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Autocrine Signaling in Cardiac Remodeling: A Rich Source of Therapeutic Targets

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ABSTRACT: The myocardium consists of different cell types, of which endothelial cells, cardiomyocytes, and fibroblasts are the most abundant. Communication between these different cell types, also called paracrine signaling, is essential for normal cardiac function, but also important in cardiac remodeling and heart failure. Systematic studies on the expression of ligands and their corresponding receptors in different cell types showed that for 60% of the expressed ligands in a particular cell, the receptor is also expressed. The fact that many ligand-receptor pairs are present in most cells, including the major cell types in the heart, indicates that autocrine signaling is a widespread phenomenon. Autocrine signaling in cardiac remodeling and heart failure is involved in all pathophysiological mechanisms generally observed: hypertrophy, fibrosis, angiogenesis, cell survival, and inflammation. Herein, we review ligand-receptor pairs present in the major cardiac cell types based on RNA-sequencing expression databases, and we review current literature on extracellular signaling proteins with an autocrine function in the heart; these include C-type natriuretic peptide, fibroblast growth factors 2, F21, and 23, macrophage migration inhibitory factor, heparin binding–epidermal growth factor, angiopoietin-like protein 2, leptin, adiponectin, follistatin-like 1, apelin, neuregulin 1, vascular endothelial growth factor, transforming growth factor β, wingless-type integration site family, member 1-induced secreted protein-1, interleukin 11, connective tissue growth factor/cellular communication network factor, and calcitonin gene–related peptide. The large number of autocrine signaling factors that have been studied in the literature supports the concept that autocrine signaling is an essential part of myocardial biology and disease.

Key Words: autocrine ■ cardiac remodeling ■ heart failure ■ intercellular communication ■ myocardium

A better understanding of heart failure pathophysiology in response to cardiac injury, also known as cardiac remodeling, is needed for the development of novel therapies for heart failure, which is still a major cause of death. The pathophysiology of cardiac remodeling involves cardiac hypertrophy, ventricular dilation, and decreased contractility at the level of the ventricle and involves cellular and interstitial changes, mainly fibrosis, at the level of myocardial tissue.

The heart is a multicellular organ consisting of endothelial cells, myocytes, fibroblasts, and inflammatory cells. Communication between these different cell types, also termed paracrine signaling, is crucial for regulation of normal cardiac function and for responsiveness of the myocardium to stressors. The best-known example of a paracrine factor in the myocardium is NO, which is produced by endothelial cells in the myocardium, plays diverse roles in cardiac remodeling, and can induce both protective and adverse reactions.[1] Endothelial cells, however, secrete many more factors, including small molecules, peptides, and proteins, that modulate cardiomyocyte contractility, growth, and survival.[2–6] Intercellular communication in the myocardium not only comprises interactions between endothelial cells and cardiomyocytes, but comprises interactions between all different cell types.

Factors secreted by cardiac cells do not only induce paracrine signaling, but also autocrine signaling (ie, they modulate the cell type that secreted the factor) (Figure 1). In autocrine signaling pathways, the cell involved will secrete both the extracellular signaling molecule as well as its receptor, whereas in

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Nonstandard Abbreviations and Acronyms

AngII	angiotensin II
ANGPTL2	angiopoietin-like protein 2
BMP	bone morphogenetic protein
CCN	cellular communication network factor
CGRP	calcitonin gene–related peptide
CNP	C-type natriuretic peptide
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EndoMT	endothelial-mesenchymal transition
ERBB	erythroblastic leukemia viral oncogene homolog
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FSTL1	follistatin-like 1
HB-EGF	heparin binding–epidermal growth factor
IL11	interleukin 11
MIF	macrophage migration inhibitory factor
NPR	natriuretic peptide receptor
NRG1	neuregulin 1
RAMP1	receptor activity modifying protein 1
RCP	receptor component protein
TGF	transforming growth factor
UCP3	uncoupling protein 3
WISP1	Wnt1-induced secreted protein-1

