The vasculature: a therapeutic target in heart failure?

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Abstract

It is well established that the vasculature plays a crucial role in maintaining oxygen and nutrients supply to the heart. Increasing evidence further suggests that the microcirculation has additional roles in supporting a healthy microenvironment. Heart failure is well known to be associated with changes and functional impairment of the microvasculature. The specific ablation of protective signals in endothelial cells in experimental models is sufficient to induce heart failure. Therefore, restoring a healthy endothelium and microcirculation may be a valuable therapeutic strategy to treat heart failure. This review article will summarize the current understanding of the vascular contribution to heart failure with reduced or preserved ejection fraction. Novel therapeutic approaches including next generation pro-angiogenic therapies and non-coding RNA therapeutics, as well as the targeting of metabolites or metabolic signalling, vascular inflammation and senescence will be discussed.

Keywords

Non-coding RNAs • MicroRNAs • Angiogenesis • Microcirculation

1. Introduction

Every organ in the human body has its own vasculature specialized for the specific needs of each organ.1,2 Historically the function of the vasculature has been described to be the transport of oxygen and nutrients to all tissues and to carry away the products of cellular metabolism in order to maintain cellular homeostasis.3 In recent years, it has been recognized that the endothelium actively controls its microenvironment regulating different processes like organ development, homeostasis, and tissue regeneration.4 The vasculature in the heart, the coronary vasculature, receives its name from the Latin word corona, meaning crown, because of the resemblance of its structure to a radiant crown. The heart is a highly vascularized organ, every cardiomyocyte is located in close proximity to a capillary5 and endothelial and associated mural cells are the most abundant cell types in the heart6,7 (Figure 1).

The question whether the endothelium might be a potential therapeutic target in cardiac disease is an old one. The Greek philosopher Aristotle already proposed in classic times that blood vessels are the frame around which the rest of the organism is built.8 Heart failure (HF) patients are characterized by systemic vasoconstriction and reduced peripheral perfusion and the therapeutic benefits from intervening with vascular tone are known long ago.9 Treatments based on the release of nitric oxide (NO) have been used in cardiac disease for a long time,10 in particular, nitroglycerine has been in clinical use for over 100 years.11 Recently, guanylate cyclase activators trials support that activation of the NO downstream signalling is of therapeutic benefit in HF.12 Another vasculature-oriented treatment are angiotensin-converting enzyme inhibitors, which inhibit the breakdown of bradykinin, which can then stimulate NO release.13

In this review, we will discuss how the vasculature interacts with different types of HF cause by myocardial infarction, maladaptive hypertrophy and the age-associated HF with preserved ejection fraction (HFpEF) (Figure 1). We will primarily focus on small vessels, and refer the readers to other review articles regarding coronary artery disease, which obviously is the cause of ischaemic HF. Based on the insights into the adaptive and maladaptive signals, we will provide a summary of possible therapeutic strategies to target the vasculature in cardiovascular disease.

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2. The vasculature of the heart—a brief introduction

Vascular development is organ specific and in the heart, historically, the epicardium was considered the source of cardiac capillaries. Epicardial cells were thought to perform epithelial to mesenchymal transition, invade the developing myocardium and give rise to the coronary capillaries. More recent studies performed using lineage tracing have now shown that only a subset of the proepicardium gives rise to a small proportion of the endothelial cells of the capillaries. The sinus venosus provides endothelial progenitors to form the cardiac capillaries in the lateral free walls of the ventricles and the septum during development, while endocardial cells are converted to capillary endothelial cells in the inner ventricular wall postnatally in a myocardial secreted VEGF-dependent manner. All these findings show that there are—at least—three different cellular sources of coronary endothelium. Interestingly, capillary density in the healthy heart is regionally different;
the number of epimyocardial capillaries is >21% as compared to endo-
cardial capillary density.25

