same class.

In another study, atorvastatin (10 mg/kg, over three days) improved EPC senescence induced by indoxyl sulfate (a protein-bound uremic toxin) in acute kidney injury mice. Its protective mechanism was activated by increasing phosphorylated eNOS and reducing ROS. Indoxyl sulfate can increase ROS via the activation of NADPH oxidase (42).

Rivaroxaban (an anticoagulant drug, 1 or 3 mg/kg/day) enhanced the eNOS, Akt, and VEGF production. Also, it decreased the percentage of EPC senescence in the hyperglycemic condition in streptozotocin-induced diabetic mice (43).

Human studies

Healthy middle-aged male volunteers were treated with 0.4 mg/day of recombinant growth hormone (GH). The GH stimulated the IGF-1 related PI3K/Akt/eNOS signaling pathway, then elevated telomerase activity which in turn reduced senescence in their EPCs (44).

Different conditions cause EPC senescence. We should consider EPC senescence in disease management and choose the agent with less adverse effects among one category of medicines for long-term use. Based on molecular pathways, we can find off-labeled indications for approved drugs to inhibit cellular senescence. We should also consider the time and dose-related impacts of therapeutic substances on cellular senescence in short and long-term prescriptions (as pointed out previously for nicotine). In some cases, A particular agent exhibits therapeutic outcomes in short-term or low-dose consumption but adverse outcomes in long-term or high-dose consumption. These reviewed medicines are potential candidates for the elderly population to enhance their EPC function and slow their cardiovascular aging process (Figure 1).

Hematopoietic stem cells (HSCs)

HSCs, characterized by expressing the tyrosine kinase receptor c-Kit (CD117) and the membrane glycoprotein Sca-1(c-Kit+ Sca-1+) without mature markers of Ter119, Gr-1, Mac-1, B220, CD4, and CD8, are located in BM. They have a high homing ability so that, even after transplantation, they can find their path to the recipient's BM. They have an unlimited self-renewal ability for homeostasis and continuous blood cell turn-over throughout life, even after insults such as infection or therapeutic ablation (45). Studies have shown that HSCs infusion had positive outcomes in different conditions. HSCs infusion in the kidney recipients reduced transplantation failure and the required immunosuppressant dose (46). After chemotherapy, they alleviated rheumatoid arthritis in drug resistance patients (47). In diabetic patients, they improved various factors such as mean fasting blood sugar, postprandial blood sugar, and HbA1c (48). Experiments indicate that lead-acetate increased ROS and mitochondrial defect so that HSCs became senescence and their repopulation ability decreased (49). Ox-LDL increased oxidative stress and reduced telomerase activity which resulted in HSC senescence (50). Autologous HSC transplantation had a better result than allogeneic HSCs transplantation for superior immunosuppression in multiple sclerosis patients. HSCs reduced the number of new T2 lesions and decreased the annual relapse rate (51). Rapid telomere shortening in transplanted HSCs speeds up their senescence. Senescent HSCs have less clonal stability and homing ability (52).

In vitro studies

Rapamycin (20 ng/ml) inhibited mTOR and increased Bmi1 expression. Hence it repressed p16 and senescence in the *ex-vivo* expansion of mice BM-HSCs (53). Bmi-1 cooperation with c-myc enhances telomerase activity which in turn decreases p16 and p19 expression, as well as cellular senescence (54). mTOR promotes cell proliferation and decreases autophagy while inhibits

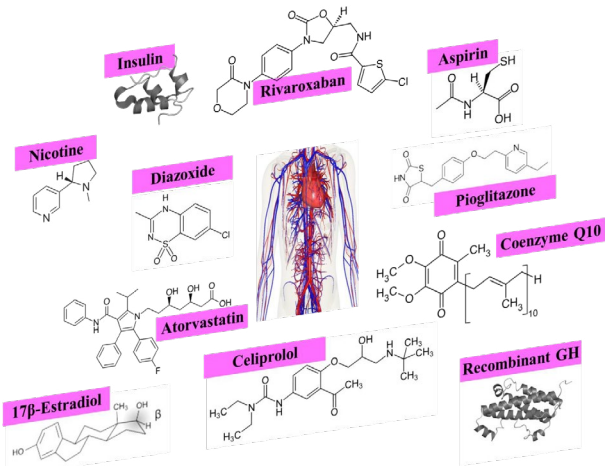

Figure 1. The chemical structure of protective agents against endothelial progenitor cell (EPC) senescence