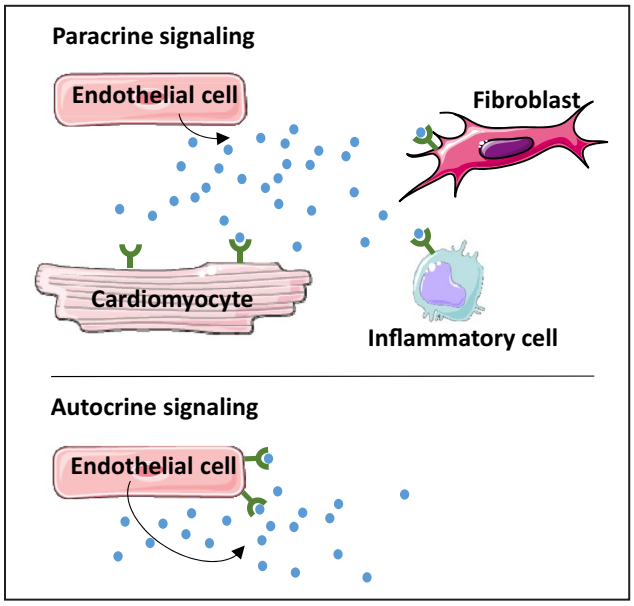

Figure 1. Paracrine and autocrine signaling in the heart. In the top panel, an example of paracrine signaling is shown. Endothelial cells secrete signaling proteins (blue dots) that target receptors on cardiomyocytes, fibroblasts, and inflammatory cells. In the bottom panel, an example of autocrine signaling in endothelial cells is shown, in which the ligand binds to receptors on the same cell type.

paracrine signaling, one cell will secrete the signaling molecule and the other cell the receptor (Figure 1). The observation that a certain cell type expresses both the ligand and the receptor for a specific signaling pathway makes autocrine signaling likely, but the relative importance of a particular autocrine signaling pathway, beyond mere expression of the ligand and its receptor, is more difficult to determine. If the expression level of the receptor is high, the likelihood that the ligand binds to the cell of origin will also be high, whereas when the expression level of the receptor is low, signaling to cell types with higher expression levels will be more important.

In this review, we focus on autocrine signaling in cardiomyocytes, endothelial cells, and fibroblasts, because they are the most abundant cell types in the heart.[7,8] However, one has to keep in mind that many other cell types populate the heart, including B cells, T cells, natural killer cells, granulocytes, dendritic cell–like cells, macrophages, Schwann cells, smooth muscle cells, and pericytes.[8] Moreover, we will focus on proteins involved in autocrine signaling, but we refer the reader to other excellent reviews on the role of autocrine NO,[9] angiotensin II (AngII),[10] and endothelin-1[11] in the heart. Also, we refer the reader interested in paracrine signaling in cardiac remodeling to other reviews.[6,12–14]

CELLULAR BIOLOGY OF AUTOCRINE SIGNALING

Autocrine signaling was first described 4 decades ago in processes of tumor growth[15] and was originally thought to be limited to states of disease. However, autocrine signaling plays a role in pathophysiology as well as in normal physiology and in embryologic development, including mammary and prostate epithelial development,[16,17] cardiac development,[18] tissue response to injury,[19] and, as will be discussed in this review, cardiac remodeling and heart failure.

Autocrine signaling can contribute to several different physiological roles (eg, negative feedback loops, positive feed-forward loops, and self-stimulation) (Figure 2). A negative feedback loop is a classic physiological mechanism in which the production of the signal is reduced in response to increased activation of its receptor. An example of feed-forward loops is the secretion of growth factors by cancer cells to limit apoptosis in the secreting cell and surrounding cells. Self-stimulation is a subset of positive