Blood vessels are formed by two biological processes.26 vasculo-
genesis is the development of de novo blood vessels by the differentiation of
angioblasts into endothelial cells.27,28 while angiogenesis is the growth of
new blood vessels from pre-existing vessels via sprouting.29–31 There are
a variety of signals implicated in correct vasculogenesis. FGF-receptor ty-
orosine kinases, and in particular FGF4, are required for the induction of
the mesoderm.32,33 Gene knock out experiments demonstrated a piv-
otal role of VEGF, VEGFR2 (KDR), and VEGFR1 (FLT3) in embryonic vas-
culogenesis.34–37 Furthermore, cell adhesion molecules, such as VE-
Cadherin, PECAM-1, and Fibronectin, also play important roles during
de novo blood vessel formation.28,38

In order to perform sprouting angiogenesis, endothelial cells need to
degrade the basal membrane, a process mediated by different prote-
inases. Proteinases also release matrix-bound angiogenic growth factors
FGF, VEGF, and TGF-β.39 An integrated feedback loop between the
VEGF and Notch signalling pathways regulates the endothelial cell deter-
mination between ‘tip’, the migratory endothelial cell that guides the
sprout, and ‘stalk’, the proliferative endothelial cells that supports vascu-
lar growth (for details see References40,41). Ischaemia-induced neovascu-
larization of the heart additionally involves clonally expanded VE-
cadherin-expressing endothelial cells.42,43 Myocardial-secreted FGF and
VEGF regulate coronary endothelial cell fate and vascular assembly.44,45
Furthermore, retinoic acid and VEGF are required for the stabilization of
the primitive network during development.46 Disruption of the pro-
cesses described above result in coronary congenital defects that may
persist after birth and can affect cardiovascular health (for review see
Reference47). The estimated prevalence of these anomalies ranges from
0.21% to 5.79% depending on the diagnostic tools48 and they can be as-
associated with shunting, ischaemia, and sudden death.49,50

There is growing evidence of a number of angiocrine signals that have
effects on cardiac remodelling.51 Examples are NRG-1 that binds and
activates Erbb receptors in cardiomyocytes promoting cardiomyocyte
proliferation and growth.52,53 or Apelin and its receptor APJ regulating
the myocardial response to infarction and cardiomyocyte hypertro-
phy.54,55 Endothelin-1, that is also produced by endothelial cells in the
heart, regulates cardiomyocyte contractility and can induce cardiac
remodelling.56 Endothelial cells and cardiomyocytes also have direct
cell–cell contact. Connexins 37, 40, and 43 are expressed both in endo-
thelial cells and cardiomyocytes and there is evidence that they are im-
portant for the spatial organization and survival of the cardiac muscle
cells.57 Although it has been suggested that they might mediate commun-
ication between endothelial cells and cardiomyocytes this needs to be
further studied (reviewed in References58,59).

Understanding the molecular mechanisms of cardiac vascular mor-
phogenesis and the interaction between endothelial cells and cardio-
myocytes is crucial to develop therapeutic strategies.48

3. The vasculature in heart failure

with reduced ejection fraction

Many studies demonstrated that heart failure with reduced ejection frac-
tion (HFrEF) is associated with impaired coronary flow reserve and
microvascular perfusion.59 Reduced perfusion and microvascular dys-
function can be primarily caused by narrowing or occlusion of the
 coronary arteries leading to reduced blood supply to the myocardium
(Figure 1). However, already in the 90s it was proposed that the
endothelium may also be a therapeutic target in dilative cardiomyopathy
and in patients without coronary artery disease.60 Risk factors, such as
metabolic syndromes and diabetes, as well as hypertension, can affect
the coronary microcirculation.59 A pig study recently confirmed a direct
impact of diabetes on cardiac microvascular dysfunction and capillary
rarefaction.60 Maladaptive hypertrophy as it occurs after pressure over-
load also is associated with reduced vessel density.61,62 Thus, microvas-
cular dysfunction can occur in the absence of obstructive epicardial
coronary artery disease in the context of cardiomyopathies or risk
factors.59,63

