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Endothelium Aging and Vascular Diseases

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1. Introduction

Aging is a biological process that causes a progressive deterioration of structure and function of all organs over the time [1]. According to the United Nation's report, the number of people aged 60 and over in the world has increased from 8% (200 million) in 1950 to 11% (760 million) in 2005, and it is estimated that this number will further increase to 22% (2 billion) in 2050. It is expected that in the US alone, the aged population of 65 and over will grow rapidly and reach 81 million by 2050 [2,3]. This rapidly increasing aging population will not only cause a decline of productive workforce but also negatively affect the country's economy. Furthermore, aging is one of the major risk factors for the development of many diseases including cardiovascular diseases [4], stroke [5] and cancer [6]. Moreover, the epidemiological data strongly suggests that more often these diseases are diagnosed in older people compared to younger individuals. In addition to the huge economical impact, these diseases also cause loss of productivity and disability in the elderly population. Therefore, it is extremely important to give high priorities to aging and age-associated disease research in order to develop novel therapies to slow the aging process as well as to prevent and /or treat the age-associated diseases more effectively. It has been found that many factors including genetics [7,8], metabolism [9], diet [10] and stress [11] can in part contribute to the aging process. Similar to other organs, the vascular system, which provides oxygen and nutrients to all the organs in the body, is also affected by the aging process and becomes more vulnerable to disease development in the elders [12,13]. For example, vascular diseases such as coronary artery disease, peripheral arterial disease, stroke and microvascular disease are more often found in the aged population. This is in part due to the structural and functional changes that occur in the vascular system of aged people. In this review, we highlighted (i) the changes that occur in the vascular system, particularly in the endothelium due to aging; (ii) the mechanisms by which the age-associated changes lead to decreased angiogenesis;

(iii) how the ubiquitin proteasome system plays important roles in regulating vascular endothelium function; (iv) the mechanisms by which the age-associated increase in oxidative stress might cause endothelial dysfunction; and finally, (iv) how the age-associated changes in the vascular system lead to the development of various vascular diseases such as coronary artery disease, peripheral artery disease and diabetic retinopathy.
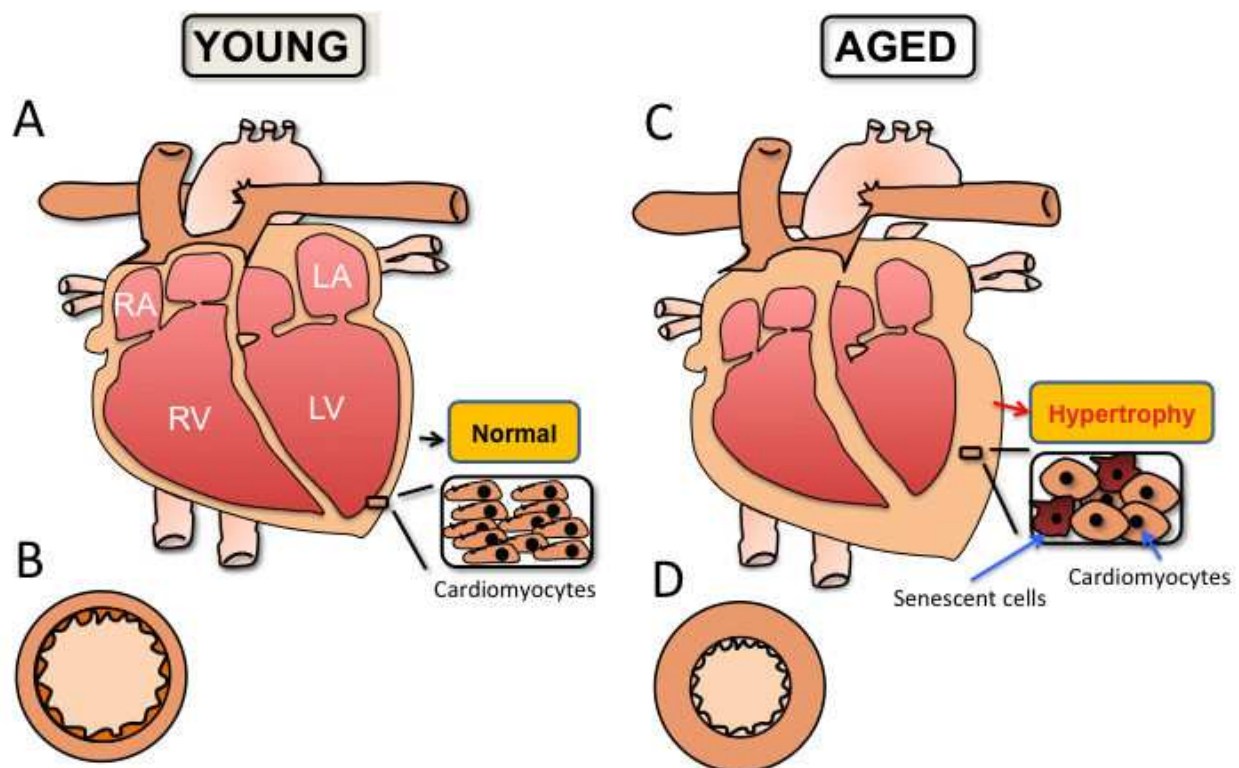
2. Age-associated changes in the vascular system

