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Review

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Capillaries as a Therapeutic Target for Heart Failure

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Prognosis of heart failure remains poor, and it is urgent to find new therapies for this critical condition. Oxygen and metabolites are delivered through capillaries; therefore, they have critical roles in the maintenance of cardiac function. With aging or age-related disorders, capillary density is reduced in the heart, and the mechanisms involved in these processes were reported to suppress capillarization in this organ. Studies with rodents showed capillary rarefaction has causal roles for promoting pathologies in failing hearts. Drugs used as first-line therapies for heart failure were also shown to enhance the capillary network in the heart. Recently, the approach with senolysis is attracting enthusiasm in aging research. Genetic or pharmacological approaches concluded that the specific depletion of senescent cells, senolysis, led to reverse aging phenotype. Reagents mediating senolysis are described to be senolytics, and these compounds were shown to ameliorate cardiac dysfunction together with enhancement of capillarization in heart failure models. Studies indicate maintenance of the capillary network as critical for inhibition of pathologies in heart failure.

Key words: **Heart failure, Capillaries, Aging, Senolysis**

Introduction

Oxygen and metabolites are delivered through capillaries, and this platform has a critical role in maintaining organ homeostasis. With aging, capillary density was reported to diminish in heart, skeletal muscle, kidney, brain, liver, subdermal or abdominal white adipose tissues, pancreas, testis, thyroid gland, and brown adipose tissue[1-5]. Aging associates with inhibition in vascular endothelial growth factor-A (VEGF-A) mediated angiogenic signaling, and this was reported to enhance aging phenotype in multiple organs[1]. Accumulation of evidence indicates a decrease in capillary density accelerates undesirable aspects of aging.

Incidence of heart failure increases with age, and the prognosis of this disorder remains poor. Aged hearts are characterized by fibrosis, inflammation, mitochondrial dysfunction, apoptosis[6], and capillary density was reported to decline with aging in rodents and humans[2, 7]. Studies indicate capillary rarefaction in the left ventricle (LV) has a close connection with functional decline in cardiac tissue[8-10]. Depending on the systolic or diastolic function of the LV, heart failure can be categorized into two groups. One is described as heart failure with reduced ejection fraction (HFrEF) and the other as heart failure with preserved ejection fraction (HFpEF). Recent studies showed sodium glucose co-transporter2 (SGLT2) inhibitors contributed to the suppression of hospitalization for heart failure both in patients with HFrEF and those with HFpEF[11-13]. Compared with HFrEF, medication for HFpEF is limited[14], and next-generation therapies for this critical disorder should be

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urgently established. HFpEF in patients increases with age, and this is associated with capillary rarefaction in LV[15]. Failing hearts based on several etiologies exhibit reduced capillary density. In this review article, we would like to focus on capillarization in cardiac tissues and discuss potential therapies for heart failure, targeting the enhancement of the capillary network in this organ.

1. Capillaries in the Heart with Various Conditions

In humans and other mammalian species, the heart is perfused through coronary circulation. Numerous bifurcations and anastomoses between capillaries create a dense vascular network, and this enables the delivery of nutrients and oxygen into cardiac tissues. Capillary rarefaction develops in hearts with aging or age-related cardiovascular-metabolic disorders, and these would be described in this chapter.

1-1. Aging

Aging is a nonmodifiable risk factor for cardiovascular diseases (CVDs). Aged heart develops functional and structural alterations[16, 17]. Vasculature is also affected with age, and rodent hearts exhibited capillary rarefaction together with diminished oxygenation capacity[7, 18, 19]. In C57BL/6J mice, capillary density was reduced in mice aged 18 months compared with those aged 2 months[19]. Young spontaneously hypertensive rats (SHRs) aged 2.5 months had a normal capillary density in their hypertrophied LV, whereas this declined in older animals aged 7 months[20]. Rakusan *et al.* compared capillary density in patients with congenital aortic stenosis (AS) among infants, children (aged 9–14 years), or adults[2]. They concluded that capillary density diminished with age in humans under LV pressure overload[2]; however, underlying mechanisms are not fully understood. Senescent endothelial cells (ECs) exhibited reduced proliferative capacity together with diminished VEGF-A production[21]. At a certain level, reactive oxygen species (ROS) is considered to mediate homeostatic effects but, beyond the physiological threshold, initiate to promote unfavorable aspects of aging[22]. ROS was reported to induce EC apoptosis and capillary rarefaction in the heart[23]. Aging leads to an increase in decoy receptors for VEGF-A in circulation and suppresses VEGF-A-mediated angiogenic signaling; this was reported to enhance physiological aging in multiple organs, but cardiac tissue was not characterized in this paper[1]. Recently, Kivela *et al.* reported transforming growth factor-β (TGF-β)/ROS/Serpinh1 axis in ECs enhanced mesenchymal features in these cells, promoting cardiac fibrosis and capillary rarefaction[19]. These studies indicate that mechanisms associated with aging have causal roles for diminished capillary density in the heart, letting this tissue become prone to develop heart failure under stressed condition.