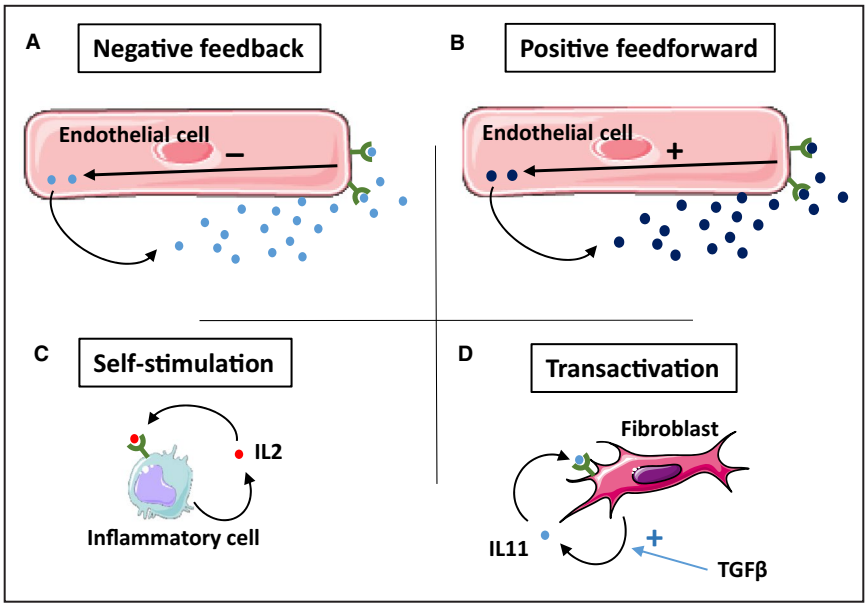

Figure 2. Cellular physiology of autocrine signaling.
Autocrine signaling can result in a negative feedback loop, in which binding of a ligand to its receptor inhibits expression of the ligand (**A**); a positive feed-forward loop, in which binding of a ligand to its receptor increases expression of the ligand (**B**); self-stimulation, which is frequently observed in immune cells (eg, interleukin [IL] 2 in T lymphocytes) (**C**); and transactivation, in which activation of a cell with a specific factor starts production of a second autocrine signaling factor (an example is production of IL11 in response to transforming growth factor [TGF] β stimulation) (**D**).

feed-forward loops and is typically used to describe the phenomenon in which immune cells secrete cytokines that lead to amplification of the initial signal. These physiological processes could, in many instances, easily be accomplished by a wide variety of intracellular signaling pathways present in mammalian cells. The fact that cells use a more elaborate process (secretion of a protein ligand and expression of its receptor) instead of using intracellular signaling pathways indicates that externalization of part of the signaling process is important. In many instances, the secreted factor will be modified by its interaction with extracellular matrix proteins, proteinases, and receptors on the surface of neighboring cells; in this manner, the autocrine signaling loop not only incorporates information from the cell itself, but also from its surroundings.

Autocrine signaling plays a major role in receptor cross talk or "transactivation" (Figure 2D). In the process of transactivation, activation of one receptor system in a given cell induces the release of an autocrine factor that activates a separate receptor. The physiological significance of transactivation has become clear in recent years, also in the process of cardiac remodeling, as its main function appears to be the integration from multiple receptor signals in complex signaling systems; examples that will be discussed are fibroblast growth factor (FGF) 23 and interleukin 11 (IL11). At the level of the cell, the 2 main processes in the myocardium that involve transactivation are induction of hypertrophy in cardiomyocytes and activation of quiescent fibroblasts into actively dividing and extracellular matrix–producing cells.

A major issue for autocrine signaling is that it is difficult to study. One reason is the circular nature of the autocrine loop; many autocrine factors enhance self-release through intracellular signaling pathways.[20] Another reason why autocrine loops are difficult to study is the spatial limits of autocrine signaling, compared with paracrine or endocrine signaling. An important consequence of spatial restriction is that ligands are often not found in the extracellular space unless their receptors are blocked.[20] As will be discussed, a third reason is that in polarized cells (eg, epithelial or endothelial cells), ligand and receptor can be on either the same or the opposite surface. For instance, both transforming growth factor (TGF) α and epidermal growth factor (EGF) bind to the EGF receptor (EGFR), but whereas TGFα and EGFR are located on the basolateral surface, EGF is located on the apical surface of epithelial cells.[21,22] The difficulty in studying autocrine signaling is also related to the complexity of autocrine signaling systems (Figure 3), which include many more entities than just one ligand and one receptor; they consist of proteinases,

signaling proteins, extracellular matrix proteins, competing ligands, competing receptors, and cellular components (Figure 3).

