Review Article

A review of pathobiological mechanisms and potential application of medicinal plants for vascular aging: focus on endothelial cell senescence

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ABSTRACT

Endothelial cell (EC) senescence plays a pivotal role in aging and is essential for the pathomechanism of aging-related diseases. Drugs targeting cellular senescence, such as senolytic or senomorphic drugs, may prevent aging and age-related diseases, but these bullets remain undeveloped to target EC senescence. Some medicinal plants may have an anti-senescence property but remain undiscovered. Deep learning has become an emerging approach for drug discovery by simply analyzing cellular morphology-based deep learning. This precious tool would be useful for screening the herb candidate in senescent EC rejuvenescence. Of note, several medicinal plants that can be found in Indonesia such as *Curcuma longa* L., *Piper retrofractum*, *Guazuma ulmifolia* Lam, *Centella asiatica* (L.) Urb., and *Garcinia mangostana* L. might potentially possess an anti-senescence effect. This review highlighted the importance of targeting EC senescence, the use of deep learning for medicinal plant screening, and some potential anti-senescence plants originated in Indonesia.

KEYWORDS artificial intelligence, cellular senescence, deep learning, endothelial cells, medicinal plants

Aging has always been a central issue globally. Although substantial researches have been conducted, the aging mechanism is still partially understood amidst the underdeveloped modern drugs.[1] Cellular senescence is pivotally involved in aging and age-related diseases.[2] In 1961, Hayflick and Moorhead established a concept of cellular senescence where primary fibroblast cells showed a deterioration of proliferation capacity after several cycles of passages, which mimics the human aging process.[3] These senescent cells are still viable but have several character alterations such as morphological and biological functions.[4] It induces organ dysfunction, organ disease, and aging phenotype in humans. The elimination of senescent cells may be a possible therapeutic for age-related diseases.[5] Age-related disease is contributed by endothelial cells (ECs) through cellular senescence.[4] Therefore, EC senescence could be a therapeutic target to prevent aging and age-related diseases.

Medicinal plants have been traditionally used for over 2,000 years to treat various diseases and conditions such as infectious diseases,[6] cancer,[7] metabolic diseases,[8] and cardiovascular diseases (CVDs).[9] Several modern commercial drugs have been successfully developed from medicinal herbs such as

aspirin[10] and digitalis[11] which have been widely used because of their safety and strong efficacy. Artemisinin, isolated from *Artemisia annua* L., has been successfully translated into malaria chemotherapy. This finding was awarded a Nobel prize in 2015,[12] suggesting that people are still enthusiastically interested in the potential of medicinal plants. However, some plants contain complex chemical compounds, including second metabolites, that are difficult to be identified.[13] To solve this issue, artificial intelligence (AI) has been used as a promising tool for drug discovery in cellular senescence through the morphological feature identification.[14–16] Therefore, AI technology could help identify anti-aging drugs derived from medicinal plants. This narrative review highlighted the rationale for targeting EC senescence, medicinal plant screening strategy for anti-EC senescence using deep learning, and some potential medicinal plants originated in Indonesia for rejuvenating vascular aging.

