

## REVIEW

# Angiogenesis and angiocrines regulating heart growth

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## Abstract

Endothelial cells (ECs) line the inner surface of all blood and lymphatic vessels throughout the body, making endothelium one of the largest tissues. In addition to its transport function, endothelium is now appreciated as a dynamic organ actively participating in angiogenesis, permeability and vascular tone regulation, as well as in the development and regeneration of tissues. The identification of endothelial-derived secreted factors, angiocrines, has revealed non-angiogenic mechanisms of endothelial cells in both physiological and pathological tissue remodeling. In the heart, ECs play a variety of important roles during cardiac development as well as in growth, homeostasis and regeneration of the adult heart. To date, several angiocrines affecting cardiomyocyte growth in response to physiological or pathological stimuli have been identified. In this review, we discuss the effects of angiogenesis and EC-mediated signaling in the regulation of cardiac hypertrophy. Identification of the molecular and metabolic signals from ECs during physiological and pathological cardiac growth could provide novel therapeutic targets to treat heart failure, as endothelium is emerging as one of the potential target organs in cardiovascular and metabolic diseases.

## Key Words

- ▶ endothelial cell
- ▶ secretome
- ▶ cardiac hypertrophy
- ▶ heart failure

## Introduction

The heart acts as a muscular pump in the hub of a closed blood vascular system to transport oxygenated blood and nutrients to various organs, and to direct the deoxygenated blood to the right ventricle to be re-oxygenated in the lungs. In addition to cardiomyocytes, the heart is composed of multiple cell types: endothelial cells, vascular smooth muscle cells, pericytes, fibroblasts, leukocytes and purkinje fibers, which are embedded in the extracellular matrix (ECM). The different cell types communicate in a multi-directional manner via paracrine factors, direct cell-cell contacts by receptor-ligand binding, adhesion molecules or by forming complexes with ECM to modulate cardiac homeostasis, growth and regeneration.

It is now recognized that the endothelium is much more than only a transport system for blood flow and that endothelial cells (ECs) play a variety of important roles during cardiac development and in growth and homeostasis of the adult heart. The adult human body is estimated to contain at least one trillion endothelial cells, which would cover a surface area of more than 3000 square meters (1). Thus, the endothelium constitutes a large organ that extends to all parts of the body forming a dynamic interface with other organs. Organ-specific ECs possess unique structural, functional and phenotypic properties via expression of specific growth factors, chemokines and adhesion molecules (2, 3). This reflects

the varying needs of different organs for example, oxygen and nutrient supply. ECs in the heart, for example, express high levels of fatty acid transporters compared to most other tissues (3).

ECs in the adult heart continuously line the coronary vasculature and cardiac chambers and are interconnected by junctional proteins forming tight junctions, cadherin junctions and gap junctions to maintain vascular integrity, homeostasis, and barrier function. ECs are metabolically active, control vasomotor tone, regulate angiogenesis, and establish a bidirectional communication between other cardiac cell types by paracrine signaling mechanisms. Indigenously, ECs are the largest cell population in the adult mouse heart by number, as the adult mouse heart was shown to be composed of endothelial cells ~42%, cardiomyocytes (CMs) ~35%, resident mesenchymal cells (fibroblasts and others) ~18% and leukocytes ~5% (4). Cardiac ECs and endocardial cells act as sensors for systemic circulating factors and for cardiac stress, and via paracrine signaling they establish an instructive niche to regulate cardiac growth and function (5).

The EC-derived signaling via the release of paracrine/juxtacrine-acting growth factors, termed as angiocrines (6), is now appreciated to be highly organ specific. Angiocrine signaling has been shown to be essential for physiological growth and homeostasis as well as regeneration in several tissues, such as liver, lungs, bone and the heart (6, 7, 8, 9, 10, 11, 12, 13). Angiocrines have an important role also in pathological conditions for example, in inflammation and cancer (14), and thus could provide novel targets to treat several diseases. So far, there is very little data on the tissue-specificity of angiocrines. It is highly likely that, like transcriptomes, also the angiocrine profiles of ECs in different tissues vary substantially. Identification of tissue-specific angiocrines could provide novel and specific targets for the treatment of various diseases such as heart failure. Endothelial cell isolation and single-cell RNA sequencing have recently provided important novel information about the heterogeneity of ECs between and within the tissues. From this rapidly expanding data, candidates for tissue-specific angiocrines can be derived. In addition, one can envision combining high-throughput metabolomic and proteomic technologies to screen for EC-derived factors in different physiological and pathological conditions. The validation and functional relevance could be then tested in cellular co-culture models and in transgenic animal models. It is likely that angiocrines work in concert with each other, and a combination of angiocrines rather than single factors would result in more effective and physiological therapies.