Many changes are known to occur due to aging in the entire vascular system that includes heart, coronary arteries, peripheral arteries and small blood vessels known as capillaries (Figure 1). There will be an increase in the overall size of the heart, due to an increase in the heart wall thickness in the aging heart. The heart valves, which control the unidirectional of blood flow, will also become stiffer. There is also deposition of the pigments known as lipofuscin in the aged heart along with possible loss of cardiomyocytes as well as cells present in the sinoatrial node (SA node). Furthermore, there is an increase in the size of cardiomyocytes to compensate for the loss of the heart cells. These changes altogether cause a progressive decline in the physiological functions of the heart in the elderly population. In addition to these changes in the heart, the blood vessels also undergo significant changes. For example, the aorta, the large artery that originates from the heart becomes thicker, stiffer and less flexible. Smaller blood vessels also become thicker and stiffer. These changes are due to alterations that occur in the cells present in the blood vessels and also in the connective tissue of the blood vessel wall. All these changes ultimately lead to hypertrophy of the heart and causes an increase in the blood pressure [14]. There seems to be an interconnection between changes in the blood vessels and changes in the heart. Changes such as thickening of the blood vessels lead to increase in the blood pressure, which further affects the heart function. In that condition, the heart tries to function more efficiently by becoming larger in size (hypertrophy) and by enhancing its pumping capacity.

3. Changes that occur in the vascular endothelium

The vascular endothelium is comprised of a layer of endothelial cells that are positioned in the inner surface of blood vessels. The endothelium forms an interface between circulating blood and vessel wall, hence has a direct contact with circulating blood. In addition to serving as a barrier, endothelial cells participate in many physiological functions. They control vascular homeostasis, regulate blood pressure by vasoconstriction and vasodilatory mechanisms and promote angiogenesis when body requires. They also secrete anti-coagulatory factors to prevent clotting [15]. Importantly, vascular endothelial cells express many important molecules such as vascular endothelial growth factor (VEGF) and its receptors vascular endothelial growth factor receptor-1 (VEGFR1), vascular endothelial growth factor receptor-2 (VEGFR2) and vascular endothelial growth factor receptor-3 (VEGFR3). VEGFR1 and VEGFR2 are expressed exclusively in vascular endothelial cells, whereas VEGFR3 is mainly

expressed in the lymphatic endothelial cells [16]. The VEGF/VEGFR2 signaling is critical for vasculogenesis as well as angiogenesis [16]. Disruption or loss of VEGF and VEGFR2 genes is associated with severe vascular abnormalities or embryonic lethality [17]. Furthermore, the endothelial cells produce other growth factors known as angiopoitins (Ang), which are required to remodel and stabilize the immature blood vessels induced by VEGF/VEGFR2. Moreover, molecules such as neuropilines are involved in modulating the binding as well as responses to VEGF receptors [16]. Furthermore, endothelial cells express endothelial nitric oxide synthase (eNOS), which produces nitric oxide (NO). NO has many important physiological functions. For example, NO promotes vasodilation [18], as well as inhibits leukocyte adhesion [19], thrombocyte aggregation [20] and smooth muscle cell proliferation [21]. Under basal conditions eNOS is found inactive, however its activity is increased by many factors including acetyl choline, bradykinin, thrombin and histamine that lead to increased production of NO.

Figure 1. Age-associated changes that occur in the heart and the vascular system. Normal young heart has highly functional cardiomyocytes, and normal atrium and ventricles (A). Young artery has normal lumen, normal arterial thickness and efficient contractile and relaxation properties (B). However, aged heart has increased thickness in the heart muscle due to hypertrophy. Specifically, cardiomyocytes from aged heart show hyperplasia along with some cardiomyocytes undergoing senescence (C). Aged artery also has increased thickness, reduced lumen and less efficient contractile and relaxation properties (D). These age-associated changes ultimately lead to reduced cardiac as well as vascular functions in the elders.

Aging also influences endothelial cells and causes a progressive deterioration of their function. Previous studies have shown that endothelium-mediated vasodilatory function progressively declines with age [22]. This is associated with decreases in eNOS expression and NO production by aging endothelial cells [23,24]. Recently, Yoon et al. have shown that decreased expression of eNOS in aged human umblical vein endothelial cells [24]. However, the precise mechanisms for the age-associated decreases of these molecules remain unknown. Interestingly, it has been observed that the aging endothelial cells produce increased amount of O_2-anions [25], which scavenge NO to form peroxinitrite, a potent form of free radical. Peroxinitrite further inactivates eNOS and decreases its activity [26]. These described mechansims in part explain oxidative stress-mediated decrease of eNOS and NO in aging endothelial cells. On the other hand, it has been suggested that the age-associated changes that occur in eNOS regulatory proteins such as caveolin-1, pAkt, and heat shock protein 90 (Hsp90) contribute to the decreased activity of eNOS in aged endothelial cells [24]. In addition to these regulatory mechanisms, several other factors also regulate eNOS activity. For example, shear stress [27], estrogens [28], and growth factors [29] could also positively regulate eNOS expression. However, as their expression levels decrease with advancing in age, these changes might cause a subsequent decrease in eNOS expression. Taken together, these alterations finally lead to both a decreased expression of eNOS and decreased levels of NO in aged endothelial cells. In addition to these changes in endothelial cells, aging also causes several other changes in vascular smooth muscle cells (VSMCs). During the aging process, VSMCs migrate from tunica media to tunica intima and start accumulating there. These cells become less functional and less responsive to growth factors such as transforming growth factor-beta1 [30]. As VSMCs are important regulatory cells that control the vascular wall by vasoconstriction and vasodilatory mechanisms, progressive loss of their physiological functions might lead to changes in vascular endothelium and impaired vascular function in the aged blood vessels.