1-2. Obesity/Diabetes and Heart Failure

Obese individuals have a higher risk for developing HFpEF than lean subjects, and obese patients with HFpEF were reported to show worse exercise capacity than non-obese patients with HFpEF[24]. Patients with HFpEF were shown to exhibit capillary rarefaction[15], and metabolic stress is reported to reduce capillary density in humans and rodents[25-27]. In obese humans exhibiting BMI >30, coronary microvascular density was lower than in non-obese individuals[25]. Wistar–Kyoto rats or C57BL6/NCrSlc mice fed with high-fat diets developed capillary rarefaction in their respective hearts[26, 27]. Mechanistically, Suda *et al.* showed metabolic stress reduced fibroblast growth factor 2 (FGF-2)/early growth response protein 1 (EGR-1)/VEGF-A signaling, and dipeptidyl peptidase 4 had causal roles for the suppression of this pathway[27]. Dietary obesity also enhanced capillary rarefaction in C57BL/6J mice, and this was mediated through the activation of TGF-β/ROS/Serpinh1 signaling in ECs, sharing similar pathogenic mechanisms with their chronologically aged mice model[19]. Individuals with unhealthy obesity, generally characterized by enrichment in visceral adiposity and systemic insulin resistance, predisposes to type 2 diabetes. Approximately 45% of patients with HFpEF were reported to have type 2 diabetes[28], and overlapping pathologies are considered to exist between diabetes and HFpEF[29]. At end-stage heart failure, cardiac capillary density became lower in patients with diabetes than in patients without diabetes[30]. The same group also showed transgenic diabetic pig developed capillary rarefaction, and this was ameliorated with the introduction of adeno-associated virus encoding *Vegfa* (AAV-*Vegfa*). Compared with control diabetic pigs, ejection fraction did not improve in the AAV-*Vegfa* group[30]. Interestingly, LV end-diastolic pressure and LV fibrosis were suppressed with the reintroduction of *Vegfa*, indicating the link between diminished capillary density and enhancement of fibrotic process[30]. Streptozotocin-induced type 1 diabetic Wistar rats showed that the duration of hyperglycemia negatively correlated with capillary density in the heart[31], suggesting glycemic overload as one of the mechanisms for promoting capillary

rarefaction under diabetic conditions.

1-3. Hypertension

In skeletal muscle, it was previously reported that an increase in mean arterial pressure is associated with a decrease in capillary density[32]. Few studies characterized cardiac capillary density in patients with hypertension. Biopsy samples obtained from patients with hypertensive heart disease (HHD), dilated cardiomyopathy (DCM), and renal failure with hemodialysis treatment (HD) showed capillary density as 1162 ± 189, 1238 ± 261, and 997 ± 183 (/mm^2), respectively[33]. In this paper, the percentage of hypertension was 100% in the HHD group, 8.9% in the DCM group, and 89.3% in the HD group. Studies analyzing capillary density in human hearts with AS showed that the capillary density of control adult hearts aged 15–30 years was 2249 ± 85 (/mm^2), which became 2102 ± 103 (/mm^2) in patients with congenital AS at similar age[2]. The capillary density of patients with acquired AS aged 51–86 years was 1671 ± 66 (/mm^2)[2]. Through these papers published from different groups, it can be speculated that hypertension is associated with capillary rarefaction in the human heart. Further studies would show direct evidence of capillarization in patients with arterial hypertension. Rodent models with hypertension including Dahl salt-sensitive hypertensive rats[34, 35] or high-salt diet[36] exhibited diminished capillary density in LV. High-salt diet, combined with Ang II administration in C57BL/6J mice, resulted in a decrease in capillary density[37]. Capillary rarefaction also developed in SHRs at 24 weeks of age, but this was comparable at 12 weeks of age[38]. SHRs were shown to exhibit reduced capillary density, but the number of smooth muscle α-actin positive arterioles increased in SHRs than in control Wistar–Kyoto rats[39]. Recent findings from Olianti *et al.* challenged previous reports by concluding that capillary rarefaction develops in an SHR model[40]. They performed 3D imaging analyses in cardiac tissues and concluded that capillary density increased in SHRs aged 4, 8, 18, and 24 weeks[40]. Microvasculature is constituted with arterioles, capillaries, and venules, and characterization of these with specific cell markers would help us comprehensively understand capillarization in LV under aortic hypertension[39, 41]. Activation of the renin–angiotensin–aldosterone system (RAAS) is one of the chief mechanisms that promote hypertension, and accumulated evidence indicates that suppression of RAAS enhances capillarization in the heart[34, 42, 43]. LV pressure overload initially increased capillarization at compensated phase, and this was followed by capillary rarefaction at decompensated phase heart failure[9]. Controlling blood pressure and suppressing afterload continues to be an important concept to suppress pathologies in the heart.

1-4. Aortic Valve Stenosis

Studies indicate the potential role of capillary rarefaction in the progression of pathologies in AS. LV outflow tract–capillary density of AS patients correlated with aortic valve area[44]. Basal anteroseptal myocardium showed reduced capillarization in severe AS patients[45]. Diminished capillary density associated with female gender, diabetes, obesity, heart failure symptoms, and low LV ejection fraction[44]. As already described, patients with congenital AS exhibited reduced capillary density with chronological aging, and adults with acquired AS had lower capillary density compared with congenital AS children[2]. The thoracic aortic constriction (TAC) model induces LV pressure overload and thereby mimics cardiac stress introduced with AS. In mice, TAC was shown to induce capillary rarefaction in LV[8-10, 23, 44]. Accumulation of p53 in cardiac tissues[9] or ECs[10] suppressed angiogenic response in LV. ROS enhanced EC apoptosis during LV pressure overload and contributed to the progression of reduced systolic function[23]. These results indicate enhancement of the capillary network in LV would become a therapy for heart failure associated with LV pressure overload.