There are excellent recent reviews on the crosstalk between endothelial cells and parenchymal cells in various tissues (14, 15, 16, 17, 18). In this review, we focus on how ECs regulate cardiomyocyte (CM) growth and cardiac homeostasis. We summarize the known mediators of the endothelial-to-cardiomyocyte interaction and discuss how angiogenesis regulates physiological and pathological heart growth. The list of cardiac angiocrines is expected to expand in the near future, as the methods to screen and identify factors produced in small amounts improve rapidly.

## Cardiac hypertrophy and vascular growth

The heart grows extensively during development via both hyperplasia and hypertrophy of the cardiomyocytes. In adulthood, the heart size remains rather stable, but the heart still retains its capacity for adaptive growth in response to chronic increase in workload to maintain required cardiac output. Cardiac hypertrophy in adults can be caused by physiological or pathological stimuli or due to genetic disorders. Physiological hypertrophy is induced by increased workload during pregnancy or exercise training (19). It is reversible and accompanied with normal or enhanced cardiac function and metabolic activity. On the other hand, pathological hypertrophy develops in response to cardiac overload due to for example, hypertension, myocardial infarction, valve dysfunction or genetic factors. It is part of the complex pathological cardiac remodeling accompanied by impaired contractile function, fibrosis, apoptosis, and upregulation of fetal gene expression, and it contributes to heart failure related mortalities (20, 21).

In healthy growth and homeostasis of the myocardium, CM growth must be accompanied by angiogenesis to expand the coronary vasculature to ensure adequate supply of oxygen and nutrients to CMs. In physiological hypertrophy, the heart preserves its oxygen supply by matching the proportional increases in cardiomyocyte size and the extent of angiogenesis (22) mainly by producing vascular endothelial growth factors, which regulate the growth of new and larger blood vessels (23). In pathological states, cardiomyocyte hypertrophy is associated with a mismatch between oxygen supply and demand, as the increased cardiomyocyte size is not matched by a corresponding increase in the vasculature, resulting in cardiac underperfusion and hypoxia (22). It has been demonstrated that inhibiting angiogenesis during overload-induced cardiac hypertrophy results in early development of heart failure (24, 25, 26). In contrast, induction of angiogenesis delays the onset of heart failure

in pressure-overload hypertrophy (27). This suggests that the mismatch between CM growth and angiogenesis promotes the transition from adaptive to pathological hypertrophy followed by progression to heart failure.

Angiogenesis in the heart is regulated by the balance of angiogenic and anti-angiogenic factors produced by cardiomyocytes, fibroblasts, inflammatory cells and endothelial cells. The CM-secreted factors are called cardiokines, among them the vascular endothelial growth factors (VEGFs), angiopoietins (Ang1 and Ang2), hepatocyte growth factor (HGF) and fibroblast growth factors (FGFs), which bind to their receptors on the EC surface and stimulate angiogenesis (28). The VEGFs and VEGF receptors play an indispensable role during embryogenesis to induce vasculogenesis, blood- and lymphangiogenesis. Until now, five VEGF ligands VEGF/VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) have been identified, and they bind to their appropriate tyrosine kinases receptors VEGFR1/Flt1, VEGFR2/KDR/Flk1 and VEGFR3/Flt4 present on endothelial cell surface (29). The VEGF receptors are dimerized and activated upon respective ligand binding. The receptors can form homo- or heterodimeric complexes to mediate their effects by auto- or transphosphorylation of the tyrosine kinase domains, to further activate the intracellular downstream targets (30).

Insufficient vessel growth and abnormal vessel regression lead to cardiac ischemia and heart failure. To treat these patients, the potential of angiogenic gene therapy and therapeutic vascular growth has been tested in several small and large animal models as well as in clinical trials (reviewed in (31)). Even though the trials have demonstrated increased vascularity, very few have resulted in improved cardiac function with clinical significance so far in human patients, which is partly due to non-optimal gene transfer efficiency and duration, but also to the fact that one single factor may not be enough for the development of functional and durable vasculature. With better understanding of the molecular mechanisms regulating the crosstalk between different cardiac cell types combined with improved gene delivery methods, we will be able to design more efficient therapies to treat cardiovascular diseases.