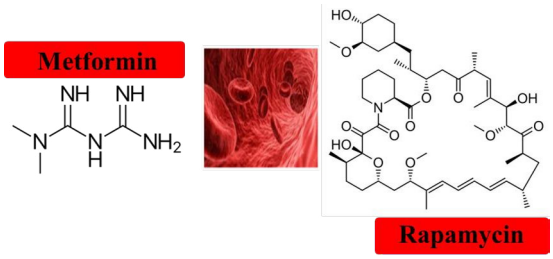

Figure 2. The chemical structure of protective agents against hematopoietic stem cell (HSC) senescence

mitochondrial MnSOD and increases ROS levels (55). The dual effect of rapamycin through mTOR, senescence induction in EPSs, and senescence inhibition in HSCs, may be dose- or cell-dependent. So, it needs further investigation.

In vivo studies

In IR-induced long-term BM injury in a total-body irradiation mice model, treatment by 250 mg/kg/day of metformin (an anti-diabetic medicine) inhibited the expression of NOX4, as well as increased the cellular level of SOD1, SOD2, CAT, and GPX1 mRNAs thus ameliorated senescence in their HSCs (56).

These protective agents could be used as preventive or curative agents in cancer patients to reduce radiotherapy or chemotherapy adverse effects related to HSCs, such as susceptibility to infections. On the other hand, less HSC senescence during the pre-transplantation procedure can guarantee more procedure efficacy. Thus, *in vitro* pre-transplantation treatment or clinical prescription after transplantation can both improve HSC therapy (Figure 2).

Mesenchymal stem cell (MSCs)

MSCs are located in various tissues and organs like BM, adipose tissue, umbilical cord blood, and Wharton Jelly, tendon, synovial, and blood circulation to maintain their homeostasis. MSCs in BM support hematopoietic stem cell niches. Moreover, they have shown immunomodulatory and anti-inflammatory activities. Their proliferation rate, however, is location-dependent, and senescence affects their homing ability toward injury or inflammatory lesions and multi-potency. Their immunophenotyping surface markers for purification are Stro1, CD73, and CD106 (57). MSC infusion has shown positive outcomes in clinical trials. Intra-articular injection of MSCs into the osteoarthritic knee improved function and pain of the knee joint (58). In addition, MSCs have been used in hepatitis B virus cirrhosis (59), Crohn's disease (60), severe diabetic foot (61), amyotrophic lateral sclerosis (62), acute myocardial infarction (63), immunomodulation after liver transplantation (64), congestive heart failure (65), blood glucose control in type-2 diabetes (66), and multiple sclerosis (67). These studies have shown the safety of MSC therapy without adverse effects. The critical issue in such procedures is that MSCs before transplantation must be expanded to provide enough cells. However, in higher passages, replicative senescence happens. There is also an age-dependent increase in MSC senescence that result in impaired proliferation capacity (68). Senescent MSCs have shown morphological changes and low self-renewal potential (69). Furthermore, elderly patients do not have enough functional MSCs for efficient autologous transplantation (70).

Moreover, different diseases or chronic medicine therapies can induce MSC senescence. Heparin (anticoagulant agent) and most of the statins increased MSC senescence (71, 72). Patients with myelodysplastic syndrome have higher senescent MSCs, so they showed lower MSC proliferation ability (73). Chronic kidney disease caused premature MSC senescence and decreased regenerative potential in rats (74). The human immunodeficiency virus (HIV-1) p55-gag protein caused MSC senescence and decreased hematopoietic activity (75). High glucose via the Akt/mTOR signaling pathway induced MSC senescence (75). Senescent MSCs negatively impress their paracrine environment, immunomodulation, cell migration, differentiation, and therapeutic capability (76, 77). Their paracrine effect can change the tissue microenvironment. This change can trigger colon cancer cell growth (43) or induce progression and metastasis of breast cancer cells (78). So, preventing MSC senescence plays an essential role in cell therapy and cancer prevention. Since they are vastly distributed in the body, there is more concern about their senescence than other cells.

In vitro studies

Isosorbide dinitrate (a vasodilator medicine, 50 μM) attenuated high glucose-induced senescence in rat MSCs. This medicine increased mir-130b expression by reversing the down-regulation of ERK phosphorylation and FOXM1 expression. MiRNA-130b is downstream of the ERK/FOXM1 pathway. MiR-130b inhibited the expression of p21, although this observation needs further studies (79).