4. Aging causes impaired angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is a physiologically an important process during growth, menstrual cycle and wound healing. Several factors are known to influence angiogenesis. The most important one is hypoxia, which activates the transcription factors such as hypoxia-inducible factor-1 alpha (HIF-1 alpha) and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) [31]. These transcription factors increase the production of VEGF and other growth factors that promote proliferation and migration of vascular endothelial cells. During angiogenesis, matrix metalloproteinases, the enzymes that degrade the capillary basement membrane and extra-cellular matrix, will be increased in order to facilitate endothelial cell migration. Therefore, angiogenesis is a complex process, and its timely induction is tightly controlled by coordination from multiple factors. Unfortunately, angiogenesis is markedly reduced by aging [32]. In keeping with this notion, wound healing, which is associated with angiogenesis, is also markedly impaired in aged mice [33] and significantly delayed and impaired in aged

individuals [34]. Several studies were attempted to find the age-associated changes that might cause impaired angiogenesis. To this end, it has been observed that aging endothelial cells are functionally less angiogenic and less responsive to growth factors [32]. Rivard et al. [32] have found that VEGF levels were markedly reduced in aging mice. During hind limb ischemia, the old mice are unable to produce sufficient VEGF levels compared to younger mice, which are critically necessary for neovascularization and proper wound healing. Furthermore, the T lymphocyte-derived VEGF also markedly reduced in old mice, which compromised the angiogenesis-mediated wound healing process during the hind limb ischemia. This study, therefore, identified loss of VEGF as one of the key factors for the impaired angiogenesis observed in aged mice [32]. Furthermore, Qian et al. found that in addition to VEGF decrease, its key receptor VEGFR2 levels were also significantly decreased in eNOS knockout old mice [35]. Since the VEGF/VEGFR2 signaling is crucial for the survival, proliferation and migration of endothelial cells, a decrease of this pivotal signaling pathway may lead to impaired angiogenesis and delayed wound healing in aged subjects. Even in the eNOS knockout mice, which produce significantly less NO, the angiogenic response was markedly less in older mice due to decreased expression of VEGFR2. This partially explains that VEGFR2 plays an important role in neovascularization even in the absence of eNOS and corresponding NO [35].

Importantly, in addition to the loss of pro-angiogenic molecules, the anti-angiogenic molecules such as thrombospondin-2 (TSP2) levels were also affected by aging. To demonstrate the significance of TSP2 in aging and wound healing process, Agah et al. created full thickness excisional wounds in TSP2 null young and TSP2 null old mice and observed the wound healing process [36]. Consistent with other groups [33], they found that regardless of TSP genetic status, the would healing is delayed in old mice in comparison with young mice. However, interestingly, they found that the wound healing was faster in TSP2 null, old mice compared to wild-type, old mice suggesting that increased TSP2 in older mice might delay the angiogenesis and wound healing process. Correspondingly, there was also impaired expression of matrix metalloproteinase-2 (MMP2) found in TSP2 null old mice. These age-associated increase in expression of TSP2 and impaired MMP2 expression in older mice together might cause impaired angiogenesis and delay the wound healing process [36]. In addition to these changes observed in older mice, there are also changes observed in cell cycle-related molecules, which may affect the proliferation of aged endothelial cells. For example, aged endothelial cells undergo senescence and cease proliferation, which may limit neovascularization. Indeed, after certain passages, human umbilical vein endothelial cells (HUVECs) known to undergo senescence and loose their proliferative capacity [37]. As NO is known to prevent endothelial cell senescence, age associated decreases in eNOS and NO may be in part responsible for the senescence observed in HUVECs. Interestingly, the telomerase reverse transcriptase (TERT), which prevents senescence by counteracting telomere shortening process is active in human endothelial cells. However, after several passages, endothelial cells display a decrease of NO and loss of TERT activity that further lead to endothelial senescence. Indeed, ectopic overexpression of TERT protects from endothelial cells from undergoing senescence and preserve the angiogenic function of endothelial cells [38]. Furthermore, TERT overexpression increased eNOS function and enhanced precursor endo-

thelial cell proliferation and migration that effectively promoted angiogenesis [39,40]. In fact, TERT expression decreased p16 and p21 activities that are significantly increased in senescent endothelial cells. These findings indicate that loss of telomerase-induced senescence also plays a role in affecting angiogenesis in aged endothelial cells. Interestingly, in a separate set of experiments, it has been demonstrated that VEGF-A, a potent pro-angiogenic factor, suppresses both p16 and p21 activities in endothelial cells, suggesting that VEGF-A could serve as an anti-senescence agent [41]. However, it remains unclear whether VEGF-A activates the VEGFR2 kinase to influence hTERT activity to exert this anti-senescence capacity. Taken together, these findings indicate that even though there is a shift between pro-angiogenic and anti-angiogenic molecules in aged endothelial cells, it remains to be determined whether increasing pro-angiogenic factors or inhibiting anti-angiogenic molecules restores angiogenesis and accelerate wound healing process especially by aged endothelial cells. Future research are therefore warranted to thoroughly address these important questions.