Vascular aging and the impact on the diseases

EC senescence has been associated with age-related diseases, including CVD, metabolic disease, and cancer, indicating the importance of targeting these senescent cells for preventing degenerative diseases, as illustrated in Figure 1. CVDs are the leading cause of mortality among the elderly and are remarkably increasing globally.[17] As referred by Dr. William Osler,[18] "*man is as old as his arteries*", vascular aging plays a role in morbidity and mortality. It is characterized as an age-associated vascular alteration in function and structure, such as loss of vascular elasticity, loss of microvasculature, and decreased vascular blood supply.[19] Importantly, it is accompanied by EC senescence.[20] As an inner layer of the blood vessels, EC plays several important roles in vascular homeostasis, such as proper nutrients and oxygen deliveries and vascular tone modulation.[21] Senescent ECs are gradually but steadily accumulated in aging tissues.[22,23] The proliferation ability of ECs is essential for new vessel formation as senescent ECs show a decreased proliferation ability.[4,24] Thus, aging-associated angiogenesis impairment is partly due to the senescence in ECs. Cellular senescence is a physiological stress response leading to irreversible cell cycle arrest. Tumor suppressor pathways, pRB/p16 and p53/p21, tightly regulate irreversible cell cycle arrest and cellular senescence.[25,26] Genome-wide association studies have been used to identify the common variants associated with human diseases. Coronary artery disease is linked with the variants at the 9p21 locus.[27,28] Interestingly, the closest genes include the aging-associated gene, *CDKN2A*, which encodes the CDK inhibitor, $p16^{INK4a}$ and the p53 regulator, $p14^{ARF}$.[29] Activation of the *CDKN2A* locus is observed in most senescent cells and plays a crucial role in their growth arrest.[30] Therefore, the senescent cell phenotype could be observed in coronary arteries and ECs in the atherosclerotic lesion.[24,31] These senescent ECs show important phenotypes such as reduced endothelial nitric oxide synthase activity, enhanced oxidative stress, and the expression of senescence-associated secretory phenotype (SASP).[32]

High glucose conditions may accelerate a senescence-like state in EC.[33] This raised the question of whether the senescent EC solely may induce age-associated metabolic dysfunction. Moreover, the senescence in EC leads to systemic insulin resistance.[4] EC senescence was detected at a cellular level at adipose tissue isolated from obese patients but not in the normoweight subjects.[34] Our previous group and the other group have independently investigated the effect of senescent EC on metabolic homeostasis *in vivo*. We generated EC-specific progeroid mice by overexpressing the telomeric repeat-binding factor 2 in the dominant negative form under the Tie2 promotor and revealed that senescent EC-mediated pro-inflammatory SASP promoted postmitotic adipocyte senescence, thus impairing systemic metabolic homeostasis under a normal feeding diet by reducing insulin receptor substrate-1 expression in these cells. We also found that interleukin (IL)-1α was highly expressed in EC senescence. This cytokine plays as a master regulator for activating SASP from senescent EC. These data suggest the potential role of senescent EC-mediated IL-1α on metabolic disease development.[4]

The incidence of cancer is increasing at an advanced age. However, the cancer prevalence in the elderly develops a dilemmatic issue since senescence cell is a proliferation arrest condition, indicating a double-edged sword of cellular senescence in aging.[35] Likewise, the hypothesis on the impact of EC senescence in cancer has been dichotomized. One theory explained whether the senescent EC could delay cancer development. As reviewed here,[36] senescent EC-mediated angiogenesis impairment was detected in the elderly and might likely inhibit cancer formation. Otherwise, neovascularization was detected in cancer,

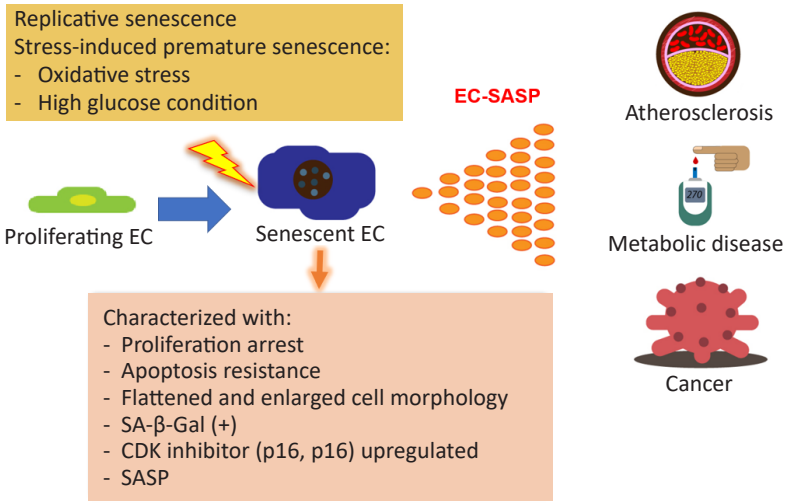

Figure 1. EC senescence induces age-related diseases by secreting pro-inflammatory factors. Both replicative and stress-induced premature (induced by oxidative stress and high glucose exposure) senescent ECs were characterized by several senescence markers including the unique cell morphology. Importantly, senescent EC might secrete various pro-inflammatory factors, called SASP, that would induce age-related diseases such as atherosclerosis, metabolic disease, and cancer. EC=endothelial cell; SASP=senescence-associated secretory phenotype, SA-β-Gal=senescence-associated beta-galactosidase