Rapamycin (100-500 nM) inhibited mTOR over-activation and senescence in the BM-MSCs of patients with systemic lupus erythematosus (80).

Coenzyme Q 10 (10 or 100 μM) suppressed the expression of p21, p16, p53, as well as the Akt/mTOR signaling pathway, so inhibited senescence induced by D-Galactose in rat BM-MSCs (81). Idebenone (the analog of coenzyme Q10 for cardiovascular disease, 10, 20, or 30 μg/ml) progressed the cell cycle and delayed replicative senescence in rat MSCs (82).

Ascorbic acid (200 μM) inhibited ROS and Akt/mTOR signaling pathway in D-galactose-induced senescence in rat MSC (83).

1,25-dihydroxy vitamin D3 (100 nM) decreased p16 and delayed senescence in h-MSCs, however, it significantly increased ROS accumulation (84).

Senescent MSCs are involved in the onset of osteoarthritis in menopausal women. 17β-estradiol (10^{-12} M) decreased senescence and improved the osteogenic ability of mini-pigs-BM-MSCs (85). Also, 17β-estradiol (10^{-7} M and 10^{-9} M) prevented telomere shortening by reducing oxidative stress and decreasing h-MSC senescence, but it did not affect p21 or SIRT1 protein expression (86).

N-acetyl-cysteine (1 mM) decreased ROS, p38, p53, and senescence in BM-MSCs obtained from prolonged

isolated thrombocytopenia (serious complication of allogeneic HSC transplantation) patients (87).

Uremic toxins in chronic kidney disease patients accelerate MSC senescence. Melatonin (100 µM) up-regulated PrPC and enhanced mitochondrial function in MSCs from CKD mice exposed to H_2O_2 and suppressed their senescence. Transplantation of these treated cells showed better activity for inhibiting necrosis and reducing the formation of collagen fibers in the ischemic area (88).

Melatonin (a natural hormone that regulates the biological clock and a sleeping pill, 10 nM, 1 µM, and 100 µM) decreased ROS levels, p53, p21, and p16. It also blocked the p53/ERK/p38 pathway and alleviated iron overload-induced senescence in mice BM-MSCs (89). Melatonin (10 nM, 1 µM, and 100 µM) up-regulated SIRT1 mRNA level, suppressed p16 and phosphorylated p38 expression, decreasing senescence in BM-MSCs exposed to H_2O_2 (56).

Nicotinamide (a form of vitamin B_3, 5 mM) delayed replicative senescence and improve differential ability in h-MSCs. Nicotinamide reduced ROS generation in mitochondria and increased cellular NAD+/NADH ratio and SIRT1 activation (90).

HIV protease inhibitor regimen (Atazanavir, Ritonavir, and lopinavir) has adverse effects on bone. H-MSCs exposed to atazanavir and lopinavir showed replicative senescence after 30 days. Pravastatin (25 µM) recovered SOD activity and ROS level in these cells and inhibited replicative senescence, improving their osteoblastic differentiation ability (91).

Since MSCs are distributed in the whole body, they can be used as accessible stem cell sources. On the other hand, their senescent secretary inflammatory factors can impair many organs or trigger cancer formation, so MSC senescence inhibition is essential for preventative and curative intervention (Figure 3).

Adipose-derived mesenchymal stem cells (ASCs)

Adipose-derived stem cells are a type of MSCs, and they have more abundant autologous sources, as well as rapid and high proliferation capacity than BM-MSCs. Also, they are cultured easier and show more genetic stability (92). Immunotolerance, cell surface molecular composition, and a high potential for multilineage differentiation of ASCs are similar to BM-MSCs (93). A comparison between BM-MSCs and ASCs of the same donors has shown that ASCs have a higher proliferation rate and a doubling population. ASCs kept their differentiation potential better than BM-MSCs in culture for a long time. Also, they expressed fewer senescence markers, so ASCs can be a good alternative for tissue reengineering (94). ASC injection could mitigate chronic pancreatitis (95), improved autonomic nervous system dysfunction in humans (96), improved multiple sclerosis (97), attenuated lungs and systemic injury induced by cigarette smoking (98)., and rescued early stages of diabetic retinopathy (99). The supernatant of ASCs culture ameliorated allergic airway inflammation through its immunomodulatory action (100) and improved wound healing (101) in clinical trials.