## Angiogenesis-mediated cardiac hypertrophy

A key observation from the angiogenic gene therapy studies in the heart was made by Michael Simons' group in 2007, when they observed that angiogenesis could induce cardiomyocyte hypertrophy in the absence of

any other external stimuli (32). In their study, temporal expression of a secreted angiogenic growth factor PR39 in cardiomyocytes resulted in vascular growth, which was associated with an increase in cardiac mass. This demonstrated that an increase in coronary vasculature in the normal heart leads to increased cardiac mass and myocardial hypertrophy paralleled by increased cardiac performance, even in the absence of a hemodynamic stimulus. The concept of angiogenesis-driven regulation of organ size was first demonstrated in prostate in 1998 (33). The paper was accompanied with an editorial by Judah Folkman, where he proposed a general mechanism by which normal tissue mass may be regulated by the paracrine effect of microvascular endothelial cells (34).

Angiogenesis-induced cardiac hypertrophy has been now observed in several studies using either cardiomyocyte-specific transgenic or adeno-associated viral (AAV) vector-treated mice overexpressing either VEGF-B or PlGF as well as in EC-specific VEGFR1 deleted mice (13, 35, 36, 37, 38, 39, 40). Cardiac hypertrophy was also reported in mice overexpressing VEGF in the heart (41). The use of VEGF as a therapeutic tool, however, has proven to be tricky, as long-term VEGF administration promotes vascular permeability and immature angiogenesis forming non-functional vessels. The same holds true for VEGF-C, whereas overexpression of VEGF-B, PlGF or VEGF-D seems to be rather safe. In our studies, we have used AAV vectors encoding all of the five VEGF family members in pre-clinical mouse and rat models. With VEGF and VEGF-C, the titration has proven to be very difficult, and even with low dose local administration, the new vessels are leaky and immature, and the mice often have to be killed (42). In contrast, overexpression of VEGF-B, PlGF or VEGF-D result in modest increase in the vasculature in many tissues, and there is no massive angiogenesis or leakage but rather an enlargement of the existing vessels (13, 37, 42, 43). Interestingly, both VEGF and VEGF-C are essential during development (44, 45, 46), while the knockout mice for VEGF-B, PlGF and VEGF-D are healthy and viable (47, 48, 49, 50). These observations indicate safer and larger therapeutic window for these three latter growth factors. Below we discuss more closely the effects of VEGF-B and PlGF, which have been shown to induce angiogenesis-related cardiac hypertrophy.

## Vascular endothelial growth factor-B

VEGF-B was discovered in 1996, and it shares high structural similarities with VEGF and PlGF (51). VEGF-B undergoes

alternative splicing to generate two isoforms VEGF-B167 and VEGF-B186. The VEGF-B167 contains heparin-binding domain and binds to heparin sulfate proteoglycans (HSPGs) on the cell surface, whereas VEGF-B186 isoform does not contain heparin-binding domain making it more soluble (52). Both isoforms bind to membrane-bound and soluble VEGFR1. The proteolytically processed form of VEGF-B186 at Arg-127 can also bind to Neuropilin 1. VEGF-B is highly expressed in metabolically active tissues such as the heart, skeletal muscle and adipose tissue (53). In the heart, one strain of the VEGF-B-knockout mice was proposed to have atrio-ventricular conduction abnormality characterized by prolonged PQ interval (48) and the other strain showed slightly decreased heart size during development (47). However, in a subsequent analysis these phenotypes were not confirmed (54). Cardiomyocyte-specific transgenic overexpression of full-length VEGF-B induced expansion of the coronary vasculature, enlargement of myocardial capillaries, mild cardiomyocyte hypertrophy, and promoted in vivo ischemia protection (13, 36, 37, 55). Importantly, the vascular effects did not include vascular leakage, unlike with VEGF overexpression. Intramyocardial adenoviral or AAV-mediated overexpression of VEGF-B167 in the rat heart prevented progression of angiotensin II associated left ventricular dysfunction (56) and promoted antiapoptotic effect in cardiomyocytes after myocardial infarction without significant effect on vasculature (40). In dogs, intramyocardial or intravascular delivery of AAV9-VEGF-B167 preserved diastolic and contractile function and attenuated ventricular chamber remodeling, halting the progression from compensated to decompensated heart failure (57, 58). Administration of AAV9-VEGF-B186 also maintained cardiac function by inducing angiogenesis and blocking apoptosis after transaortic constriction in mice (59). It has also been suggested that VEGF-B directly improves myocardial contractility and CM survival by binding to VEGFR1 on CMs (40). Recent studies, however, have demonstrated that the expression of VEGFR1 and VEGFR2 are restricted to ECs in the heart, and there is very low expression in CMs (13, 37, 60). In the heart, increased VEGF-B expression levels activate AKT/mTORC1 and ERK1/2 MAPK pathways, likely in both ECs and CMs, resulting in vascular and cardiomyocyte growth (13, 37, 42).