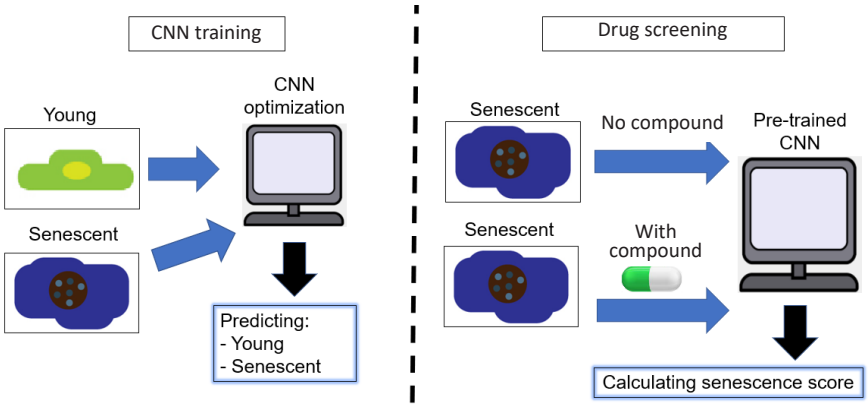

Figure 2. Deep learning system development for drug screening for EC senescence**.** A deep learning system (CNN) was initially trained with every single image of young or senescent ECs. Moreover, EC senescence was incubated with various compounds from the drug library and analyzed with the pre-trained CNN. The machine would further calculate the senescence score for each compound. The lower senescence scores would be identified as the candidate for anti-senescence drugs and validated in the EC senescence with several senescence marker analyses. CNN=convolutional neural network; EC=endothelial cell
This system was adapted and had granted permission from Kusumoto D, Seki T, Sawada H, Kunitomi A, Katsuki T, Kimura M, et al. Anti-senescent drug screening by deep learning-based morphology senescence scoring. Nat Commun. 2021;12:257

indicating the need for angiogenesis for cancer progression.[37] However, cellular senescence produces the stoichiometry of the senescence-associated cytokine system, which induces carcinogenesis in its surrounding healthy cells.[38] Moreover, Orjalo et al[39] revealed that IL-1α orchestrated SASP secreted from a senescent cell including IL-6 and IL-8, which is responsible for metaplasia in healthy cells. Consistently, a previous study also explained that IL-1α was highly expressed in EC senescence, suggesting EC senescence will promote cancer cells.[4] However, specific investigation using EC-specific senescence mice is necessary to investigate further vascular aging in cancer development.

Medicinal plant screening for vascular aging rejuvenation by analyzing cellular morphology phenotype

To develop a herb screening system for EC senescence, it is important to identify the cellular senescence status by well-established markers (Figure 1).[25] One of the most common senescence markers

is senescence-associated beta-galactosidase (SA-β-Gal) activity staining.[40] β-galactosidase is a lysosomal enzyme and is highly expressed in senescent cells with increased lysosomal activity, but it is weakly expressed in proliferating cells, quiescent cells, or terminally differentiated cells.[41] Therefore, the senescent cells can be differentially identified by SA-β-Gal staining. However, this staining has several limitations. Over incubation and over confluent cell may increase the false-positive results, which requires careful monitoring to detect senescent cells properly.[25] In addition, non-senescent macrophages also show SA-β-Gal activity under physiological condition,[42] since high lysosomal activity plays an essential role in macrophage function.