The limitation of cell therapy with ASCs is cellular senescence (102). Besides this, highly distributed

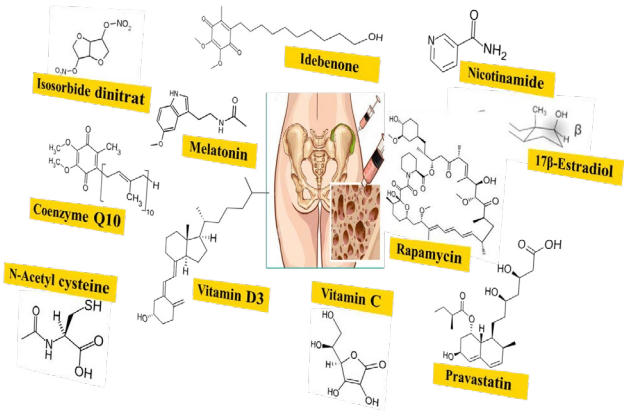

Figure 3. The chemical structure of protective agents against mesenchymal stem cell (MSC) senescence

senescent adipose tissue in the body secretes inflammatory cytokines (103), increasing insulin resistance and obesity in metabolic syndrome disease (104). Furthermore, inflammatory IL-6 and IL-8 can produce more senescent cells (105).

In vitro studies

Vitamin C up-regulated Jhdm1a/b, c-Myc, Klf4, and down-regulated p21, so increased proliferation, postponed senescence, and transformation in higher passages of the mouse ASCs. Vitamin C did not have a significant effect on p53. Jhdm1a/b belongs to the Jumonji family proteins that are responsible for the demethylation of 3K36me2/3. Demethylated 3K36me2/3 can promote cell cycle progression. c-Myc and Klf4 have paramount roles in cell proliferation and differentiation, required for the self-renewal of ASCs (106).

L-carnitine (a natural substance that helps the body with energy production, and, as a supplement sold in the market, 0.2 mM) decreased senescence by its anti-oxidant effects in rat ASCs. However, its detailed mechanism needs further investigation (107).

Because of the increased use of the electromagnetic field for domestic and industrial purposes, different studies proved its influence on biological systems, including anti-oxidative enzymes, cell proliferation, and differentiation of stem cells. The electromagnetic field of 50 Hz and 20 milliTesla, could prevent the growth and metabolism of h-MSCs. $ZnSO_4$ (0.14 µg/ml) increased TERT gene expression and decreased senescence in rat ASCs exposed to the highly low-frequency electromagnetic field of 50 Hz and 20 milliTesla (108).

Melatonin (100 µM) protected h-ASCs against uremic toxin *p*-cresol-induced senescence (*p*-cresol found at high concentrations in the serum of CKD patients). Melatonin-induced phosphorylation of Akt activated the Akt signaling pathway, thus increased catalase activity and reduced ROS. Melatonin also decreased the level of phosphorylated mTOR by reducing AMPK. ROS elevation is strongly related to the AMPK activity. Melatonin could increase SMP30, which is involved in the anti-aging process (109). In another study, melatonin (10 µM), as an anti-oxidant, reduced replicative senescence in mice ASCs and preserved their differential potential. Melatonin in higher passages decreased NADPH oxidase

content and, consequently ROS generation (110).

In vivo studies

ASCs isolated from mice treated with 2.8 mg/d of oral metformin for eight weeks had increased SOD activities and lessened ROS, NO, and cellular senescence. Metformin also enhanced its osteogenic properties and bone density (111).

Human studies

In a study, diabetic kidney disease patients took oral administration of dasatinib (a tyrosine kinase inhibitor for leukemia, 100 mg) and quercetin (a plant pigment, 1000 mg) for three days. After eleven days, senescent and pre-senescent adipocyte progenitors in their bodies had decreased, and circulating inflammatory factors were removed (112).

Senescent cells are functionally impaired and, by releasing inflammatory molecules, create a harmful microenvironment for other cells, resulting in more senescent cells and finally damaged organs. Fat tissue is highly distributed in the body, so the prevention of ASC senescence via pharmacotherapy can attenuate different diseases related to obesity, such as insulin resistance and diabetes, and improve the quality of patients' life. The inhibition of ASC senescence in cell culture is beneficial for harvesting more functional cells for cell therapy purposes (Figure 4).

Other adult stem cells

In vitro studies

Myeloblasts or muscle satellite cells are involved in muscle regeneration and aging declines their population. Rapamycin (10 nM) inhibited mTORC1, diminished senescence, and improved differentiation potential in muscle-derived stem/progenitor cells isolated from progeroid mice (113) (Figure 5).