One has to be aware of the fact that in the spatial operation of an autocrine loop, a fraction of the ligand will be captured on the receptor present on the cell that produces the ligand. This fraction of ligand captured on the producer cell will determine the spatial range over which the ligand can travel before binding to its receptor. When this "local capture fraction" is close to 1, the spatial domain of the autocrine loop will be limited to the cell dimension, whereas when the capture fraction is close to 0, the spatial domain of the autocrine loop will be much larger than the cell dimension. Also, the local capture fraction of the ligand in an autocrine loop will be dependent on the production rate of both ligand and receptor in the cell. This has been elegantly demonstrated for EGF and EGFR production in fibroblasts using a engineered system in which ligand production was controlled by a tetracycline-operon expression system.[20] As the ligand production rate increases relatively to the receptor production rate, which determines the cell surface receptor number, the fraction of ligand captured on the producing cell decreases and as a consequence more ligand will be available for signaling over a longer distance (ie, paracrine signaling).[20]

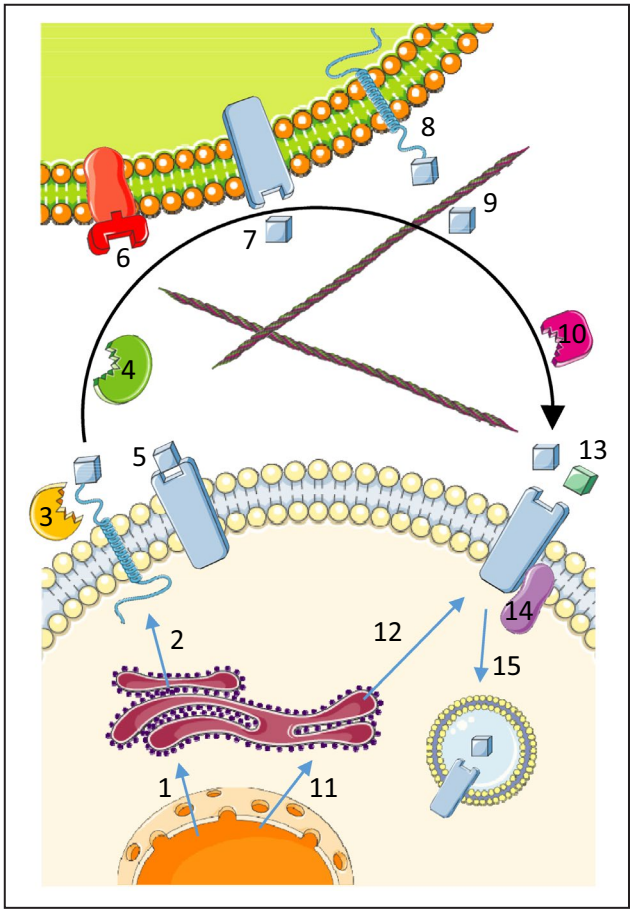

Figure 3. The complexity of autocrine signaling systems. Autocrine signaling is influenced by (1) ligand production rate (transcription); (2) ligand production rate (translation); (3) ligand release from transmembrane domain by proteinases; (4) ligand activation by release of inactivating complexes; (5) ligand capture on cell surface receptors; (6) ligand interaction with different receptors; (7) ligand binding to receptors on other cells (paracrine signaling); (8) ligand production by other cells/cell types; (9) ligand interaction with extracellular matrix proteins; (10) ligand inactivation by proteinases; (11) receptor production rate (transcription); (12) receptor production rate (translation); (13) competition with other ligands; (14) receptor interaction with intracellular signaling proteins; and (15) receptor internalization.

The spatial operation of an autocrine loop is not only dependent on production rates of ligand and receptor, but also dependent on the structure of the ligand. For instance, all ligands of the EGF family, which includes EGF, heparin binding–EGF (HB-EGF), TGFα, and neuregulin 1 (NRG1) to neuregulin 4,[3,23–25] among others, are initially produced as transmembrane proteins, of which the N-terminal extracellular domain can be released by proteolysis at the cell membrane (Figure 3, step 3). The extracellular domain can also be cleaved by different proteases, resulting in ligands of different sizes and properties. An important example is HB-EGF, which, in contrast to EGF, contains a heparin binding domain that allows binding to glycosaminoglycans. This binding with various glycosaminoglycans on the cell surface and in the extracellular space dramatically reduces the spatial range over which HB-EGF can exert its actions.[20]