1-5. Chronic Kidney Disease

Chronic kidney disease (CKD) is an independent risk for CVD[46]. The hearts of experimental uremic Sprague–Dawley rats (5/6 nephrectomy (5/6 NX)) exhibited cardiac hypertrophy, interstitial fibrosis, and reduced capillary density[47]. Nephrectomy-induced capillary rarefaction in the heart was completely inhibited by the administration of moxonidine, a central sympatholytic agent, but not by the calcium antagonist nifedipine[48]. Another report showed transcript *Vegfa* reduced in the heart with 5/6 NX, and this was ameliorated with selective renal sympathetic denervation[49]. These findings indicate that renal dysfunction accelerates capillary rarefaction through sympathetic nervous activation. Another study showed that treatment with an endothelin receptor antagonist normalized microvascular density[50]. Amann *et al.* tested two angiotensin-converting enzyme (ACE) inhibitors and concluded that ramipril[51], but not trandolapril[50], inhibited a decrease in capillary density in a uremic rat model. Additionally, the combination of erythropoietin and ACE inhibitor (enalapril) increased microvascular density in 5/6 NX rats[52]. Mechanistically, a decrease

in oxidative stress and apoptotic signaling contributed to maintaining LV capillary network under uremic condition[52]. Di Marco *et al.* demonstrated the level of calcineurin increased in the heart with nephrectomy, and an inhibitor for this molecule normalized microvascular density in 5/6 NX rats[53]. They showed that calcineurin inhibitor increased angiogenic or stem cell-related molecules including vascular endothelial growth factor 2 (VEGFR2), survivin, cKit-1, and stem cell factor[53]. In CKD patients, the estimated glomerular filtration rate showed a negative correlation with soluble Flt-1 (sFlt-1) in plasma, and the level of sFlt-1 correlated with heart failure symptoms and early mortality[54]. Rats administrated with sFlt-1 had reduced LV capillary density, lower systolic and diastolic function, increase in TUNEL positive cells, and fibrosis in the heart compared with controls[54]. In this paper, inhibition of sFlt-1 increased capillary density, myocardial blood volume, and ameliorated diastolic dysfunction in the 5/6 NX model. Several complex mechanisms have roles in reducing capillaries in the heart under renal dysfunction, and further studies are needed to understand pathologies of cardiorenal syndrome.

1-6. Amyloidosis

Cardiac amyloidosis is characterized by the extracellular deposition of insoluble fibrils composed of misfolded proteins called amyloid. The layers of the walls of intramural vessels are progressively infiltrated by the fibrils, eventually leading to microvascular obstruction[55]. The amyloid deposits surrounding myocytes induce oxidative stress and affect the contractile function of these cells[56]. Reduced stroke volume and compliance of the LV wall contribute to decreasing diastolic flow in the intramural vessels. In symptomatic patients with AL or transthyretin amyloidosis, myocyte blood flow became lower than in hypertensive patients exhibiting LV hypertrophy[57]. Recently, Kim *et al.* showed direct evidence of reduced capillary density in AL amyloidosis patients[58]. They analyzed cardiac biopsy samples and showed capillary density had a negative correlation with NT-proBNP level, amyloid load, LV diastolic, and systolic function[58]. In this paper, the patients exhibiting capillary density of $>220/mm^2$ were shown to have a better prognosis than those exhibiting $<220/mm^2$ [58]. Together with therapy targeting removal of amyloid deposition, whether maintaining capillarization becomes a therapy for amyloidosis remains an open question to be explored.

1-7. Hypertrophic Cardiomyopathy

Myectomy samples showed that capillary density decreased in patients with hypertrophic cardiomyopathy (HCM) compared with control individuals, and interestingly, this showed a negative correlation with left ventricular outflow tract (LVOT) pressure gradient[59]. HCM patients with moderate hypertrophy and LVOT obstruction also showed reduced capillaries in LV compared with control subjects[60]. DBA/2J mouse carrying variants in the two most susceptible genes—Mybpc3 and Myh7—demonstrates the features of HCM, and vessel density was shown to diminish in this mouse model compared with C57BL/6J wild type mice[61]. The fundamental question that remains to be answered is whether enhancing the capillary network reverses or slows down the pathologies in HCM.

2. Role of Senescence-Related Molecules in the Cardiac Capillary Network

Aging or age-related disorders are associated with capillary rarefaction in the heart. Studies indicate age-related mechanisms have causal roles for reducing capillarization in the heart, and these are described in this chapter.

2-1. p53

Chronological aging is associated with a higher prevalence of age-related diseases including heart failure, diabetes, and atherosclerotic disorders. Mechanisms of aging and age-related disorders are complex; however, studies indicate the crucial role of cellular senescence in the progression of these disorders[9, 10, 22, 62-68]. The p53 protein, which is often described as the "guardian of the genome," is a transcriptional factor involved in genomic stability. This molecule mediates the protective effect through coordinating DNA repair, cell-cycle regulation, and apoptosis. p53 also has a central role in inducing cellular senescence, and this promotes pathogenesis in age-related disorders. Previous reports showed p53 increased in the aged[69] or failing heart[9]. In mice, LV pressure overload induced capillary rarefaction together with enhanced p53 level in cardiac tissue[9, 21]. Mechanistically, p53 enhanced ubiquitination and proteasomal degradation of hypoxia-inducible factor 1α (HIF-1α), thereby suppressing VEGF-A mediated angiogenesis[9, 70]. Another study showed EC-p53 enhanced capillary rarefaction, tissue fibrosis, and inflammation in the heart during LV pressure overload[10, 66]. Impairment of cardiac angiogenesis by p53 was also observed in other models such as angiotensin II (Ang II)-infused mice[71]. In diabetic rodents, pharmacological inhibition of p53 led to stabilization of HIF-1α, enhanced capillarization, and

ameliorated cardiac dysfunction[72]. Inhibition of p53 would contribute to increasing the capillary network in the heart and suppress pathogenesis in the failing heart.