5. Aging-induced oxidative stress and vascular endothelial dysfunction

Oxidative stress is implicated in causing aging of endothelium and endothelial dysfunctions. In turn, aged endothelium produces increased free radicals, which might further accelerates aging. Based upon biomarkers of oxidant damage, increased levels of nitrotyrosine were observed in human aged vascular endothelial cells [42], Moreover, oxidative stress markers were also observed in the arteries of aged animals [26,43], suggesting that aging is indeed associated with increased formation of reactive oxygen species (ROS). Many different mechanisms are responsible for causing oxidative stress in endothelial cells that includes mitochondria-mediated production of ROS, decreases in free radical scavengers and increased susceptibility of macromolecules to free radical damage. Similar to other cells, oxidative stress damages proteins, lipids and DNA in vascular endothelial cells, thus causing loss of endothelial cell function. One of the major free radicals is super oxide anion (O_2^-), which is produced by aging mitochondria due to increased mitochondrial DNA damage. It has been demonstrated that NADPH contributes to O_2^- generation in vascular endothelial cells. Usually, the O_2^- anions are detoxified to H_2O_2 by manganese super oxide dismutase (MnSOD), which is present in the mitochondria. However, in the presence of NO, O_2^- leads to formation of a potent free radical known as peroxinitrite ($ONOO^-$) that further damages macromolecules in the endothelial cells. It has been demonstrated that $ONOO^-$ can inactivate both MnSOD and eNOS in the endothelial cells [44]. The switch of eNOS from an NO generating enzyme to an O_2^- generating enzyme (NO synthase uncoupling) leads to increased production of O_2^- and enhanced oxidative stress in aged endothelial cells (Figure 2). Taken together, NADPH and eNOS are important contributors for O_2^- generation in aged endothelial cells, since inhibition of NADPH and eNOS attenuates O_2^- production in the aorta of aged Wistar-Kyoto rats [25].
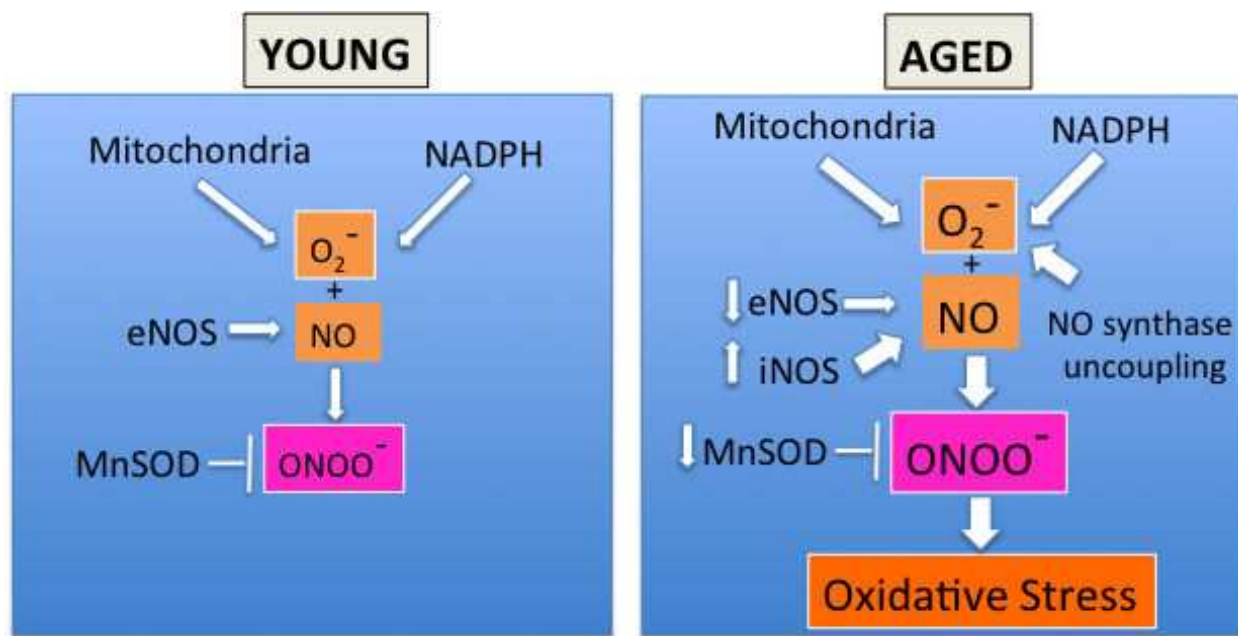
Figure 2. Oxidative stress in aged endothelial cells. Compared to younger endothelial cells, aged endothelial cells produce increased levels of free radicals. In the presence of nitric oxide (NO), which is originated from iNOS in aged endothelial cells, O_2^- lead to formation of a potent free radical known as peroxinitrite (ONOO$^-$). These changes lead to increased oxidative stress that damages macromolecules and ultimately lead to loss of endothelial cell function in aged cells.

The potential role of oxidative stress in vascular endothelium aging is also evident from the experiments carried out with antioxidants. For example, Vitamin C has been shown to decrease telomere shortening and increase the longevity of endothelial cells in culture [45]. N-Acetylcysteine, a potent antioxidant known to decrease endothelial cell senescence by preserving TERT activity and preventing its nuclear export [46]. Interestingly, it has been demonstrated that p66shc deletion protects endothelial cells from aging-associated vascular dysfunction [43] and sirtuins decrease the p66shc expression [47]. Although human clinical trials with antioxidants such as Vitamin C and E have not yielded beneficial effects on improving cardiovascular function [48,49], future studies with other antioxidants such as N-acetylcysteine may yield positive results in improving endothelial dysfunction associated with aging and oxidative stress.