AUTOCRINE SENSING OF THE CELLULAR ENVIRONMENT

The different physiological roles, negative feedback, positive feed forward, self-stimulation, and transactivation, commonly presented in the literature on autocrine signaling do not amount to a complete list of roles. Theoretically, autocrine signaling might provide cells with a sensory tool that operates by secreting a signal into the extracellular milieu combined with real-time sampling of the signal (Figure 4). This sensory tool could enable cells to monitor their surroundings in an intricate manner, because the amount of transmitted signal to be sensed by the source cell will be altered by the number of surrounding cells and their proximity. In essence, this sensory system could be analogous to echolocation used by bats, with an important difference that cells are not limited to transmission of a single signal but could transmit 10s of signals simultaneously. Autocrine signaling could not only enable cells to monitor the number and proximity of neighboring cells, but also their expression of both ligand and receptor, which could provide the cells with clues on the identity of their neighbors, especially when a cell

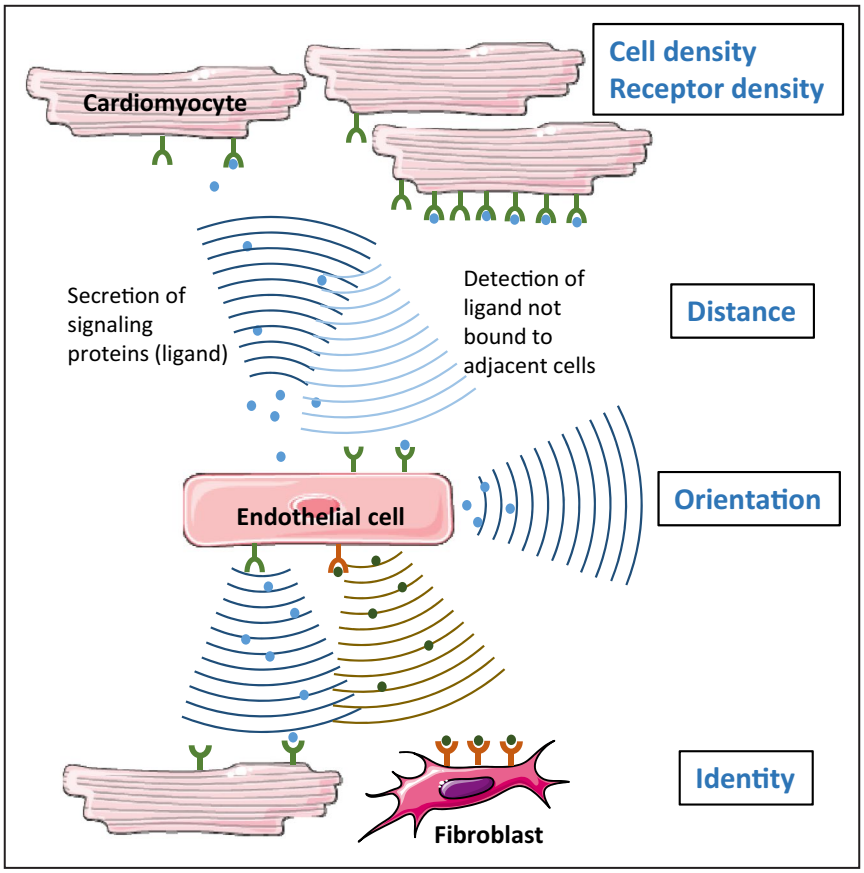

Figure 4. Autocrine signaling as a sensory tool for cells in the myocardium.
When a particular cell, in this case an endothelial cell shown in the center of the figure, expresses a ligand-receptor pair, this autocrine signaling pair can potentially serve as a sensory tool. When this endothelial cell is in close proximity to cardiomyocytes that express large amounts of a receptor for the same ligand, the amount of ligand bound to the receptors on the source cell will be lower. The "returning signal" or "echo" will be dependent on the number of cells, the receptor level on these cells, and their distance from the source cell. Polarization in expression of either the ligand or the receptor will allow the source cell to determine the location of the neighboring cell and, therefore, determine its relative orientation to other cells. Expression of ligands is not a continuous process but is highly variable over time, which allows the source cell to sample its surroundings in the time dimension as well. Cells do not express a single autocrine ligand, but 10s of different autocrine ligands at the same time. One can speculate that cells could gather information on the identity of their neighbors by differences in returning signals, based on differences in receptor expression in neighboring cells.

combines 10s of signals in real time. This sensory system could also enable the cell to determine the relative orientation of the other cells in relation to its own shape; this feature will help cells to determine their relative position in layered organs (eg, blood vessels or intestines).