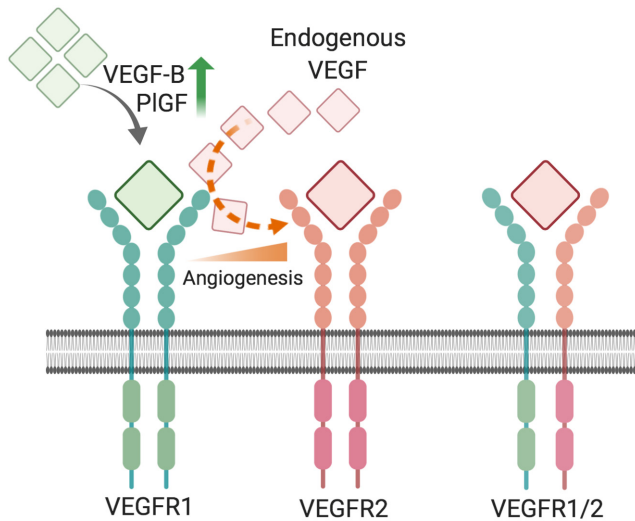
### Placental growth factor

PlGF is encoded by the *PGF* gene. The mice lacking PlGF are viable and develop normally during embryogenesis, but under pathological conditions such as ischemia,

inflammation, wound healing and cancer the PlGF-deficient mice had defective blood vessel growth (49). In humans, PlGF is expressed as four isoforms PlGF1, PlGF2, PlGF3, PlGF4, whereas in mice only one isoform (PlGF2) is expressed (61). Initially, PlGF was found to be expressed in human placenta and its expression is also detected for example, in lungs, skeletal muscles and the heart (53, 61). Both PlGF and VEGF-B have high binding affinity for VEGFR1, but PlGF is able to promote VEGFR1 phosphorylation effectively, whereas VEGF-B has very little effect (62, 63). This together with their different tissue expression patterns might explain the differences in biological functions. In the mouse heart, PlGF has been found to induce angiogenesis and to mediate cardiac hypertrophy via nitric oxide (NO)-dependent Akt/mTORC1 pathway (38). In another study, PlGF was found to induce pressure overload-mediated cardiac angiogenesis and adaptive hypertrophy through paracrine signaling between CMs, ECs and fibroblasts (39).

### VEGFR2-ERBB signaling in cardiac hypertrophy

Notably, exogenous administration of PR39, VEGF-B or PlGF failed to induce hypertrophic effect on cultured neonatal cardiomyocytes highlighting that vasculature growth and paracrine signaling from EC to CM are essential (32, 39). PlGF-induced CM growth was shown to be partly attenuated by nitric oxide blocking, indicating that NO is one of the mediators of the EC-CM crosstalk. In our recent study, we demonstrated that VEGF-B or PlGF-induced cardiac angiogenesis and hypertrophy were completely blocked by genetically or pharmacologically inhibiting VEGFR2 signaling, but in contrast, further enhanced by VEGFR1 deletion (13). VEGFR2 activation is known to lead to NO production by ECs (64), thus VEGFR2 activation could explain the partial effects of NO blocking. The observations comply with the proposed role for VEGFR1 as a decoy receptor for VEGF (65, 66, 67). When the extracellular domain of the VEGFR1 is deleted or its ligands VEGF-B and PlGF are overexpressed, the binding of VEGF to VEGFR1 is prevented and this renders more endogenous VEGF available to bind and activate VEGFR2, the main angiogenic receptor (Fig. 1). However, the important difference between this indirect activation of VEGF-VEGFR2 signaling and VEGF overexpression models is that indirect VEGFR2 activation is restricted by endogenous VEGF levels, preventing immature vessel growth and leakage seen with supraphysiological VEGF doses.