Impaired perfusion was detected in HF patients of different aetiologies
by various techniques including the measurement of coronary flow re-
serve, magnetic resonance imaging, positron emission tomography,
single-photon emission computed tomography, or contrast echo in
humans and by assessing capillary density by histology. However, surpris-
ingly little is known regarding the structural and molecular changes that
occur in the microcirculation in HF. In experimental models, acute myo-
cardial infarction (with or without reperfusion) or aortic banding in-
sues an initial increase in capillary density, which is mainly mediated by
hypoxia-induced augmentation of angiocrine signals (e.g. VEGF) in car-
diomyocytes that induces a pro-angiogenic response.64 However, at later
time points, capillary density is reduced leading to a mismatch of oxygen
supply to the hypertrophic myocardium.61,62 (Figure 1). Interestingly,
a detailed histological study of vessel morphology in rats after aortic band-
ing and ischaemia/reperfusion followed with aortic debanding describes
that the coronary vasculature volume increased in this HF model.64 This
study also reports striking effects on capillary morphology: whereas in
control hearts, capillaries were uniformly arranged with a linear orienta-
tion and consistent shape, they exhibit irregular arrangements, significant
augmentation of diameter and a curvy, distorted, inconsistent shape in
HF. They found extremely narrow capillary branches (<3 μm) that ap-
ppear to bridge between larger capillaries and contribute to the increased
microvascular density in this study. Since these small capillaries likely do
not allow erythrocytes to pass through, it is unlikely they contribute to
cardiac tissue oxygenation. The results of this study suggest that a careful
assessment of vascular structures is very important and that just counting
of capillary density or area may not necessarily correlate with perfusion
or the provision of appropriate microenvironmental factors.

Recent studies additionally show that the vasculature participates not
only in the regulation of local blood perfusion by also controls the me-
tabolic exchange between the blood and the tissues.65 The metabolic re-
quirements of the heart in order to fulfill its pumping function are immense.66
In a healthy heart, most of the energetic requirements of the heart are
fulfilled by fatty acid metabolism but the heart also can use other sources
for generation of ATP.67 During HF, but also during ageing, cardiomyo-
cytes have been described to present a metabolic shift, from fatty acids
to glucose.68 Altered nutrient delivery from endothelial cells to the car-
diomyocytes might play a role as one potential cause for these metabolic
changes in HF. Thus, specific inhibition of endothelial Notch signalling
pathway impairs fatty acid delivery to the cardiomyocytes and induces
metabolic and vascular remodelling in the heart.69 Treatment of mice
with Delta-like 4 neutralizing antibodies impaired fractional shortening
and ejection fraction by reducing the expression of CD36 and FABP4
and the increased expression of ANGPTL4, an inhibitor of lipoprotein li-
pase.70 Furthermore, vascular Eph/ephrin signalling controls the function
of caveolae and lipid transport. Loss of functions analysis revealed that
caveolae are required for CD36 traffic to the membrane and fatty acid
uptake by endothelial cells.71 CD36 is required in the endothelial cells
for the uptake of circulating fatty acids into muscle tissue.72 Failure to do
so results in dilated cardiomyopathy-like defects: reduced ejection fraction and increased diastolic and systolic volumes. These studies situate the vasculature in a central position regulating cardiac metabolism and, thus, protecting it against HF. However, given that the heart is considered as a ‘metabolic omnivore’ and can use multiple sources to produce ATP, further studies are essential to provide more insights whether endothelial nutrient transport can directly influence cardiomyocyte metabolism and how this may contribute to cardiomyocyte failure.

The cause of endothelial microcirculatory dysfunction in HFrEF is diverse. Impaired capillary growth in the infarct and border zone even after appropriate reperfusion may be one primary cause particularly in aged and diabetic patients. The underlying coronary artery disease, however, is also associated with induction of reactive oxygen species (ROS), reduces NO bioavailability and inflammatory activation in the microvasculature. Microvascular rarefaction may occur under conditions of continuous stress exposed by risk factors (such as diabetes) or the noxious environment of the scar tissue. The shedding of the vascular endothelial glycocalyx, which is the fragile inner layer of the endothelium composed by a network of different glycosaminoglycans and proteoglycans via activation of matrix metalloproteases, may further contribute to the impairment of endothelial function.