Of all cells present in the myocardium, the concept of cellular orientation and polarity is most applicable to endothelial cells, because these cells display a clear apicobasal polarity with an apical/luminal and a basolateral/abluminal surface.[26] Apicobasal polarity of endothelial cells has been studied mostly in the brain, where interesting observations have been made. For instance, when vascular endothelial growth factor (VEGF) is applied to the apical/luminal surface, cytoprotective pathways are activated through VEGF receptor 1, whereas when VEGF is applied to the basal/abluminal surface, endothelial permeability is increased through VEGF receptor 2.[26] Another example is disturbed apicobasal polarity in endothelial cells induced by multiple sclerosis; disturbed apicobasal polarity leads to increased chemokine (C-X-C motif) ligand 12 (commonly referred to as stromal cell-derived factor-1) expression and increased infiltration of inflammatory cells.[27] The study of the role of apicobasal polarity in endothelial cell function in the myocardium has yet to be started. The same is true for the study of the interaction between apicobasal polarity and autocrine signaling. It is conceivable that for several ligand-receptor pairs, of which expression is confirmed by RNA

sequencing, quantitative polymerase chain reaction, or Western blot experiments, the ligand is expressed on one side, whereas the receptor is expressed on the other side.

The concept of autocrine sensing has not been widely studied in multicellular organisms, but a similar process has been studied in bacteria and has been termed quorum sensing.[28] Bacterial quorum sensing involves chemical signals, produced by bacteria, that accumulate in the local environment; when a threshold level is reached, transcription of specific genes is activated.[28] Quorum sensing occurs in gram-positive and gram-negative bacteria and involves many different signals, including small molecules and peptides. Quorum sensing allows bacteria to determine population density and the need of producing extracellular materials (eg, biofilms).[28] If bacteria use a complex system like quorum sensing, it can be expected that more evolved cellular life forms, which demonstrate spectacular specialization and cooperation in tissues, use at least similar signaling systems, but in effect probably more complex autocrine signaling systems than bacteria.

AUTOCRINE SIGNALING IS A WIDESPREAD PHENOMENON

One might assume that most ligands expressed by mammalian cells act on receptors expressed on different cells and thus that they only function as paracrine signals. This assumption has been contradicted by a systematic interrogation of the expression of ligands and receptors on 144 different human cell types.[29] This systematic study showed that most human cell types express hundreds of ligands and receptors, confirming the existence of complex intercellular communication in tissues. But more surprisingly, this study also showed that two thirds of these ligands are potentially involved in autocrine signaling because ≥1 of their receptors is also expressed.[29] Therefore, this study indicates that autocrine and paracrine signaling exist in parallel in most human cell types.

Systematic study of ligand-receptor pairs in cardiac cells (cardiomyocytes, endothelial cells, and fibroblasts) has not been performed. Therefore, we searched for ligand-receptor pairs in gene expression data from RNA-sequencing experiments performed in our own laboratory (endothelial cells)[30,31] and from public resources (cardiomyocytes and fibroblasts).[29] For this search, we used the ligand-receptor pair database that was constructed by Ramilowski and coworkers[29] and that contains 2422 ligand-receptor interactions. The ligands in this database are all present in the extracellular space but belong to different functional classes (eg, growth factors, signaling proteins, cytokines, chemokines, matricellular proteins, structural proteins, proteoglycans, proteases and their inhibitors, enzymes, coagulation factors, proteins involved in complement activation, and proteins involved in lipid transport, among others). For primary isolated human cardiomyocytes, which have not been cultured in vitro, and primary cultured human cardiac fibroblasts, we used the FANTOM5 expression atlas[32] and selected 10 tags per million as the expression threshold, which has been validated previously.[29] We considered ligand-receptor pairs as potentially autocrine when both ligand and receptor met the 10 tags per million threshold and when the ratio of both was <20 in both directions, because we assumed that when either the ligand or receptor displays a much higher expression than the other, autocrine signaling is less likely. In this manner, we identified 257 potentially autocrine ligand-receptor pairs in cardiomyocytes and 326 in cardiac fibroblasts (Data S1). For cardiac endothelial cells, we used 2 RNA-sequencing experiments previously performed in our laboratory: one on freshly isolated rat cardiac endothelial cells[30] and another on cultured human cardiac endothelial cells.[31] In this manner, we identified 272 potentially autocrine ligand-receptor pairs in rat cardiac endothelial cells in vivo and 286 in human cultured cardiac endothelial cells in vitro (Data S1). There is substantial overlap between freshly isolated rat cardiac endothelial cells and cultured human cardiac endothelial cells, but obviously there are also differences because they are derived from different species and, more important, because in vitro culture of cells induces substantial phenotypic changes.