6. Ubiquitin-proteasome system regulates endothelial cell function

The ubiquitin-proteasome system (UPS) plays important roles in a variety of key cellular functions including cellular protein homeostasis, signal transduction, cell cycle control, immune function, cellular senescence and apoptosis. This system targets specific proteins in the cell for degradation via ubiquitination-mediated destruction mechanism by specific ubiquitin E3 ligases [50,51]. Two major complexes, Skp1-Cul-1-F-box protein complex (SCF) and Anaphase Promoting Complex/Cyclosome (APC/C) are involved in the regulation of

cell cycle as well as other key regulatory processes in the cell. Dysfunction of UPS leads to development of many diseases including cancer and cardiovascular disease. Therefore, how UPS regulates endothelial cell function and endothelial cell cycle is crucial in order to understand the underlying mechanisms involved in vascular disease development, and will also provide important insights into developing novel therapies for many vascular diseases associated with aging. Increasing evidence suggests that UPS regulates endothelial function by specifically regulating the key proteins present in endothelial cells. For example, the half-lives of both eNOS and inducible nitric oxide synthase (iNOS) are regulated by proteasome-dependent degradation [52,53]. Furthermore, the von Hippel-Lindau protein (pVHL) regulates HIF-1 alpha, which is a critical factor involved in regulating angiogenesis [54] (Figure 3). Consistent with the key role of UPS in endothelial function, treatment with low doses of proteasome inhibitor increases endothelial cell function [55]. These findings further suggest that UPS could be a potential target to improve the physiological functions of vasculature, hence may be utilized as a valuable drug target to develop novel treatments for aging-associated vascular diseases. However, the specific E3 ligase complexes and the molecular mechanisms that are involved in the regulation of endothelial cell cycle and endothelial cell function remain unknown.
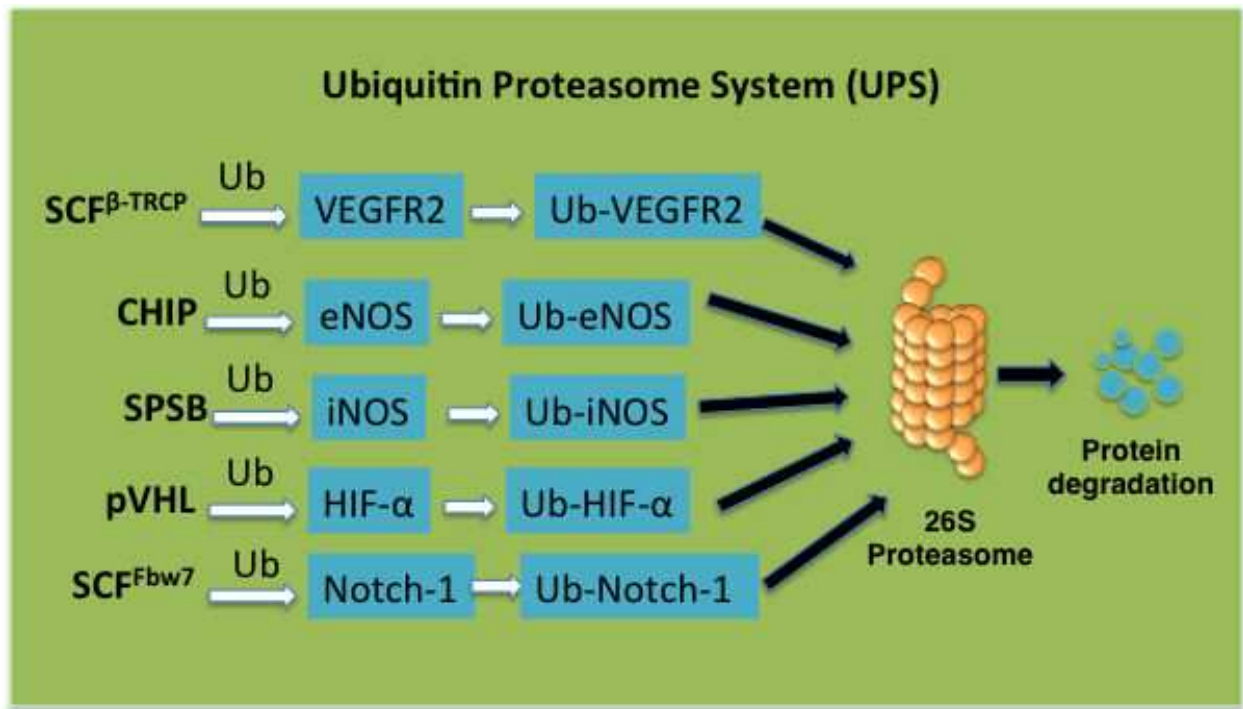
Figure 3. The ubiquitin proteasome system (UPS) regulates the stability of various key proteins in endothelial cells. The E3 ubiquitin ligases such as SCF$^{\beta\text{-}TRCP}$, C-terminus of Hsp70-interacting protein (CHIP), SOCS box-containing protein [ECS(SPSB)] and pVHL, target VEGFR2, eNOS, iNOS and HIF-1 alpha, respectively, for proteasome-dependent degradation. These E3 ligases recognize their respective substrates once the substrates are properly phosphorylated at the critical phosphodegrons by one or more kinases. This is an important regulatory mechanism by which UPS controls the half-lives of various key proteins in endothelial cells to influence the angiogenesis process.