The occurrence of microcirculatory dysfunction may not necessarily be causally related to the development of HF and it may represent a consequence or an epiphenomenon: cardiomyocyte death and dysfunction may lead to fibrosis and impairment of cardiomyocyte–endothelium communication pathways, which subsequently may induce endothelial cell dysfunction. In this situation, simply reverting endothelial dysfunction to restore oxygen supply may not be sufficient to rescue the dysfunctional cardiomyocytes. Thus, an approach to target the cell intrinsic cardiomyocyte dysfunction and a restoration of the overall metabolic milieu and paracrine environment may be required to heal the failing heart. However, microvascular dysfunction may contribute to a vicious cycle by further promoting cardiac inflammation and limiting local oxygen or nutrient supply. This may further deteriorate cardiac tissue homeostasis, and as such treating the cardiac microvasculature may be, independent of it being a cause or a consequence of the cardiac disease, a valuable therapeutic approach to reduce progression of HF. In this sense, recovering microvasculature function might be of especial interest for patients that present a stunned or hibernating myocardium.

### 4. The vasculature in HFrEF

HFrEF is becoming the predominant form of HF in ageing societies. Pre-clinical and clinical evidence support an important link between coronary microcirculatory dysfunction and HFrEF. Clinically, several studies showed a high prevalence of impaired coronary microvascular dysfunction in patients with HFrEF. Specifically, the PROMIS-HFrEF study showed an impaired coronary flow in HFrEF patients. Histological analysis of autopsies confirmed a significant reduction of capillary density in subepicardial, midmyocardial, subendocardial, and papillary muscle of patients with HFrEF.

A recent experimental study further showed that cardiac microvascular endothelial cells regulate the relaxation profile of cardiomyocytes. Experimentally, various studies suggest that impaired endothelial function induced by reduced endothelial NO bioavailability plays a causal role in HFrEF. Reduced NO availability results in reduced PKG activity in cardiomyocytes and contributes to the development of cardiac hypertrophy. The microvasculature in the HFrEF myocardium shows an increased expression of adhesion molecules, migration of activated leukocytes and elevated levels of active oxygen species. Interestingly, the protective effects of the cardiac microvasculature on the relaxation profile of cardiomyocytes is lost when endothelial cells are exposed to pro-inflammatory cytokines, supporting a critical role of vascular inflammation in the pathogenesis of HFrEF. Therefore, inflammation and endothelial dysfunction with impaired NO–cGMP signalling axis cause a reduction of the activity of the down-stream kinases PKG and PKA. These alterations lead to an excess of diastolic Ca2+ and sensitivity to it by troponin C and hypophosphorylation of titin. This leads to myocardial delayed relaxation and increased stiffness. The final consequence of a deficient NO–cGMP signalling pathway is a concentrically remodelled left ventricle with diastolic dysfunction. A crucial and causal role of lack of NO in the pathogenesis of HFrEF is supported by Shiattarella et al, who demonstrated that inhibition of constitutive NOS using N(omega)-nitro-L-arginine methyl ester in combination with high fat diet induces many of the clinical features of HFrEF in mice. However, attempts to augment NO by either increasing its synthesis or bioavailability by interfering with ROS had have not yet materialized in clinically effective therapies.

### 5. Therapeutic strategies

Although ample evidence supports a critical role of the vasculature and particularly the endothelium in controlling cardiac function, therapeutic approaches are so far sparse. The field had been suffering from failures of pro-angiogenic gene therapies and limited clinical success of cell therapeutic approaches. However, increasing understanding of the processes of regulating vessel growth and new insights regarding ‘angiocrine’ signals that mediate protective and pro-regenerative functions of endothelial cells open new avenues for therapeutic approaches (Figures 2 and 3).