2-2. p21

Protein p21, coded by cyclin-dependent kinase inhibitor 1a (*Cdkn1a*), is a cell-cycle regulator and locates downstream of p53 [73]. Activation of p21 triggers cellular senescence and apoptosis[74]. C57BL/6 mice aged 24 months had a higher level of p21 in cardiac tissues compared with 4-month-old mice[69]. Controversies exist regarding the role of p21 in capillarization. Systemic depletion of p21 diminished capillary density together with reduced cardiac systolic function[75]. Another report showed radiation increased breast cancer type 1 susceptibility protein homolog (BRCA1) and p21 level in heart[76]. EC-specific *Brca1* depletion resulted in reduced p21 and capillary density, indicating the protective effect of EC-BRCA1/p21 mediated DNA repair response in heart[76]. In the dental pulp, the knockdown of p53 or p21 has led to an increase in capillary density, and studies showed p53/p21 signaling reduced Bmi-1-mediated vasculogenic differentiation of dental pulp stem cells[77]. It was also reported that p21 negatively regulated the proliferation of old endothelial progenitor cells (EPCs) *in vitro* and *in vivo* settings[78]. Another study showed that starvation-induced NADPH oxidase (Nox2) upregulation and ROS production promoted EC cycle arrest and apoptosis via the p21 pathway[79]. The role of p21 in capillarization may be context dependent and different among organs, cells, and disorders.

2-3. p16

p16-INK4a is a cell-cycle regulator encoded by the *Cdkn2a* gene. p16 is widely used as a marker for senescent cells and has been reported to increase with age in cardiomyocytes[80], ECs[81], and cardiac progenitor cells[82]. The level of p16 in the heart increased in aged mice[69]. In humans, the protein levels of p53, p21, and p16 increased in ECs from older individuals (~60 years old), and those were shown to reduce in older exercising adults (~57 years old)[81]. Protein canopy2 (CNPY2) stimulates cell proliferation, and a previous report showed CNPY2-mediated p16 inhibition enhanced capillarization and tissue repair in the heart after myocardial infarction[83]. Another study showed p16 deletion enhanced capillarization and promoted kidney regeneration after kidney ischemia–reperfusion injury[84]. Further studies are needed to show the role of p16 in cardiac capillarization.

2-4. Sirtuins

Sirtuins (Sirt1-7) are a family of nicotinamide adenine dinucleotide (NAD) dependent histone deacetylases. NAD reduces with age and NAD administration is considered as a promising target to reverse aging phenotype[85]. It is well known that sirtuins get activated by NAD, and sirtuins are known to have roles in aging and capillarization[86]. SIRT1 inhibition induced premature cell senescence in human umbilical vein ECs (HUVECs), whereas overexpression of this molecule prevented them from premature senescence-like phenotype[87]. The level of NAD was reported to decline with age in skeletal muscle ECs, and this had a causal role in reducing SIRT1 and capillary rarefaction in this organ[88]. EC-specific Sirt1 knockout mice exhibited capillary rarefaction in the heart and developed diastolic dysfunction[89]. EC-specific Sirt3 depletion also resulted in diminished capillarization in the heart together with diastolic dysfunction[90]. Administration of Ang II-induced fibrosis diminished vascular density in the heart, and this was further enhanced with depletion of Sirt3 [91]. The role of other sirtuins in cardiac capillarization continues to be an interesting topic to be explored.

2-5. AKT

The serine/threonine protein kinase AKT-mediated signaling has critical roles in the regulation of cardiac hypertrophy, contractile function, and coronary angiogenesis[8, 92]. Mice aged 24–26 months showed high AKT levels in the heart compared with mice aged 3–4 months[93]. In cardiac tissues, the role of AKT is bidirectional, and a summary of previous papers indicates that the level of AKT signaling should be kept under a certain physiological level for maintaining homeostasis of the heart[94]. Short-term AKT activation induced physiological hypertrophy with maintained vascular density, suggesting that coronary angiogenesis is enhanced to keep pace with the growth of the myocardium[95]. By contrast, prolonged AKT activation introduced pathological cardiac hypertrophy, characterized by enhanced fibrosis and capillary rarefaction[95]. LV pressure overload upregulated AKT signaling, and heterozygous depletion of AKT ameliorated cardiac dysfunction by inhibiting excessive hypertrophic response in the heart together with an increase in vascular density[8]. In ECs, AKT promotes cell proliferation, survival, permeability, release of nitric oxide (NO), and cell migration[96] and is essential for VEGF-mediated angiogenesis[97]. Gain of AKT signaling in ECs contributed to an increase in endothelial nitric oxide synthase (eNOS)/NO

pathway, and this contributed to the suppression of EC apoptosis together with enhanced capillarization in LV[23]. By contrast, EC-AKT is also reported to enhance senescent phenotype. Senescent primary cultured human ECs exhibited a high level of phospho-AKT, and overexpression of this molecule suppressed population doubling and increased p53/p21 levels in these cells[98]. These studies indicate AKT activation must be cell-targeted and within a certain physiological level.