Irreversible cell cycle arrest is also an important hallmark of cellular senescence. pRb and p53 play a role in the cell cycle arrest of senescent cells. The upregulation of cyclin-dependent kinase inhibitors, such as $p16^{INK4a}$ and $p21^{WAF1/Cip1}$, induces persistent activation of the Rb family and cell cycle arrest.[25] These markers are widely used for estimating the degree of senescence.[4,24] As with the SA-β-Gal assay, p16 is highly expressed in some non-senescent cells such as macrophage and mesenchymal cells.[42,43]

Cellular senescence may induce chronic inflammation and damage their surrounding healthy tissues through SASP that consists of various humoral factors including cytokines, chemokines, growth factors, and extracellular vesicles secreted from senescent cells.[38] Coppé et al,[44,45] firstly identified SASP in culture conditioned medium of senescent cells. Recently, the senescence-associated secretory communication network can also be evaluated in human plasma and subsequently can be a therapeutic target for senescence cells.[46]

Among those markers, no single marker is likely to be used as the gold standard for identifying the senescent cell. However, most senescent cells, including ECs, have typical cellular morphology, characterized by the flattened and enlarged form independently from the younger ones.[4,24] Interestingly, any stress-induced senescence also shows the typical cellular morphology in EC.[4,24,31] When the senescent cells become larger, the ratio between DNA and cytoplasm is decreased. This imbalance would lead to uncoupling RNA and protein synthesis from cell volume, which contributes to damaged cellular function in senescent cells.[47] Altogether, these evidence show that recognizing the senescence cells phenotype through analyzing their unique morphological features will be the easiest and most attractive way to identify EC senescence.

AI technology has been widely used in the medical field. The machine learning approach, as one of the advanced methods in AI, can be used as a promising tool for drug discovery with an anti-aging effect using image data cells.[48–50] Moreover, this method does not require a high facility or high cost.[51] We have previously developed a deep learning system for drug screening for EC senescence.[16] This system could identify senescent cells, and a quantitative scoring system based on the established network could evaluate the state of ECs. Briefly, we initially trained the deep learning system, called convolutional neural networks, with the microscopic images of young and senescent ECs. The senescent ECs were treated with various compounds from the drug library for the drug screening procedure. Each cell image was captured and compared to differentiate either the senescent or younger cells. The senescence score of each candidate compound was calculated and further selected as the potential anti-senescent drug candidates. Subsequently, we validated those candidates in the EC senescence by analyzing various senescence markers of those cells (Figure 2).[16]

High-throughput screening is an ideal large-scale screening assay to discover a novel natural product with anti-aging properties.[52] An accumulative research indicated that there should be some medicinal plants with a strong anti-senescent effect.[53] However, it remains challenging to identify the anti-senescence effect in medicinal plants. Several concerns that should be addressed are the guidelines on identifying medicinal plant candidates, key molecules, including secondary metabolites (if any) within those medicinal plants, and the number of medicinal plants documented in the medicinal plant library.[53,54] Although some trials have identified the key molecule in medicinal plants for the pharmacologic target, it mostly remains unclear which molecules have therapeutic effects. It is still difficult to determine the specific molecules within the medicinal plants.[13] These issues are important to develop a modern type of anti-senescence drugs derived from medicinal plants. Alternatively, instead of investigating the key molecules from the plants, identifying the transition of cellular morphology after medicinal plants' exposure to senescent EC by simply using AI will be a promising tool for medicinal plant screening in these senescent cells.[16]

The potential medicinal plants with the "anti-EC senescence" effect

Natural product-derived medicines have been historically practiced. *Commiphora myrrha* (T.Nees) Engl had been documented as a medicine for cough and cold with anti-inflammatory properties in the Mesopotamia era and is still used in the community.[55] In ancient documents, Sumerians used willow bark or *Salix purpurea* L. as an anti-inflammatory medicine for rheumatic disease.[56] Importantly, Johann Buchner purified its active ingredient and named it salicin in 1828 and later found as an anti-inflammatory and antipyretic drug by Thomas Maclagan in 1874.[57,58] A pharmaceutical company chemically synthesized acetylsalicylic acid, also known as aspirin, by acetylating the salicylic acid isolated from meadowsweet leaves; and later John Vane figured out how aspirin works as anti-inflammatory reagents by inactivating cyclooxygenase (COX) that produces prostaglandin (PG).[59] Afterward, aspirin became the most famous and common drug based on its potent analgesic and antipyretic effects and is currently being widely used as an antithrombotic agent for cerebrocardiovascular disease.[10] Previous reports showed that COX/PGE2 axis-mediated inflammation would induce senescence, and aspirin may be used as an anti-senescence drug.[60–62] However, a clinical study suggested that aspirin did not prolong the survival rate in older healthy adults.[63,64]