Recent studies indicate that F-box proteins such as SCF^{Fbw7} and SCF$^{\beta-TRCP}$ are potentially involved in regulating endothelial cell function. For example, mice lacking Fbw7 die early (embryonic day 10.5) with developmental defects in vascular and haematopoietic system as well as heart chamber maturation [56,57]. As Fbw7 regulates the key cell cycle regulators including Notch, cyclin E, c-Myc and c-Jun, deletion of Fbw7 leads to accumulation of these substrates in the endothelial and /or hematopoitic cells. Indeed, elevated Notch protein levels were observed in Fbw7-deficient embryos that lead to the deregulation of the transcriptional repressor, Hey1, which is an important factor for cardiovascular development [56]. Therefore, these findings suggest that Fbw7 is an important E3 ligase governing the timely destruction of the key substrates involved in cardiovascular development. Furthermore, our laboratory has recently identified SCF$^{\beta-TRCP}$ as an E3 ubiquitin ligase that is potentially involved in regulating VEGFR2 protein levels in microvascular endothelial cells [58]. As stated in above sections, VEGFR2 is the major regulator of angiogenesis. Increased angiogenesis is associated with certain cancers, whereas angiogenesis is markedly decreased in aging individuals. Our study, for the first time, revealed that deregulation of β-TRCP leads to stabilization of VEGFR2 and subsequent increases in angiogenesis, whereas increased β-TRCP activity leads to decreased VEGFR2 levels and reduced angiogenesis. Mechanistically, casein kinase-I (CKI)-induced phosphorylation of VEGFR2 at critical phospho-degrons leads to its ubiquitination by β-TRCP, and subsequent degradation of VEGFR2 through the 26S proteasome [58]. However, we are just beginning to understand the critical role of UPS in endothelial function, future studies are therefore warranted to unravel the important role of various E3 ubiquitin ligases in the regulation of vascular system, which may ultimately, help to prevent vascular diseases in the elderly population.

7. Aging and vascular diseases

Aging vascular endothelium is susceptible to the development of various vascular diseases including cardiovascular disease (CVD) (coronary artery disease; atherosclerosis and hypertension), peripheral vascular disease (PVD), diabetic retinopathy, renal vascular disease and micro-vascular disease. Importantly, aging-associated changes that occur in the blood vessels are the major cause for the development of these diseases. Therefore, identifying the molecular changes that occur in the aging-endothelium and elucidating the underlying molecular mechanisms responsible for vascular disease development lead to the development of novel therapies to treat various vascular diseases.

## 7.1. Cardiovascular and peripheral vascular diseases

Cardiovascular disease (CVD) is the number one cause of human death in the US as well as in the world. CVD mostly occur in the aged population [59], and according to the World Health Organization, an estimated 17.3 million deaths occurred due to CVD in 2008. Coronary artery disease (CAD) is the major form of CVD, which occurs when coronary arteries are blocked due to atherosclerosis. Aging endothelium is very susceptible for plaque formation that leads to progressive blockage of the coronary arteries. This causes reduced blood

supply (decreased supply of oxygen and nutrients) to the affected area of the heart. Although partial blockages may cause symptoms such as angina, complete loss of blood supply leads to heart attack, and if not treated immediately, may lead to sudden death. It has been observed that several age-associated changes in the endothelium-derived factors are responsible for plaque formation in the arteries. Importantly, endothelin (ET), a vascular endothelium-derived growth factor was found to be significantly increased in the aged endothelium [60,61,62]. ET mainly acts through its receptors ET-A and ET-B present on endothelial as well as vascular smooth muscle cells (VSMCs). ET-A activation leads to the constriction and proliferation of VSMCs, whereas ET-B activation leads to increased production of NO, which leads to vasodilation and inhibition of platelet aggregation. Studies indicate that ET-A receptor is mainly involved in the development of atherosclerosis, as inhibition of ET-A receptor prevents atherosclerosis in apolipoprotein-E deficient mice [63]. More importantly, endothelin-1 also decreases eNOS in vascular endothelial cells through ET-A receptor activation [64], suggesting that aging-induced increases in ET-1 as well as increased activation of ET-A receptor are potentially involved in causing atherosclerosis. Furthermore, the aging-induced increased expression of various adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) also contribute to the ongoing process of atherosclerosis [65].

Inflammation, another major factor that is also known to increase with aging potentially contribute to the process of atherosclerosis [66]. Consistently, the incidence of atherosclerosis is found much higher in patients with autoimmune diseases such as rheumatoid arthritis [67,68] and systemic lupus erythematosus [69]. Several different immune cells and increased expression of adhesion molecules also play a major role in developing atherosclerotic plaque. For instance, adhesion molecules ICAM-1 and VCAM-1 not only facilitate the binding of immune cells such as monocytes and T-cells, but also help to transport these cells into the arterial wall. Once inside, the monocytes differentiate into macrophages, and ultimately become foam cells by taking up the oxidized LDL. The proteoglycans present in the extra cellular space of the intima bind with the oxidized LDL molecules. Moreover, the activated T-cells secrete several different cytokines that promote inflammation and activate VSMCs to proliferate. Altogether, this ongoing inflammatory process accelerates the process of atherosclerosis and damages the coronary arterial wall [70] (Figure 4).