2-6. Inflammation

Levels of circulating pro-inflammatory molecules, such as interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein increase with aging[99-103] or in patients with HFpEF[104]. Endothelial activating markers including ICAM-1 and E-selectin became higher in myocardial samples of a ZSF-1 HFpEF rat model or patients with HFpEF[105]. Murine LV pressure overload model showed that ICAM-1 has a critical role in the infiltration of inflammatory macrophages into cardiac tissues[66]. On a cellular level, TNF-α and IL-6 activate NADPH oxidase (NOX) in the coronary arteriolar wall, and this enhances the production of superoxide anion, decreases NO availability, and inhibits vasodilation[106].

Studies analyzing type 1 diabetic mice models concluded that hyperglycemia increased TNF-α in cardiac tissues, and this promoted capillary rarefaction by enhancing cell death in ECs[107]. IL-10 was reported to suppress inflammatory response and promote capillary density through the activation of STAT3 [108] or via the upregulation of heme clearance pathway[109] in a murine myocardial infarction model. Controversies remain for the role of inflammation in cardiac capillarization. In an Ang II high salt-induced hypertension model, IL-6 knockout mice had better cardiac ejection fraction, reduced macrophage infiltration, and fibrosis in LV, but capillary density was comparable between the genotypes[37]. IL-6 knockout mice had reduced capillary density in the heart, together with fibrosis and LV dilatation[110]. In patients with myocardial infarction, circulating monocytes had a high level of semaphorin3A at day 30 after the onset of this disease[111]. Heterozygous depletion of semaphorin3A suppressed cardiac inflammation and improved cardiac function; however, no change in capillary density was observed in the knockout mice[111]. The role of inflammation in the capillary network seems complex and mediates a bidirectional or neutral effect for capillarization in the heart.

2-7. ROS

ROS has central roles in the progression of pathologies in aging or age-related disorders. ROS including superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), peroxynitrite ($OONO^-$), and the hydroxyl radical ($HO^{\bullet}$) are produced through physiological biological activities. At certain levels, they have roles as signaling mediators, however, become toxic in excess and thereby described as oxidative stress. Under physiological conditions, oxidative balance is tightly regulated by prooxidant and antioxidant systems. ROS has been shown to regulate multiple cellular functions in vessels including angiogenesis, EC apoptosis, and vascular tone[112, 113]. ROS was reported to activate VEGFR2 in ECs and promote angiogenesis[114]. Low-density lipoprotein (LDL) induced endothelial dysfunction through the activation of lectin-like-oxidized LDL receptor-1 (LOX-1) and excessive ROS, and these led to a decrease in eNOS uncoupling[115]. ROS derives from several sources including mitochondrial respiratory chain enzymes, xanthine oxidases, lipoxygenases, and NOX proteins. With aging, complex I activity in mitochondria decreased in rats, and this was associated with the capacity of this organelle to produce H2O2 [116]. NOX2 and NOX4 are predominant forms of NOXs expressed in the heart[117]. NOX2-derived ROS mediates the antiangiogenic effect, whereas NOX4-derived ROS was reported to have an angiogenic effect. In the brain, Nox2 activation was shown to enhance age-related cerebral capillary rarefaction[118]. In the hind limb ischemia model, Nox2 depletion resulted in a decrease in ROS level together with an increase in VEGF-A expression[119]. Studies using endothelial-specific Nox2 overexpression showed NOX2 activation promoted superoxide-driven cardiovascular dysfunction, macrophage recruitment, and adverse remodeling[120, 121]. By contrast, NOX4 is constitutively active at a low level and induces protective effects in the heart under chronic stress[122]. Systemic Nox4 knockout mice showed impaired angiogenesis in a hind limb ischemia model[123]. The Nox4 knockout model exhibited cardiac capillary rarefaction and LV systolic dysfunction during LV pressure overload[122]. Cardiomyocyte-specific Nox4 overexpression enhanced capillarization and improved LV systolic function[122]. Mechanistically, Nox4 was shown to increase HIF-1α/VEGF-A under hypoxic condition[122].

3. Pharmaceutical Approach for Enhancing Cardiac Capillary Network

Several types of drugs are reported to suppress pathologies in heart failure, contributing to the inhibition of hospitalization for heart failure, amelioration of symptoms, and improvement in LV

ejection fraction[124, 125]. In this chapter, we would mainly focus on cardioprotective drugs and show their effects against the capillary network in LV.

3-1. ACEi/ARB/ARNI

Activation of RAAS mediated mainly through Ang II/Ang II receptor type 1 (AT1R) signaling induces myocardial hypertrophy and fibrosis and contributes to the progression of heart failure. RAAS inhibitors reduce HFrEF-related morbidity and mortality[126-128]. Accumulation of evidence indicates suppression of RAAS leads to enhancement of capillarization in the heart. In type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rat model, an AT1R blocker candesartan ameliorated LV capillary rarefaction and improved diastolic function at 36 weeks of age[129]. Another study by de Boer *et al.* showed that microvascular density after myocardial infarction (MI) was decreased by cardiomyocyte-specific AT1R overexpression, and one of the AT1 receptor blockers losartan increased LV capillarization without an increase of VEGF[130]. Another AT1 receptor blocker irbesartan suppressed oxidative stress and EC apoptosis, ameliorated cardiac hypertrophy, and increased capillary density in Dahl salt-sensitive (DS) rats[131]. An ACE inhibitor ramipril enhanced LV capillarization in an SHR[43]. Another ACE inhibitor perindopril increased LV capillary density in DS rats[34]. Perindopril was also reported to enhance LV capillarization in obese Zucker rats[42]. The proangiogenic effect of ACE inhibition is considered partly mediated via the activation of bradykinin receptor/eNOS signaling in ECs[43, 132, 133]. Interestingly, several studies indicate the proangiogenic effect of RAAS.