Metformin is a long-lasting first-line drug for type 2 diabetes mellitus (T2DM). Among several types of anti-diabetic drugs, metformin has been widely used for several advantages, including good efficacy, less severe side effects, inexpensiveness, and availability in most countries.[65] Metformin mainly improves insulin sensitivity and suppresses hepatic gluconeogenesis, and it mechanistically inhibits the mitochondrial respiratory chain complex 1 and activates AMP-activated protein kinase (AMPK).[66] The cardiovascular benefits of metformin have been widely shown in T2DM patients.[67] Metformin also shows several potentials in non-CVDs such as fibrotic lung disease,[68] cancer,[69,70] and neurodegenerative disease.[71,72] These data indicate the potential role of metformin in health span improvement. Interestingly, several studies also showed that metformin prolonged the lifespan in animal models.[73] In terms of the human lifespan, a retrospective study of metformin or sulfonylurea medication in T2DM patients showed lower mortality rates in metformin groups than those treated with sulfonylurea.[74] Importantly, a multicenter, double-blind, and randomized clinical trial (targeting aging with metformin trial) was conducted to identify the longevity effect of metformin on nondiabetic older subjects.[75]

In Southeast Asia, Indonesia has an abundance of biodiversity, consisting of the heterogeneous flora and fauna collection spreading out in up to 17,000 islands in the country.[76] Of note, Indonesia is the second country with the highest amount of authentic medicinal plants after the Amazon rain forests.[77] As with the undeveloped area, the exploration of indigenous medicinal plants from Indonesia would be a potential drug for several diseases. In this section, we introduced some herbs that would potentially possess an anti-senescent effect (Table 1).

***Curcuma longa* L.**

Curcuma longa L. or turmeric is traditionally used mainly in Southeast and East Asia as the main source of active compound curcumin. Curcumin has a wide variety of effects, such as inflammation,[78] cancer,[79] and metabolic diseases,[80] possibly due to their antioxidant and anti-inflammatory properties. It improves the degree of atherosclerosis and extends the lifespan in experimental animals.[81,82] Importantly, curcumin is unlikely to induce senescence in cancer cells,[83,84] which suggests that it can be used as an anti-senescent drug for humans without severe side effects.

Javanese long pepper

Javanese long pepper (*Piper retrofractum* Vahl.) or *cabe Jawa* in Indonesian is a medicinal herb that originated in Indonesia. Instead of the seasoning purpose, the abundant antioxidant property was used for various diseases including obesity and hepatoprotection.[85] Piperlongumine, one of the active compounds in long pepper, has been potentially identified as a senolytic agent that selectively eliminates senescent cells. Piperlongumine induces apoptosis in senescent cells induced by various stimuli.[86,87] This compound is relatively safe and has good efficacy when administrated orally.

***Guazuma ulmifolia* Lam.**

Guazuma ulmifolia Lam., known as *mutamba* (or *daun jati Belanda* in Indonesian), is a traditional herb medicine in South America and Asia.[88] This plant contains proanthocyanidin as phenolic compounds