**Figure 1**

Indirect activation of VEGFR2 by VEGF-B and PIGF and decoy function of VEGFR1. VEGFR1 has 10-fold higher binding affinity to VEGF than VEGFR2, but much weaker activation, thus it has been shown to act mainly as a decoy receptor for VEGF. Increase in levels of VEGF-B or PIGF, which only bind to VEGFR1, displace VEGF from VEGFR1 increasing the availability of endogenous VEGF to bind to and activate VEGFR2, which leads to angiogenesis. Note that this activation of VEGF-VEGFR2 signaling is limited by the endogenous levels of VEGF, and thus does not induce immature vessel growth and leakage compared to overexpression models of VEGF.

In addition to NO, what else could mediate the VEGFR2 signaling effect from EC to CM? We screened for potential mediators by identifying secreted factors with endothelial expression from the transcriptomics data of the VEGF-B overexpressing hearts. We tested several candidates, and out of them we found increased levels of heparin-binding EGF-like growth factor (HB-EGF) and Adam12, a metalloprotease required for HB-EGF cleavage. Enhanced ERBB1/EGFR and ERBB4 phosphorylation was coupled with increased endothelial VEGFR2 activation during angiogenesis-induced physiological hypertrophy (13). *In vitro* and *in vivo* experiments demonstrated that VEGF-B or VEGF stimulation increased the expression and/or shedding of (HB-EGF) and neuregulin-1 (NRG-1), and enhanced the phosphorylation of EGFR, ERBB4 and AKT.

Neuregulins, HB-EGF and their ERBB tyrosine kinase receptors (ERBB1-4) have been shown to play crucial roles in both the developing and adult cardiovascular system by regulating cardiac development and stress responses. NRG-1 and HB-EGF are released from cardiac microvascular endothelial cells and endocardial endothelium, and they act as paracrine factors via the ERBB receptors expressed in cardiac myocytes. In animal models, deletion of NRG-1, ERBB2, or ERBB4 results in embryonic lethality because of the failure of proper cardiac ventricular and valvular

development (68, 69, 70). Most of the HB-EGF-deficient mice die during the first postnatal week and the survivors develop severe heart failure with enlarged ventricular chambers and cardiac valves (71). In adult human heart, left ventricular dysfunction is observed in cancer patients treated with an antibody against ERBB2 (trastuzumab), suggesting cardioprotective effects of ERBB signaling also in humans (72). During the last decade, NRG-1 has been studied extensively in relation to cardiac homeostasis and regeneration. Below we summarize the functions of NRG-1 and HB-EGF in the context of cardiac hypertrophy.

### Neuregulin-1

In heart failure, enhanced activity of NRG-1 emerges as one of the adaptive mechanisms counteracting cardiac remodeling and disease progression (73). The endogenous source of NRG-1 in the heart has been shown largely to be the microvascular and endocardial endothelium (74, 75), and ECs have been demonstrated to be a crucial source of NRG-1 for cardioprotection against ischemic insult (76). NRG-1 induces protein synthesis and hypertrophy of cardiomyocytes through activation of downstream signaling cascades including the ERK1/2 mitogen-activated protein kinase, PI3-kinase, AKT-kinase, mTOR, and FAK (77). These molecular events promote hypertrophic and, at least during development, also mitotic cardiomyocyte growth, as well as cardiomyocyte resistance to apoptosis (77, 78). However, in pathological hypertrophy induced by for example, angiotensin II-hypertension model or myocardial infarction, NRG-1 reduces the increase of cardiomyocyte cross-sectional area (73). NRG-1/ERBB signaling is also activated in physiological hemodynamic overload during pregnancy (79) and HB-EGF/NRG-1 expression and shedding are induced by VEGFR2 signaling in angiogenesis-induced physiological cardiac hypertrophy (13). Taken together, NRG-1 induces physiological growth of CMs, while suppressing pathological myocyte growth.

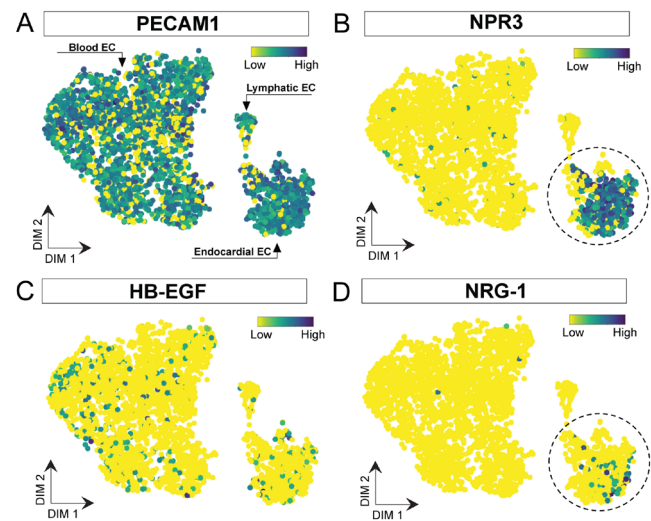
Translational studies have evaluated the potential of NRG-1-ERBB signaling activation in ameliorating ischemia-reperfusion injury and in enhancing cardiac regeneration. In 2017, a human phase 1 clinical study using cimaglermin (NRG-1 $\beta$ 3) reported improvement in left ventricular function in heart failure patients three months after single-infusion treatment (80). Previously, phase 2 human trials had reported recombinant NRG-1 administration to be safe and to improve cardiovascular function in patients with heart failure (81, 82). The effects