Neural stem cells that generate new glial cells and neurons in the hippocampus and sub-ventricular zone of the lateral ventricles in the brain play an essential role in learning and memory (114). Anti-retroviral medicines (Tenofovir, Emtricitabine, Ritonavir, and Darunavir) increased ROS generation by mitochondria, induced telomere shortening, and decreased SIRT3 protein expression in mouse neural progenitor cells

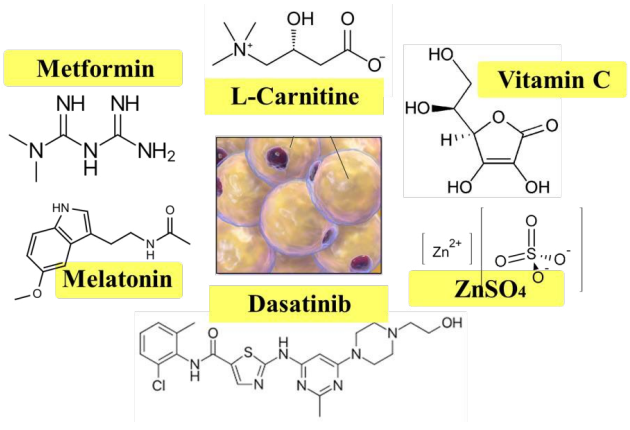

Figure 4. The chemical structure of protective agents against adipose-derived stem cell (ASC) senescence

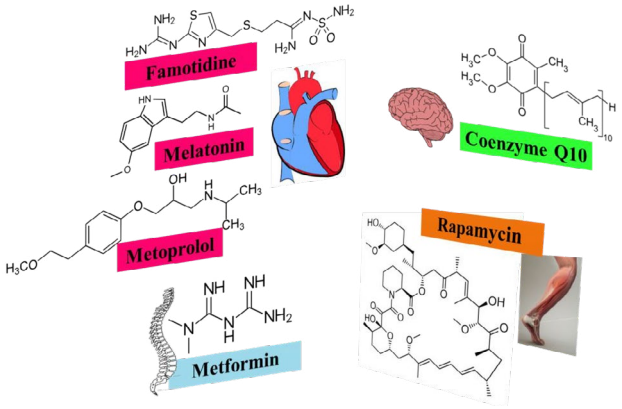

Figure 5. The chemical structure of protective agents against other adult stem cell senescence

(NPCs). Mitochondria-targeted CoQ10 therapy (500 nM) reversed these effects and reduced cellular senescence (115).

Disc degeneration is an age-related disease associated with cellular senescence (116). It is one of the reasons for lower back pain and is very common in the elderly population (117). Metformin (1 mM) decreased LPS-induced-senescence and inflammatory factors in rabbit annulus fibrosus stem cells (118).

Cardiac stem cells (CSCs) and cardiac progenitor cells (CPCs) are involved in myocardial regeneration and repairment (119). Melatonin (10 µM) via long noncoding RNA H19 increased mir-675. Mir-675 overexpression down-regulated p21 and p53, which inhibited H_2O_2-induced senescence in CPCs, also reduced IL-6 secretion (120).

In vivo studies

Treating with famotidine (a histamine-2 receptor antagonist, 30 mg/kg/day) for two months improved self-renewal ability, proliferation capacity, and migration in CSCs of spontaneously hypertensive rats. This agent also decreased the ROS level and cellular senescence in these cells (121). However, the exact anti-oxidant mechanism was not investigated. Also, eating with metoprolol (50 mg/kg/d) for two months decreased ROS level and cellular senescence in CSCs of spontaneously hypertensive rats (122).

Induced pluripotent stem cells (iPSCs)

Although adult stem cells seem to be useful for regeneration, their sources, differentiation, and expansion potential are limited. In addition to this, elderly patients have fewer stem cells than their younger donors. In contrast to adult stem cells, pluripotent stem cells, such as embryonic stem cells, have unlimited potential to proliferate and differentiate into all cell types (123). Various cells from different tissues can be reprogrammed to iPSCs then differentiated to other cell types. Since these cells are autologous, they are a preferable option for cell therapy (124, 125). There are different induction factors such as Oct4, Sox2, Klf4, c-Mys (OSKM) to reprogram adult stem cells into pluripotent stem cells. IPSCs provide a basis for personalized stem cell therapies and autologous transplantation, which