Atherosclerosis is also occurs in other arteries other than coronary arteries. If atherosclerosis occurs in the peripheral arteries then it is called peripheral vascular disease or peripheral arterial disease (PAD). PAD is also influenced by aging and mostly occurs in elderly population. The prevalence increases with age from 3% under 60 years of age to 20% in aged 70 years and over [71]. Several factors influence the development of PAD that includes smoking, dyslipidemia, hypertension, diabetes and platelet aggregation. Advanced atherosclerosis in coronary arteries leads to angina and heart attack, whereas in cerebral arteries leads to stroke or transient ischemic attacks. If atherosclerosis occurs in peripheral arteries, that will lead to pain during walking or exercising (claudication), and this condition causes defects in the wound healing or ulcers. Preventing or slowing down the age-associated changes that

occurs in the vascular system will protect the aged population from developing various vascular diseases.
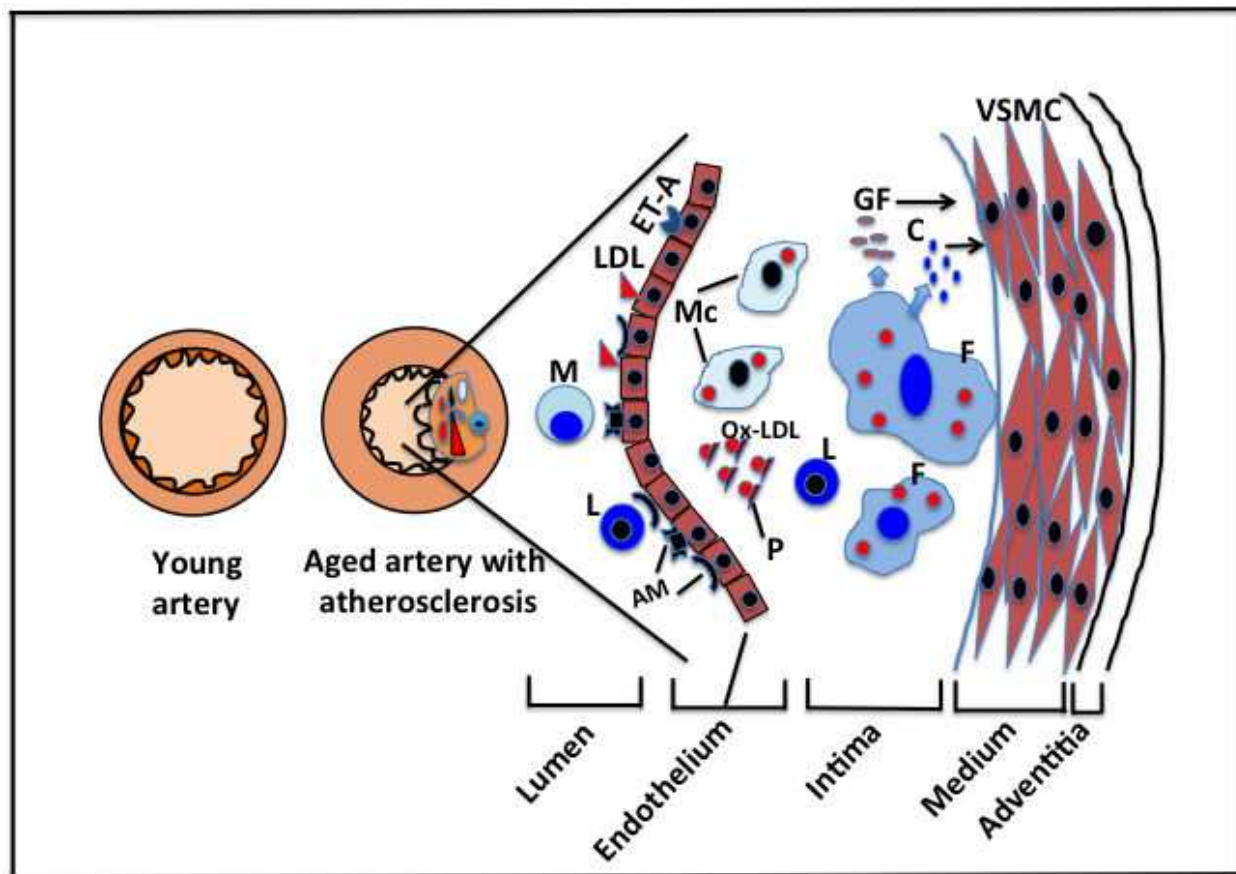
Figure 4. Atherosclerosis in the aged artery. Aged endothelial cells express various adhesion molecules (AM), which facilitate the binding as well as transportation of various inflammatory cells, including monocytes (M) and lymphocytes (L) into the intima. Oxidized low density lipoproteins (OxLDL) play a major role in the formation of foam cells (F). The foam cells secrete several growth factors (GF) and cytokines (C) that lead to increased proliferation of vascular smooth muscle cells (VSMCs). Increased expression of endothelin-1 facilitates atherosclerosis through ET-A receptor activation. The lymphocytes also play a critical role in causing inflammation in the endothelium. Altogether, these changes facilitate the plaque formation in the blood vessels of aged populations.