of ERBB signaling in the heart are remarkably pleiotropic with effects on endothelial cells, fibroblasts, macrophages and neuronal cells (73). Thus, the benefits reported with NRG-1 therapy may involve multiple cell types and mechanisms of action in addition to myocyte growth and/or proliferation.

## Heparin-binding EGF-like growth factor

HB-EGF is expressed in several cell types, including ECs, and its activity can be regulated either by expression levels or shedding from the cell surface by metalloproteases (83, 84, 85, 86). The first evidence that secreted HB-EGF regulates cardiomyocyte growth came from studies in isolated adult cardiomyocytes, which were shown to exhibit nearly a two-fold increase in protein content when stimulated with conditioned media from HB-EGF overexpressing COS cells (87). Later, shedding of HB-EGF by ADAM12 was demonstrated to play an important role in the development of cardiac hypertrophy induced by phenylephrine, angiotensin II or endothelin-1 (88). Studies in HB-EGF-null mice provided evidence that HB-EGF is essential for normal cardiac function, as the mice deficient of HB-EGF had enlarged hearts, hypertrophic cardiomyocytes, and abnormal cardiac valves, which led to dilation of cardiac chambers and decreased systolic function (71). The importance of endothelial HB-EGF was demonstrated by its EC-specific deletion, which resulted in cardiac hypertrophy and heart valve malformations, postnatal lethality, resembling the phenotype of HB-EGF-null mice (89). Furthermore, mice expressing uncleavable form of HB-EGF developed dilated cardiomyopathy and severe heart failure, emphasizing the importance of HB-EGF shedding and soluble HB-EGF in the maintenance of cardiac homeostasis (90). On the other hand, cardiomyocyte-specific HB-EGF overexpressing mice demonstrated activation of cardiac fibroblasts and increased fibrosis, but no effect on cardiomyocyte size was observed (91).

To analyze the expression of HB-EGF and NRG-1 in different cardiac EC types, we used the recently published murine EC single-cell RNA sequencing database (<https://www.vibcancer.be/software-tools/ec-atlas>) from the Carmeliet lab (92). In the EC atlas database, we first categorized the cardiac EC cell type clusters as blood EC (lyve1- Prox1- Npr3-), Lymphatic EC (lyve1+ Prox1+) and Endocardial cells (Npr3+) based on the previously published markers for different ECs. Then the expression of HB-EGF and NRG-1 were verified in these clusters.



**Figure 2**

Expression of NRG-1 and HB-EGF in cardiac vascular and endocardial ECs. t-distributed Stochastic Neighbor Embedding (t-SNE) plot showing. (A) The expression of a pan-endothelial cell marker PECAM1 in the blood, lymphatic and endocardial ECs in the adult mouse heart; (B) Endocardial-specific marker NPR3 in the endocardial cluster (inside the dashed-lines); (C and D) HB-EGF and NRG-1 in the vascular and endocardial ECs. To analyze the expression of HB-EGF and NRG-1 in different cardiac EC cell types, a nonlinear dimensionality reduction method was applied to reduce and visualize the multi-dimensional big data in two- or three-dimensions (t-SNE). Based on the gene expression profile of single cells, the algorithm clusters the cardiac EC cells with similar and dissimilar gene expression pattern in nearby and distant points with high probability. Our analysis indicates that HB-EGF is expressed throughout all EC clusters, whereas NRG-1 is mainly restricted to endocardial cells. For the analysis, we have used the publicly available single cell mouse cardiac EC atlas (<https://www.vibcancer.be/software-tools/ec-atlas>) (92).