#### 5.1 Growth factors

The potential of therapeutic angiogenesis has been discussed already in the last two decades. Several studies have been using pro-angiogenic factors with the objective to induce neo vascularization in patients with ischaemic heart disease. VEGF-A has been tested in different clinical trials using different delivery systems, adenosviruses or naked plasmid, injected intramyocardially or via catheter directly into the coronary arteries. Unfortunately, the results of these trials have been disappointing. Nonetheless, a recent study has revealed the importance of correct dosing as high mitogenic stimulation of the endothelial cells can arrest angiogenesis, thus, it might be important to reconsider those clinical trials and define the correct dosing and delivery method(s). VEGF-D induces both angiogenesis and lymph angiogenesis. It has been tested in patients with refractory angina pectoris and it increases the myocardial perfusion.

Angiogenic VEGF-B has been shown to induce the expression of genes related to myocardial contraction and metabolism protecting the muscular cells from apoptosis and ischaemic damage in mice. Despite its promising perspectives, high doses of VEGF-B can induce ventricular arrhythmias and thus, as with VEGF-A, dose and delivery method will be crucial for the development of potential clinical trials. Intracoronary adenoviral gene transfer of FGFr4 has been shown to improve cardiac perfusion in post-menopausal women although it had no effect on men. Furthermore, FGF is a mediator of the physiological repair of the glycocalyx. Enhancing this repairing signal would be a potential therapeutic approach to be explored in cardiac disease.
Where viral and plasmid gene therapies have had limited success,\textsuperscript{104} mRNA therapy with transcripts prepared using naturally occurring modifications might be more successful. Modified mRNA with N1-methylpseudouridine and further optimization by purification and capping\textsuperscript{105} enhances translation\textsuperscript{106} and has improved cardiac delivery.\textsuperscript{107} A single injection of modified VEGF-A mRNA in the cardiac apex of mice after myocardial infarction increased myocardial capillary density, reduced infarct size, and significantly improved survival even after a year.\textsuperscript{108} Modified mRNA has also been tested in large animal models. Carlsson et al. have shown that human VEGF mRNA injections into the swine heart improves cardiac function when given 1 week post-myocardial infarction.\textsuperscript{109} In addition, injection of modified IGF-1 modified mRNA has been shown to reduce apoptosis in the infarct border zone.\textsuperscript{110}

Alternative to overexpression of angiogenic and endothelial-protective factors, one may envision inhibiting harmful mediators of vascular dysfunction. A prominent example is angiopoietin-2, which is induced by cardiac injury and mediates pericyte detachment, vascular leakage, increased adhesion molecular expression, and degradation of the glycosylcalyx.\textsuperscript{111} Inhibition of angiopoietin-2 by gene deletion or by blocking antibodies preserved cardiac function.\textsuperscript{111}

Finally, collateral induction may augment cardiac tissue perfusion, particularly in patients with vessel occlusions.\textsuperscript{112} Historically activated monocytes and monocyte-derived growth factors were considered as therapeutic approaches.\textsuperscript{113,114} A recent study by Das et al. have shown that collateral induction improves neonatal regeneration after myocardial infarction in a CXCL12-CXCR4 dependent manner.\textsuperscript{115} A single CXCL12 injection at the time of infarction induced collateral formation 14 days after the ischemic damage.\textsuperscript{115} Although the mechanisms of collateral formation in the adult human heart remain unclear, to induce arteriogenesis in combination with angiogenesis is a potential interesting approach to treat ischaemic HF.