Subhypertensive dose Ang II/AT1R signaling increased VEGF-A/eNOS pathway and mediated angiogenic response in ischemic hindlimb model[134]. Ang II/VEGF-A axis was also shown to induce the proliferation of EPCs[135]. Enarapril or candersartan both inhibited VEGF-A-induced myocardial angiogenesis and capillarization in the normal heart of C57BL/6 male mice, but functional studies were not conducted to test LV function[136]. LV capillary density was increased at 20 weeks of age in the OLETF diabetic rat model, and candesartan reduced vascular expression of VEGF-A, HIF-1α, and capillary density in these rats[137]. Exercise increased capillarization in the heart, but Ang II infusion showed a trend to reduce this in LV of Sprague–Dawley rats; however, this did not show statistical significance[138]. The roles of RAAS in angiogenic response seems context dependent and are different among models, organs, and time frame of analyses.

Neprilysin (neutral endopeptidase, NEP) cleaves and inactivates peptide hormones such as glucagon, bradykinin, angiotensin, endothelin-1, and natriuretic peptides[139]. PARADIGM-HF trial-tested angiotensin receptor–neprilysin inhibitor (ARNI) against enalapril in HFrEF patients, and the ARNI group showed a significant decrease in the risk of death and heart failure hospitalization[140]. In an experimental MI rat model, ARNI increased transcript *Vegfa* in LV, but this did not associate with an increase in capillary density[141]. Suematsu *et al.* showed ARNI ameliorated cardiac dysfunction together with an increase in transcript *Vegfa* in hearts in ApoE KO mice, but capillary density was not characterized in this paper[142]. Further studies are needed to show the role of NEP inhibitors in cardiac capillarization.

3-2. Beta-Blockers and Ivabradine

β-blockers continue to be the first-line therapy for heart failure[124, 125]. Studies indicate the proangiogenic effect of this type of drug in cardiac tissues. A negative correlation between heart rate (HR) and capillary density indicates bradycardia promotes angiogenic response[143-145]. Bradycardia stimulated VEGF production and facilitated angiogenesis in heart[146]. Reduced HR prolongs diastolic filling, and this triggers an increase in the stretch-associated release of VEGF[147]. Cardiac capillary density was reduced in the renal failure model generated in Sprague–Dawley rats, and metoprolol was shown to ameliorate this[148]. Either ivabradine or metoprolol enhanced LV capillary density in MI rats[145]. Alinidine, another bradycardiac drug, increased VEGF and capillary density in hearts subjected to myocardial infarction[144]. Ivabradine inhibits pacemaker current and reduces HR. Ivabradine was reported to reduce hospitalization of heart failure[149]. In the MI rats, ivabradine administration enhanced capillary density in LV and improved cardiac systolic function[145, 150].

3-3. SGLT2 Inhibitor

Sodium glucose cotransporter 2 inhibitors (SGLT2i) were initially generated as a therapy for type 2 diabetes, and this class of drugs was shown to significantly reduce the risk of hospitalization for heart failure[11, 12, 151, 152]. SGLT2i also reduced the risk of cardiovascular death or hospitalizations for heart failure in patients without diabetes[153, 154]. Biological effects of SGLT2i are considered multifactorial, and these include a decrease in ROS and inflammation, increase in ketone production, and inhibition of sodium–hydrogen exchange[155]. Several reports focused on the role of SGLT2i in cardiac capillaries. SGLT2 inhibition with empagliflozin (EMPA)

improved coronary microvascular function in prediabetic ob/ob mice[156]. Another study revealed that EMPA inhibited the mitochondrial fission via AMPK signaling and rescued cardiac microvascular EC injury via ROS inhibition[157]. Recently, Nakao *et al.* demonstrated that EMPA maintained LV capillarization and improved cardiac function in a murine LV pressure overload model[23]. EMPA administration reduced ROS-mediated apoptosis in ECs, and together with the activation of AKT/eNOS/NO pathway, EMPA enhanced capillarization in LV under pressure overload[23]. Whether SGLT2i also enhances capillary density in the human heart continues to be an interesting topic to be explored.

3-4. MRA

The Randomized Aldactone Evaluation Study demonstrated inhibition of mineralocorticoid receptor (MR) led to a 30% decrease in mortality rates in patients with heart failure after myocardial infarction[158]. Aldosterone is the principal ligand for MR and is known to associate with increased risk for cardiovascular events[159]. MR activation induces cardiac hypertrophy, inflammation, and fibrosis, and these were blocked via MR inhibition[160-163]. In a murine MI model, cardiomyocyte-specific MR depletion increased LV capillary density, reduced interstitial fibrosis and inflammation, and improved systolic function[161]. Deoxycorticosterone acetate administration induced hypertension and LV capillary rarefaction in WT mice, and this was ameliorated in EC-specific MR KO mice[162]. Another report showed EC-specific MR depletion improved systolic function, but capillary density was comparable between the genotype[164]. Smooth muscle cell-specific MR knockout mice showed increased capillary density, diminished fibrosis in the heart, and improved cardiac function during LV pressure overload[163]. Obesity and aging are associated with a dysregulation in MR signaling[165, 166]. Together with evidence from preclinical studies, MR signaling continues to be an important therapeutic target for heart failure.