The results demonstrate that HB-EGF is expressed in all blood ECs, lymphatic EC clusters as well as in endocardial cells, whereas NRG-1 is expressed almost exclusively in the endocardial cells of the adult mice heart (Fig. 2). This suggests that HB-EGF is acting throughout the heart, whereas NRG-1 is mainly involved in the endocardium-myocardium crosstalk.

## Other angiocrines regulating cardiomyocyte growth

In addition to NO and the ERBB ligands, several secreted factors have been shown to regulate cardiomyocyte growth (Table 1). We screened the previously reported factors against our RNA sequencing database from isolated and purified adult mouse cardiac ECs to select those factors, which are produced by ECs. It should be noted that many of these angiocrines are also produced by other cell types and they can also act on non-myocytes, highlighting the

**Table 1** Angiocrines regulating cardiac hypertrophy.

Angiocrine	Effects on cardiomyocytes	References
Periostin	<ul style="list-style-type: none"> <li>Periostin overexpression in the heart protected the mice from rupture following MI and induced hypertrophy with aging.</li> <li>In an 8-week TAC mouse model, deletion of periostin decreased cardiac hypertrophy and fibrosis.</li> <li>During the first 10 days after myocardial infarction the mice lacking periostin were susceptible to ventricular rupture but the surviving mice showed decreased fibrosis and better ventricular function.</li> </ul>	(99, 100)
TSP-1	<ul style="list-style-type: none"> <li>TSP-1 levels were increased due to pressure-overload in mice.</li> <li>TSP-1 null mice increased MMP3 and MMP9 expression, exhibited sarcomeric loss, sarcolemma disruption and cardiomyocyte degenerative changes following pressure overload.</li> <li>Loss of TSP-1 in mice induced early cardiac hypertrophy and promoted delayed dilation in response to pressure overload.</li> </ul>	(101, 102)
ADM	<ul style="list-style-type: none"> <li>Adrenomedullin is a pleiotropic peptide that inhibits cardiomyocyte hypertrophy and cardiac fibroblast proliferation in acute rat myocardial infarction models and in humans with cardiac disease.</li> </ul>	(103)
Midkine	<ul style="list-style-type: none"> <li>Plays an important role in vascular integrity and angiogenesis.</li> <li>Midkine levels are elevated in heart failure (HF) patients and acts as a marker for stratifying the risk in HF patients.</li> <li>Cardiac-specific overexpression of Midkine promoted severe cardiac hypertrophy, dysfunction and decreased the survival rate of the mice after TAC.</li> </ul>	(104, 105)
BMP-4	<ul style="list-style-type: none"> <li>BMP-4 levels are upregulated during pathological cardiac hypertrophic models and induces cardiomyocyte hypertrophy and apoptosis.</li> </ul>	(106)
FSTL-1	<ul style="list-style-type: none"> <li>Acute or chronic overexpression of FSTL-1 increased the AMPK activation in the myocardium and prevented the TAC-induced hypertrophy and cardiac failure.</li> </ul>	(107)
CTGF	<ul style="list-style-type: none"> <li>CTGF activates ERK1/2, p38 MAPK, JNK and Akt signaling cascades and promotes cardiac hypertrophy.</li> </ul>	(108)
IGF-1	<ul style="list-style-type: none"> <li>High dose of IGF-1 induced physiological cardiac hypertrophy and positive inotropic effect without altering the expression of fetal and myocardial gene expression.</li> </ul>	(109)
APLN	<ul style="list-style-type: none"> <li>Apln acts as a negative regulator of cardiac hypertrophy during ANG II infusion and promotes myocardial remodeling, fibrosis and results in cardiac dysfunction.</li> <li>Exogenous administration of pyr1-apelin13 increases the ACE2 expression, reduces cardiac hypertrophy and fibrosis in the ANG II-infused ApoE knockout mice.</li> <li>Loss of Apln receptor decreases chronic pressure overload induced cardiac hypertrophy and heart failure.</li> <li>Myocardial Apln receptor is needed to promote stretch induced increase in contractility.</li> </ul>	(110, 111, 112)

The list consists of secreted factors produced by cardiac endothelial cells and, which have been demonstrated to regulate cardiomyocyte hypertrophy. The list was first retrieved from the meta-analysis by Segers et al. (17) and the expression in EC was confirmed using our own RNAseq database from isolated adult mouse cardiac ECs.

complexity of multicellular paracrine signaling in the heart.