\textbf{Figure 2} Schematic of the different mechanisms regulating vascular homeostasis in the heart and the molecular players involved in them. There mechanism specific for the maintenance of vascular homeostasis like the endothelial metabolism, angiogenesis and the formation of collaterals, or the signals involved in atherosclerosis and inflammation. Other mechanisms like nutrient transport or the effect on cardiomyocyte relaxation have an effect directly on cardiomyocytes.
5.2 Pro-angiogenic cell therapies

Cell therapies aiming at restoring the vasculature by delivering progenitor cells came into the focus in 1997, when Asahara and Isner described circulating cells expressing endothelial and hematopoietic markers. Meanwhile various cell populations isolated from the circulating blood or the bone marrow but also of other sources (e.g. fat tissue) have been experimentally and clinically been used in patients with cardiovascular disease (for review see References 116,117). Although promising in many experimental studies and in patients with refractory angina,118 the overall clinical success has been limited. This may be due to multiple issues including challenges in cell delivery, which is even more complicated in a chronic disease state, such as HFpEF or HFrEF, which ultimately would require repetitive treatment. Autologous cell therapy is additionally compromised by the impaired functionality of cells derived from elderly and diseased patients.119,120 The new discovery of a high incidence of mutations in hematopoietic stem cells driving clonal expansion in elderly patients with cardiovascular disease and HF (up to 20–30%) may have additional impact.121,122 Since such mutations are associated with profound alterations in inflammatory and other signatures, autologous cells of carriers with such mutations may have different (likely impaired) functions.123,124 Recent larger scale clinical trials using bone marrow-derived mononuclear cells for the treatment of acute myocardial infarction (the BAMI trial) failed to enrol sufficient patients to finally clarify the potential of bone marrow mononuclear cells.125 A next generation of vessel forming cells may include iPSC- or ESC-derived cells, for which several protocols have been developed.126 However, such strategies may be more likely to be successful in combination with tissue engineering.

Mural cells, such as pericytes, may also have a reparative potential in the heart.127 Pericytes cover the capillaries, which grant them a privileged position to control and modulate the vasculature. Transplantation of different populations of pericytes has been shown to increase cardiac function and increased vascularization in infarcted mice but also in large animal models like the swine.127,128 But all these approaches rely on transplantation of external pericytes and their capacity to adapt to the environment they are transplanted into. To understand the molecular mechanisms that govern pericyte biology in the heart, and the response to injury from the local cardiac pericytes will be crucial to develop pericyte-based therapies or to therapeutically modulate pericyte functions and phenotypes in the future.

Figure 3 Graphical scheme of the different strategies to improve microcirculatory dysfunction in the HF.
5.3 Non-coding RNAs

The advent of deep sequencing technologies led to the identification of a considerable amount of non-coding RNA transcripts, which are increasingly recognized for their functions in controlling endothelial and vascular functions. MicroRNAs have already been studied for a decade and several microRNA were shown to either protect or harm endothelial cells. In the context of vascular functions in the heart, examples included miR-92a, which can be targeted by antimiRs to augment neovascularization in mice and pigs. Moreover, miR-126 was shown to improve endothelial cell functions, promote vessel growth and prevents atherosclerotic lesion formation. Other examples include miR-21, which impairs pro-angiogenic cell functions and augments fibrosis, and members of the miR-23/24/27 cluster, which regulates angiogenesis and endothelial apoptosis in cardiac ischaemia. Some miRNAs were already further considered for pre-clinical development. AntimiR approaches against miR-92a were shown to be safe and efficient in a recent human Phase I study. AntimiRs directed against miR-155 (cobo-marsen), which might also provide an atheroprotective effect, are currently applied in patients with multiple haematological malignancies.

Other non-coding RNAs, such as circular RNAs, YRNAs, or long non-coding RNAs (lncRNAs), are currently gaining increasing attention (for review see References). Among the many angiogenesis-regulating lncRNAs, the lncRNA RNAs Meg3 may be an interesting candidate to therapeutic development. The inhibition of this age-induced lncRNA reduces endothelial senescence and improves neovascularization in the context of aging. It is also particularly highly expressed in fibroblasts and GapmeR-mediated inhibition of Meg3-reduced cardiac fibrosis and cardiac hypertrophy. The combination of vascularprotective and anti-fibrotic effects may be advantageous in the context of HFrEF.