7.2. Diabetic retinopathy, a vascular disease of the eye

Diabetes affects approximately 200 million people around the world and almost 20 million in the United States. Diabetic retinopathy (DR) is a microvascular disease of the eye and most commonly seen in elderly population [72]. Type I as well as Type II diabetes lead to the development of DR. Importantly, microvessels of the eye are mostly affected by hyperglycemia. Several changes in the blood vessels have been observed including loss of pericytes, thickening of the basement membrane and increased permeability of blood vessels in DR. Furthermore, as DR progresses from non-proliferative DR to proliferative DR, the new blood vessels start to grow (neovascularization) to compensate for the affected blood vessels. Although the molecular mechanisms by which diabetes affects blood vessels of the eye

remain not completely understood, it is evident from several studies that hyperglycemia directly plays a major role in causing DR. The highly elevated blood glucose activates aldose reductase pathway in certain tissues, which converts the sugars into alcohols, mainly sorbitol. The increased formation of sorbitol further affects the intramural pericytes present in the blood vessels of the retina to cause loss of function of pericytes [73]. As pericytes inhibit the endothelial cell function in occular blood vessels, loss of pericytes function leads to the formation of microaneurysms and ultimately lead to neovascularization. This pathological condition is mostly observed at the borders of retina and occurs along the vascular arcades as well as at the optic nerve head. The newly formed blood vessels do not directly affect the retina, however, the blood vessels are susceptible to vitreous traction and lead to hemorrhage into the vitreous cavity or preretinal space. If not treated, this condition may ultimately lead to vision loss. Many studies were attempted to understand the underlying molecular mechanisms by which neovascularization occurs in DR. Like in other pathological conditions described above, it is in part due to aging-associated defects in angiogenesis. Specifically, increased shear stress causes enhanced permeability of the blood vessels. On one hand, the blood vessels constantly remodel to adapt such changes induced by shear stress. On the other hand, the increased shear stress also causes activation, proliferation and migration of endothelial cells that ultimately cause neovascularization [74]. Furthermore, shear stress also known to cause vasodilatory effects by inhibiting endothelin1, a potent vasoconstrictor and increasing the levels of eNOS and prostaglandins which are potent vasodilators. Increased shear stress also increases matrix production by the endothelial cells, which causes basement thickening. Increased secretion of tissue-type plasminogen activator causes thrombosis and affects microcirculation [75]. Once blood vessels are obscured, the hypoxia generated inside will cause increased dilation of nearby vessels and leads to increased production of growth factors that further promote increased neovascularization.

Among the various growth factors, VEGF-A seems to be potentially involved in promoting angiogenesis in DR. In fact, Miller et al. demonstrated that increased VEGF-A levels correlate with enhanced angiogenesis in ocular tissue [76]. Moreover, high affinity receptors for VEGF-A have also been identified in endothelial cells as well as the pericytes of blood vessels located in the eye. This clearly suggests that VEGF-A-induced signaling pathway might play a potential role in promoting angiogenesis in DR. Furthermore, as angiogenesis is precisely regulated both by pro-angiogenic and anti-angiogenic factors, Funatsu et al. conducted studies to evaluate whether the balance between these two types of molecules is critical in causing angiogenesis in DR [77]. They simultaneously measured pro-angiogenic (VEGF-A) as well as anti-angiogenic molecules (endostatin and PF4) in the vitreous and in the plasma samples to correlate with DR. Interestingly, these studies revealed that vitreous VEGF-A and endostatin levels clearly correlate with the severity of DR, however, no correlation was found between DR and plasma levels of VEGF-A and endostatin [77]. Therefore, this study suggested that loss of balance between pro- and anti-angiogenic molecules might be responsible for the neovascularization observed in DR.

Several drugs were investigated to inhibit neovascularization associated with DR. For example, Ruboxistaurin, a protein kinase C inhibitor tested for efficacy. This is based upon the

effects of hyperglycemia on diacylglycerol, which is known to be elevated in DR. Diacylglycerol is a potent activator of protein kinase C, and in turn protein kinase C increases VEGF-A secretion. The protein kinase C inhibitors are known to have some beneficial effects on DR. Furthermore, as VEGF-A levels are increased in DR, anti-VEGF-A compounds were also developed to specifically inhibit neovascularization associated with DR [78].

8. Conclusion

Aging is one of the major risk factors for the development of various vascular diseases such as cardiovascular disease, peripheral vascular disease and vascular diseases of the eye. Although exact molecular mechanisms are not clearly known, several molecules are known to be altered in aged endothelial cells. Importantly, reduced expression of eNOS and decreased production of NO, a potent vasodilator, have been observed. Furthermore, decreased expression of VEGF and VEGF receptors, and conversely, increased expression of TSP2, a potent angiogenesis inhibitor, have been observed in aged endothelial cells as well. The imbalance between the pro-angiogenic and the anti-angiogenic molecules seems to be responsible for the decreased angiogenesis observed in aged endothelial cells. Importantly, it has been also demonstrated that aging-induced oxidative stress is one of the major contributing factors for the loss of endothelial cell function in advanced age. In this regard, novel antioxidants may prevent aging-induced oxidative stress and thereby improve endothelial cell function in aged cells. As most of the pro-angiogenic and the anti-angiogenic molecules are unstable, recent studies have also established a potential role of UPS in regulating endothelial cell function. However, further thorough investigations are required to pinpoint the precise role of UPS in regulating the aging-associated decline of angiogenesis in the endothelial cells. To this end, it is critical to identify the age-associated molecular signature changes in different cells present in the endothelium such as endothelial cells, smooth muscle cells and pericytes in order to understand how these changes ultimately lead to the loss of endothelial function. This critical information will not only help to identify the crucial signaling pathways through which aging process affects the angiogenesis, but also will aid to develop novel therapies to combat various vascular diseases associated with aging.

Acknowledgements

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