### Endocardium-myocardium crosstalk during heart development

Endocardium is formed by specialized endothelial cells that line the inner surface of the cardiac chambers. Endocardium is a source of instructive signals for the development of the heart valves and chambers, and the endocardium–myocardium communication is essential for myocardial trabeculation and compaction. Notch signaling pathway has been identified to coordinate cellular interactions during heart development by communicating with other pathways (93), such as

NRG-1 – ERBB (94). HB-EGF has also been identified as an endocardial Notch target and an essential angiocrine regulating cardiac development (95). Hippo signaling is an important regulator of mammalian heart size by controlling cardiomyocyte growth in a cell-autonomous manner (96). Recently, the constituents of Hippo signaling, transcription factors Yap and Taz, were demonstrated to play also an instructive, non-cell-autonomous role in the heart during development. Endocardial Yap and Taz regulated myocardial growth through the release of NRG-1 as a paracrine factor from endocardium to cardiomyocytes (97). Yap/Taz act as mechanical sensors in ECs. They transduce the signals exerted by for example, blood-flow, extracellular matrix stiffness and cardiomyocyte contraction, triggering cellular signaling and angiogenesis (98). It is suggested that mechanotransduction of Yap/

Taz mediates pathological effects of disturbed blood flow in vascular diseases (98). It is likely that changes in the mechanical cues to ECs in cardiovascular diseases would affect their angiocrine profiles as well. These data indicate that the same pathways are acting both in the developing heart as well as during adult heart growth, however, the role of endocardial paracrine signaling in the adult heart is currently not well understood.

## Conclusions

Considerable knowledge has accumulated over the recent years regarding angiocrine signaling and its role in the regulation of tissue growth, regeneration and homeostasis. Cardiac angiogenesis plays a central role in the development of physiological vs pathological heart growth and the transition to heart failure. It has now been appreciated that it is not only the transport function of the vasculature that is important, but also its active role in paracrine signaling and crosstalk between other cardiac cell types. The understanding of cardiac angiocrines important for cardiomyocyte growth, metabolism and function is of utmost importance for the identification of novel therapeutic targets. In this review we have focused on angiocrines and signaling pathways known to

modulate CM growth (Fig. 3). In addition to angiocrines, micro RNAs (miRNA) and long non-coding RNAs (lncRNA) are produced and secreted by endothelial cells. These can be taken up by cardiomyocytes, where they can regulate growth and function. Not much is currently known about specific miRNAs and lncRNAs regulating EC-CM interaction, thus we have not covered them in this review. The challenge in the field is still to decipher the fundamental differences in intercellular communication between physiological and pathological cardiac growth, and how the latter proceeds to heart failure, which is a major health problem.

From a clinical perspective, identification of heart-specific angiocrine factors, which would either be produced only by cardiac ECs or have function/activity restricted to cardiomyocytes, could provide specific therapies without side effects in other tissues and cell types. For this, we need advanced studies to learn more about heart-specific ECs and angiocrines.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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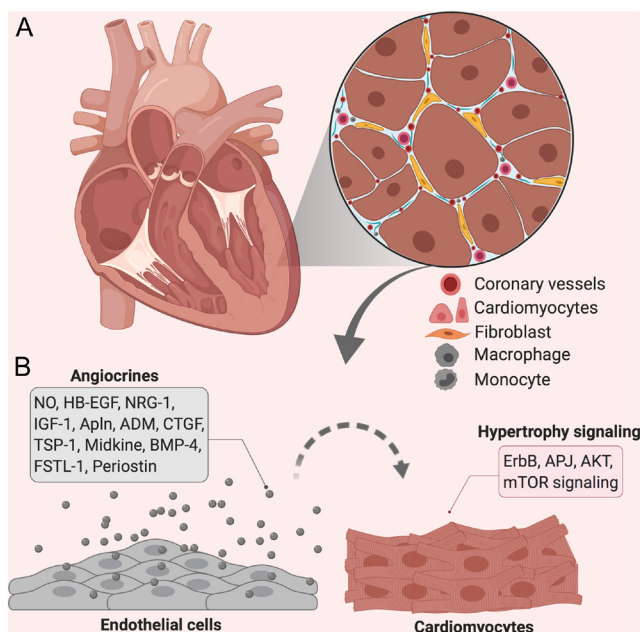
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**Figure 3**

Cardiac cell types and angiocrine-mediated regulation of cardiomyocyte hypertrophy. (A) Tissue architecture and the major cell types present in the adult heart. (B) Angiocrines secreted from cardiac ECs known to participate in hypertrophic signaling and cardiomyocyte growth.



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