Another well studied lncRNA is MALAT1, which is induced by hypoxia and is known to be important for vascularization. It controls vascular and cardiac inflammation. However, a therapeutic approach would require overexpression, which is particularly challenging due to the excessive length of the lncRNA. Understanding its molecular mechanism of actions, however, might lead to the identification of downstream signals, which might be easier accessible.

5.4 Extracellular vesicles

Extracellular vesicles and particularly the <100 nm small exosomes gained increasing attention for augmenting vascular repair. These vesicles come in different sizes and flavours depending on the cellular origin and the way they are released in response to physiological stimuli or cell death. Their putative therapeutic activity including the increase in angiogenesis has been shown in many different mice models. It is believed that the pro-angiogenic activity might be due to the delivery of growth factors, mRNAs or non-coding RNAs. Particularly the transport of pro-angiogenic microRNAs was shown to induce vessel growth and improve cardiac function (e.g. ). A potential disadvantage of endogenously derived extracellular vesicles is the lack of specificity and the complex cargo. This may be circumventing by the engineering of recombinant vesicles that can be loaded with a defined mixture of molecules and might be linked to specific adaptor to control delivery. As a first step, targeting inflamed endothelial cells was reported by using leucocyte-inspired biodegradable particles that selectively adhere to inflamed endothelium.

5.5 Targeting vascular inflammation

Endothelial cells of patients with HF are characterized by increased expression of vascular adhesion molecules (e.g. E-selectin and intercellular adhesion molecule-1 (ICAM1)), which promotes adhesion and invasion of pro-inflammatory cells into the heart. Since recent studies suggest that invasion of bone marrow-derived inflammatory cells, particularly monocytes, replace tissue-resident reparative macrophages, and thereby contribute to chronic inflammation and HF, targeting of monocyte adhesion may be a strategy to prevent chronic inflammation. Indeed, deletion of ICAM1 reduced infiltration of immune cells including T-cells and improved cardiac function in experimental models of HF.

In addition, systemic anti-inflammatory therapies, most prominently TNFalpha inhibitors, were developed and tested in patients with HF. However, anti-TNF antibodies as well as other general anti-inflammatory strategies (e.g. pentoxifylline and methotrexate) revealed mixed results (for summary of clinical studies see Reference). Additional approaches include more specific targeting of inflammatory mediators to prevent endothelial activation. For example, targeting myeloperoxidase, which is released by neutrophils and profoundly augments vascular inflammation and dysfunction, was shown to prevent ischaemic HF. In addition, preventing the shedding or restoration of the protective endothelial glyocalyx, as it occurs during HF, may ameliorate vascular inflammation and preserve endothelial functions, such as NO release. Heparanase inhibition or sulodexide, a mixture of heparin and dermatan sulphate, which have been shown to preserve endothelial glyocalyx in different diseases, or growth factors (such as FGF or anti-angiopoietin-2) may be useful to restore endothelial cell functions also in the context of HF.

5.6 Targeting endothelial metabolism for vessel normalization

The importance of endothelial metabolism for proper endothelial cell functioning and the role of endothelial cells in nutrient transport and the metabolic control of tissues have been increasingly recognized in the last years. Interestingly, several metabolic pathways have been identified as targets to prevent pathological angiogenesis. Inhibition of carnitine palmitoyltransferase 1, a regulator of fatty acid oxidation, or glutaminase 1, which hydrolyses glutamine into ammonia and glutamate, both impaired angiogenesis. Likewise, silencing of asparagine synthetase reduces vessel sprouting in vitro. The transcription factor FOXO, which regulates various targets including the inhibition of c-myc, controls endothelial quiescence. Whether modulations of these pathways can be used to augment or normalize cardiac microvasculature is currently unknown.