3-5. DPP4 Inhibitor

Dipeptidyl peptidase-4 (DPP4) inhibitor is a class of oral antidiabetic agents. The extracellular catalytic domain of DPP4 is responsible for the enzymatic degradation of several peptides, including incretins GLP-1 and GIP, neuropeptides, chemokines, and endogenous growth factors[167]. Studies indicated DPP4 inhibitors cannot suppress pathologies of heart failure in humans[168-170]; however, preclinical studies suggest this type of drug enhances cardiac capillary density. In a dietary obese mice model, Suda *et al.* showed linagliptin ameliorated capillary rarefaction in the heart through the activation of the FGF-2/EGR-1 pathway[27]. The membrane-bound form of DPP4 was activated in diabetic rats, and this led to a decrease of myocardial stromal cell-derived factor-1α (SDF-1α) concentrations and angiogenesis in hearts[171]. In these rats, DPP4 suppression reversed cardiac fibrosis, diastolic dysfunction, and increased capillary density together with a restoration of SDF-1α[171]. Another study also demonstrated that DPP4 inhibitor (PKF275-055) suppressed the biological inactivation of SDF-1, increased mobilization of EPCs, and regenerated cardiac capillaries in ob/ob mice[172]. Studies analyzing rodents showed DPP4 inhibitors as promising therapies to combat heart failure; however, clinical trials could not show the cardioprotective effect of these drugs[168-170]. This may be due to differences among humans and rodents, relative differences in the dose of DPP4 inhibitors showing beneficial biological effects among species, or other unknown factors.

3-6. Senolytics

Senescent cells increase in aged tissues, and this is considered to associate with the progression of age-related cardiovascular-metabolic diseases[22, 62, 63, 173, 174]. Senescence-associated molecules such as p53, p21, and p16 increase in heart with aging, LV pressure overload, myocardial ischemia, and diabetes[9, 21, 66, 69, 72]. Recent studies testing genetic, as well as pharmacological models, showed elimination of senescent cells, described as "Senolysis," reverses aging phenotype[80, 175-177]. This approach provides an attractive therapeutic option for age-related CVDs. Senolytics are agents that can selectively target prosurvival proteins in senescent cells and induce cell death. Dasatinib, Quercetin, and Navitoclax are three major senolytics widely studied *in vivo* and *in vitro*[178]. Besides the genetic senolytic model, these reagents have been shown to improve vascular function[179], reduce detrimental features of cardiac aging[80] and restore the regenerative capacity of hearts in murine myocardial ischemia, Ang II-infused cardiac hypertrophy, diabetic, or aged model[80, 82, 180, 181]. Dookun *et al.* showed that clearance of senescent cells with Navitoclax improved cardiac recovery including enhanced angiogenesis after cardiac ischemia–reperfusion injury[177]. Yu *et al.* showed Quercetin suppressed cardiac vascular rarefaction and improved systolic dysfunction in high-fat diet-fed mice[182]. The therapeutic potential of senolytics is now tested in humans. Justice *et al.* showed administration of Dasatinib + Quercetin in patients with idiopathic pulmonary fibrosis can improve physical function[183].