Table 1. The potential Indonesian medicinal herbs with anti-senescence effect

Medicinal plants	Major compounds	Diseases	Anti-senescence	Availability in Indonesia
Curcuma longa L. (turmeric)	Curcumin	Anti-inflammation,[78] cancer,[79] and metabolic diseases[80]	Anti-senescence: - *Drosophila melanogaster*: antioxidant (increased superoxide dismutase activity and extended the lifespan)[81] - Mice: antioxidant and SIRT1 activator (eliminated senescent cell in atherosclerosis)[82] Pro-senescence effect (induced proliferation arrest in cancer cells)[83,84]	Yes
Piper retrofractum Vahl. (Javanese long pepper)	Piperlongumine	Obesity and hepatoprotection[85]	Anti-senescence: - In senescent human fibroblasts: as senolytic agent (induced apoptosis in senescent cells)[86,87]	Yes
Guazuma ulmifolia Lam.	Proanthocyanidin	Hypertension[88]	Anti-senescence (probably): - Proanthocyanidin effect as anti-senescence in grape seed: antioxidant (prevented the senescence phenotype in ovarian aging and degenerative retinopathy)[89,90] - Proanthocyanidin effect as anti-senescence in persimmon: antioxidant (prevented aging phenotype in H_2O_2-induced cellular senescence)[91]	Yes
Centella asiatica (L.) Urb. (*gotu kola*)	Asiaticoside, asiatic acid, madecassoside, and madecassic acid	Diabetic neuropathy,[92] cognitive impairment,[92] and dermatology problem[93]	Anti-senescence: - In senescent human dermal fibroblast: antioxidant (prevented aging phenotype in $H_2O_2^-$ induced cellular senescence)[94,95]	Yes
Garcinia mangostana L. (mangosteen)	Alpha mangostin and gamma mangostin	Cancer[97] and liver fibrosis[98]	Anti-senescence: - In senescent HUVEC: SIRT1 and AMPK activator (prevented aging phenotype in high glucose-induced cellular senescence)[55]	Yes

AMPK=AMP-activated protein kinase; HUVEC=human umbilical vein endothelial cells; SIRT1=sirtuin 1

and shows an anti-hypertensive effect.[88] Likewise, proanthocyanidin plays an emerging role in cellular senescence. Proanthocyanidin extracted from grape seed prevents senescence induced by oxidative stress.[89,90] Otherwise, proanthocyanidin isolated from persimmon prevents cellular senescence induced by H_2O_2.[91]

Centella asiatica (L.) Urb.

Centella asiatica (L.) Urb. or *gotu kola* is a medicinal plant cultivated in tropical regions. It has been traditionally used since 3,000 years ago and has been widely recognized in recent years.[92] *C. asiatica* contains triterpenoids, such as asiaticoside, asiatic acid, madecassoside, and madecassic acid. This herb has been widely investigated in several diseases, such as skin diseases, diabetic neuropathy, and vascular cognitive impairment.[93] Two independent studies investigated the effect of *C. asiatica* in the senescent human fibroblasts induced by oxidative stress treatment showed that both single or combination treatment with *Moringa oleifera* Lam. ameliorated oxidative exposure, thus preventing the senescence phenotype in those cells.[94,95]

Garcinia mangostana L.

Garcinia mangostana L. or mangosteen is widely discovered in Southeast Asia. The native Indonesians have practically used it for fever, wound healing, and diarrhea.[8] Mangosteen produces several xanthones as secondary metabolites. Among them, alpha (α)- and gamma (γ)-mangostin are dominantly found in this purple fruit.[96] The α-mangostin efficiently increases apoptosis in cancer and fibrotic cells.[97,98] This

compound may also improve the pathologic findings in hypertension, dyslipidemia, obesity, and diabetes.[8] A single report showed that α-mangostin rejuvenated hyperglycemia-induced premature senescent EC by modulating sirtuin 1 and AMPK signaling,[55] indicating the emerging role of α-mangostin in EC senescence.

In conclusion, ECs play a critical role in aging and age-related diseases, and EC senescence can be a target for drug discovery. Medicinal plants have been historically practiced during human civilization and possess a strong potential for various diseases. The deep learning-based cellular morphology approach could open new insight into drug discovery, particularly in identifying the unexplored medicinal plants as a novel anti-senescence drug. Therefore, the discovery of herbal drugs targeting cellular senescence may prevent aging and age-related diseases in the future. Several medicinal plants originated in Indonesia such as *Curcuma longa* L. (turmeric), *Piper retrofractum* Vahl. (Javanese long pepper), *Guazuma ulmifolia* Lam. (*mutamba/daun jati Belanda*), *Centella asiatica* (L.) Urb. (*gotu kola*), and *Garcinia mangostana* L. (mangosteen) might have an anti-senescence property in the EC senescence effect. However, advanced research is needed to investigate those candidate plants in senescent EC in the future.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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