Endothelial metabolism can also be a target in metabolic disease, such as hyperglycaemia. Hyperglycaemia can trigger the production of ROS that can then uncouple eNOS. Because glycolytic intermediates feed into the pentose phosphate pathway, it was proposed that increasing the pentose phosphate pathway and away from glycolysis would reduce the levels of the damaging metabolites and be protective upon hyperglycaemia.

Metabolites of arachidonic acid or other polysaturated fatty acids are known for their vascular effects. For example, coronary endothelial function is controlled by thromboxane A2, prostacyclin, and prostaglandin H2. Recent studies now identified additional lipid metabolites that control vascular functions. Hu et al. demonstrate that the inhibition of the soluble epoxide hydrolase, which reduces the formation of the diol 19,20-dihydroxydocosapentaenoic acid improves vessel integrity by reducing pericyte loss and breakdown of endothelial barrier function in
the retina.\textsuperscript{164} It might be interesting to employ these new insights in the context of the cardiac vasculature during aging or HF.

Finally, first studies in mice showed that endothelial cell specific modulation of Notch or EphB4 signalling leads to altered nutrient supply and cardiac dysfunction.\textsuperscript{70,71} Although evidence for a dysfunctional metabolic nutrient supply by endothelial cells in human HF is so far sparses, one may speculate that controlling endothelial nutrient transport capacity may be used as future therapeutic option.

5.7 Others

5.7.1 Targeting endothelial cell senescence

Senescence is a protective response from the organisms against stress that limits the proliferation of aged non-functional cells.\textsuperscript{165} However, senescent cells accumulate in fibrotic regions\textsuperscript{166} and there is increasing accumulative evidence that senescence is closely related to cardiovascular disease.\textsuperscript{167,168} Indeed, endothelial cell senescence is associated with an augmented dysfunction and vascular inflammation.\textsuperscript{169} Recent studies further demonstrated that endothelial senescence contributes to HFpEF.\textsuperscript{170}

Senolytics, which selectively target senescent cells have been shown to reverse pathological changes in post-infarction remodelling and HF.\textsuperscript{171,172} Also the genetic senolytic model, which allows the inducible elimination of p16\textsuperscript{NK4a}\textsuperscript{166} senescent cells reduced the size of fibrotic area in the heart of old mice.\textsuperscript{168} Of note, these approaches not only target endothelial cells but also cardiomyocytes and other mural cells, which may together contribute to the therapeutic benefits.

5.7.2 Vaccination

Therapeutic vaccines for non-infectious diseases are currently in development for the treatment of various disorders including cardiovascular diseases, such as hypertension or atherosclerosis. Vaccination may be an attractive therapeutic strategy because of its high specificity and potential long-term effects. Although not yet directly explored for the treatment of the vasculature in HF, one may consider to apply some of the recent strategies shown to be effective in pulmonary arterial hypertension.\textsuperscript{173,174} This study used a passive vaccination approach to inhibit ET-1 signalling by targeting a 10-amino acid peptide sequence in the second extracellular loop-domain of the G-protein coupled ETA receptor.\textsuperscript{173} The inhibitory effects were similar to a clinically used pharmacological approach.

6. Conclusions

The cardiac vasculature plays a crucial role in maintaining oxygen and nutrient supply to the cardiac tissue and supports a healthy microenvironment. HF is well known to be associated with changes and functional impairment of the microvasculature. Experimental studies with specific ablation of protective signals in endothelial cells demonstrate that the induction of microcirculatory dysfunction is sufficient to induce HF. Therefore, the restoration of a healthy endothelium and microcirculation represent a valuable therapeutic strategy. Even if a sole targeting of the microcirculation may not reverse HF, it may prevent the further progression of the disease. Examples for such potential interventions are described above and include growth factors, cells, biologicals, non-coding RNAs, and others. Since HF comes in different flavours and not all HF patients will primarily suffer from microcirculatory dysfunction, the challenge will be to define the subgroup of patients that will likely profit from such treatments. Precision imaging and/or biomarkers will be necessary for the successful clinical development of microcirculation targeting therapies.

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