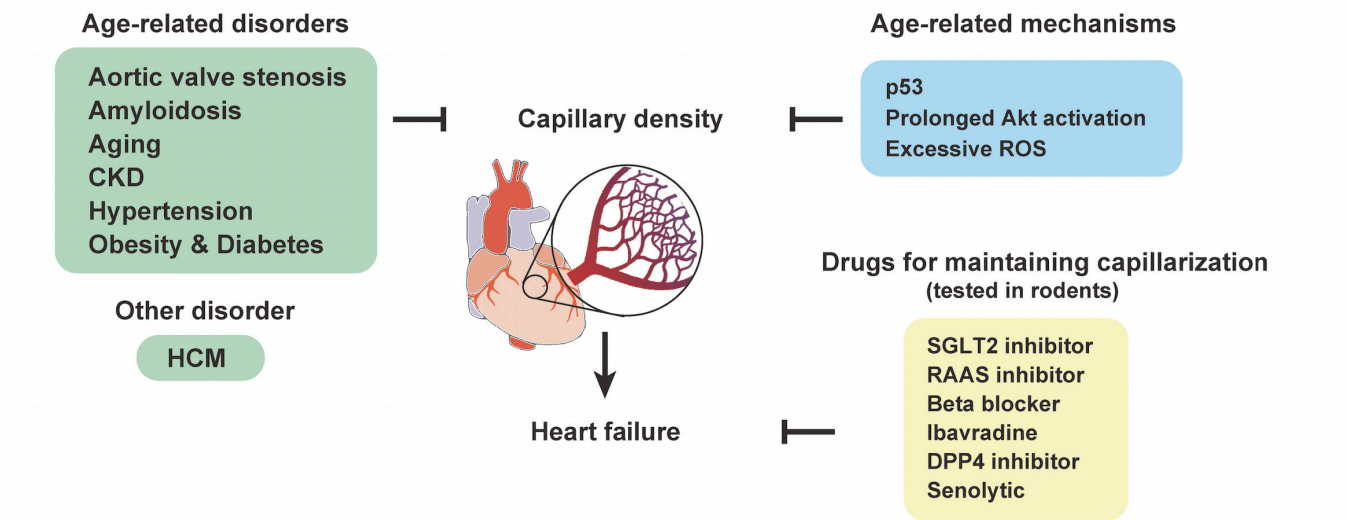

Fig. 1. Disorders and mechanisms for reduced capillary density in the heart

Age-related disorders including aortic valve stenosis, amyloidosis, aging, chronic kidney disease (CKD), hypertension, obesity, and diabetes diminish capillary density in the heart. Hypertrophic cardiomyopathy (HCM) is also associated with capillary rarefaction. Age-related mechanisms such as p53, prolonged AKT activation, and excessive ROS reduce capillary density. Drugs tested in rodents to enhance capillarization are also demonstrated. Class and name of drugs, tested species and models, analyzed organs, and effects are demonstrated.

Hickson *et al.* tested this combination of drugs in patients with diabetic kidney disease and found senescent cells reduced in subcutaneous adipose tissue[184]. Recently, Suda *et al.* showed senolytic vaccination therapy reversed aging phenotype in obesity or atherosclerotic model[185]. The senolytic approach would open new avenues for therapeutic options for age-related cardiovascular disorders.

4. Discussion

In this review article, we outlined capillarization in the heart under several pathogenic conditions. Capillary rarefaction develops with aging or age-related disorders, and studies indicate that this accelerates the functional decrease in cardiac tissues letting this organ prone to develop heart failure. Age-related mechanisms including p53-mediated cell senescence, sirtuins, AKT signaling, RAAS, chronic inflammation, and excessive ROS had critical roles for maintenance or suppression of capillarization in the heart. Drugs used as first-line therapies for patients with heart failure were reported to contribute to the maintenance of capillarization in the heart. Accumulating evidence indicates β-blockers, RAAS inhibitors, and SGLT2 inhibitors improve the prognosis of heart failure, and studies with rodents show these drugs also contribute to enhancing capillarization in the heart. Recently, approaches with senolysis are highlighted in aging and cardiovascular research. Studies showed specific depletion of senescent cells ameliorated cardiac dysfunction in heart failure models, and interestingly, capillary density was also shown to improve with senolysis. Senolytics are now tested in humans, and results have shown a decrease in senescent cells and improvement in clinical symptoms. Many of the senolytics are classified as cancer drugs, and potential side effects are issues to be considered. Exploration of less toxic senolytics continues to be one of the interesting and important research topics. With chronological aging, capillary rarefaction develops in systemic organs, and this is considered to accelerate a functional decrease in these tissues. Exploration of reagents contributing to the enhancement of capillarization would help us find new drugs for age-related disorders including heart failure **(Fig. 1 and Table 1)**.

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Table 1. Drugs, tested models, and their effects in cardiac capillarization

Class of drugs	Name of drugs	Tested species	Models	Organ	Effect	Ref
ACEi/ARB/ ARNI	Losartan	Rat	Myocardial infarction	Heart	Increased LV capillary density	de Boer *et al.*, 2003
	Irbesartan	Rat	Hypertension	Heart	Increased LV capillary density	Nako *et al.*, 2012
	Candesartan	Rat	Type 2 diabetes	Heart	Increased LV capillary density at 36 weeks of age	Hayashi *et al.*, 2003
	Candesartan	Rat	Type 2 diabetes	Heart	Reduced capillary density at 20 weeks of age	Jesmin *et al.*, 2002
	Candersartan or Enarapril	Mouse	Wild type mice injected with Vegfa plasmid	Heart	Reduced capillary density	Siddiqui *et al.*, 2005
	Ramipril	Rat	Hypertension	Heart	Increased LV capillary density	Zhu *et al.*, 1997
	Perindopril	Rat	Hypertension	Heart	Increased LV capillary density	Yazawa *et al.*, 2011
	Perindopril	Rat	Obesity	Heart	Increased LV capillary density	Toblli *et al.*, 2004
	Sacubitril/ valsartan	Rat	Myocardial infarction	Heart	Increased transcript Vegfa, but no change in capillary density	Pfau *et al.*, 2019
	Sacubitril/ valsartan	Mouse	Atherosclerosis	Heart	Increased transcript Vegfa, but capillary density was not characterized	Suematsu *et al.*, 2021
Beta blocker	Metoprolol	Rat	Myocardial infarction	Heart	Increased LV capillary density	Ulu *et al.*, 2009
	Metoprolol	Rat	Renal failure	Heart	Increased LV capillary density	Amann *et al.*, 2006)
Bradycardiac drug	Alinidine	Rat	Myocardial infarction	Heart	Increased LV capillary density	Lei *et al.*, 2004
	Ivabradine	Rat	Myocardial infarction	Heart	Increased LV capillary density	Ulu *et al.*, 2009
SGLT2 inhibitor	Empagliflozin	Mouse	LV pressure overload	Heart	Increased LV capillary density	Nakao *et al.*, 2021
DPP4 inhibitor	Linagliptin	Mouse	Obesity	Heart	Increased LV capillary density	Suda *et al.*, 2017
Senolytics	Navitoclax	Mouse	Myocardial infarction	Heart	Increased LV capillary density	Dookun *et al.*, 2020b
	Quercetin	Mouse	Obesity	Heart	Increased LV capillary density	Yu *et al.*, 2021

Class and name of drugs, tested species and models, analyzed organs, and effects are demonstrated.

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