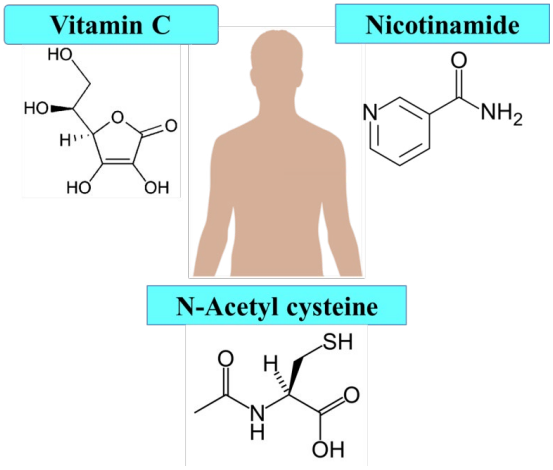

Figure 6. The chemical structure of protective agents against induced pluripotent stem cell senescence

does not have immunologic issues like transplant rejection (123). However, senescence is one of those key barriers to successful reprogramming (126) (Figure 6).

In vitro studies

N-acetylcysteine (1mM) alleviated oxidative stress and senescence in iPSCs and improved their hematopoietic differentiation (127).

Vitamin C reduced p21, p53, and iPSCs senescence and improved their generation (128).

The OSKM gene induces ROS, p21, p53, p27, and p16 proteins during cell reprogramming. Nicotinamide (a biologically active amid of nicotinic acid, one mM) reversed these effects and inhibited senescence in human iPSCs (129).

Discussion

Stem cell senescence has been studied in aging, diseases, adverse drug effects, and as a challenging phenomenon in cell therapy. The most investigated types of these cells are EPCs, HSCs, and MSCs. Other investigated kinds include CPCs, myeloblasts, and iPSCs. EPCs are involved in vascular homeostasis and new blood vessel regeneration (130). The decrease in their functional cell number is associated with aging and atherosclerotic processes (20). HSCs are involved in blood coagulation, oxygen transportation, and immune system function, so their senescence leads to blood dysfunction (52). MSCs exist in many tissues, including bone marrow, adipose tissue, the bloodstream, and cord blood (57). MSCs have high self-renewal capacity and the ability to differentiate into other kinds of cells, such as adipocytes, chondrocytes, and osteoblasts, depending on their host organ (131). Although adult stem cells appear to be valuable sources for regeneration, they have limited sources, differentiation, and expansion potential (123). However, differentiated cells can be reprogrammed to iPSCs and then differentiated to desired cell types (124, 125).

As reviewed in this paper, most of these protective agents increased telomerase activity or decreased oxidative damage via various anti-oxidant mechanisms, which ultimately inhibited cellular senescence.

Senescence prevention in the body results in health and longevity. Various medicines inhibit senescence through different mechanisms. As mentioned in this review, 17β-estradiol, melatonin, metformin, rapamycin, coenzyme Q10, N-acetyl cysteine, and vitamin C were the most studied agents in different kinds of stem cells (Figure1). Although most of these studies were *in vitro,* we can consider these agents in cell therapy to increase the shelf life and the functional cell number of donated stem cells before transplantation to achieve more clinical success. Moreover, *in vitro* studies are the first step towards clinical studies. Although more studies are necessary for clinical application, these reviewed agents have been used in the clinical setting for different purposes for a long time; therefore, we only need to evaluate their systemic anti-senescence effects and effective anti-senescence dosages.

Conclusion

Off-label use of approved medicines and supplements is a convenient, safe, and economical approach to prevent stem cell senescence both *in vitro* and *in vivo*. These agents provide a wide range of options based on targeted cells. Since all of them have passed substantial safety trials, we only need to determine their effective dosage to prevent stem cell senescence. Maybe it seems that heterogeneity of administration, patients, and diseases, can make repurposing inefficient and time-consuming. Still, in comparison with discovering new anti-senescence agents, this approach is much more economical and accessible. Moreover, performing retrospective studies for each medicine can address these issues.

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Authors' Contributions

Study conception or design: H T; Data analyzing and draft manuscript preparation: H T; Critical revision of the paper: BM R, and H H; Supervision of the research: BM R, and H H; Final approval of the version to be published (the names of all authors must be listed): H H.

Conflicts of Interest

The authors declare not to have any conflicts of interest.

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