To provide the reader with a general overview of potentially autocrine ligand-receptor pairs, we present a selection of them in Table 1 and Tables S1 and S2; only ligand-receptor pairs are shown for which the primary function of the ligand is intercellular signaling (these include signaling molecules, cytokines, growth factors, and chemokines). Table S1 shows potentially autocrine ligand-receptor pairs of isolated human cardiomyocytes, Table 1 of freshly isolated rat cardiac endothelial cells, and Table S2 of cultured human cardiac fibroblasts. The ligand-receptor pairs in which the ligand has a different primary function (eg, structural proteins or proteases) can be found in Data S1.

A first conclusion that can be drawn from these data is that cardiomyocytes express less ligand-receptor pairs (79 pairs), with a primary function in intercellular signaling, than endothelial cells (124 pairs) or fibroblasts (131 pairs). Cardiomyocytes appear to be less talkative, which is not surprising considering their muscular function, than the other 2 cell types, because they express 36 ligands, which meet the expression threshold, compared with 66 ligands expressed by endothelial cells and 54 ligands expressed by fibroblasts. More surprising is that 22 (of the 36 ligands expressed by cardiomyocytes) are expressed by all 3 cell types (Table 2) and that

Table 1. Autocrine Ligand-Receptor Pairs Expressed by Isolated Rat Cardiac Microvascular Endothelial Cells

Gene Pair	Ligand	Receptor
ADM_CALCRL	Adrenomedullin	Calcitonin receptor like
ADM_GPR182		G protein–coupled receptor 182
ADM_RAMP2		Receptor activity modifying protein 2
ANGPT2_TEK	Angiopoietin 2	TEK tyrosine kinase, endothelial
ANGPT2_TIE1		TK with immunoglobulin- and EGF-like domain 1
ANGPTL4_TIE1	Angiopoietin-like 4	TK with immunoglobulin- and EGF-like domain 1
ANXA1_DYSF	Annexin A1	Dysferlin
APLN_APLNR	Apelin	Apelin receptor
BMP2_ACVR1	Bone morphogenetic protein 2	Activin A receptor, type I
BMP2_ACVR2A		Activin A receptor, type IIA
BMP2_ACVR2B		Activin A receptor, type IIB
BMP2_BMPR2		Bone morphogenetic protein receptor, type II
BMP4_ACVR1	Bone morphogenetic protein 4	Activin A receptor, type I
BMP4_ACVR2A		Activin A receptor, type IIA
BMP4_ACVR2B		Activin A receptor, type IIB
BMP4_BMPR2		Bone morphogenetic protein receptor, type II
CCL5_SDC1	Chemokine (C-C motif) ligand 5	Syndecan 1
CCL5_SDC4		Syndecan 4
CTGF_ITGA5	Connective tissue growth factor	Integrin, α 5
CTGF_LRP1		LDLR-related protein 1
CTGF_LRP6		LDLR6-related protein 6
CXCL10_SDC4	Chemokine (C-X-C motif) ligand 10	Syndecan 4
CXCL12_ACKR3	Chemokine (C-X-C motif) ligand 12	Atypical chemokine receptor 3
CXCL12_CXCR4		Chemokine (C-X-C motif) receptor 4
CXCL12_ITGB1		Integrin, β 1
CYR61_CAV1	Cysteine-rich, angiogenic inducer, 61	Caveolin 1
DHH_PTCH1	Desert hedgehog	Patched 1
DKK2_LRP6	Dickkopf WNT signaling pathway inhibitor 2	LDLR6-related protein 6
DLL1_NOTCH4	Δ-like 1	Notch 4
DLL4_NOTCH1	Δ-like 4	Notch 1
DLL4_NOTCH4		Notch 4
GDF11_ACVR1B	Growth differentiation factor 11	Activin A receptor, type IB
GDF11_ACVR2A		Activin A receptor, type IIA
GDF11_ACVR2B		Activin A receptor, type IIB
HBEGF_CD44	Heparin-binding EGF-like growth factor	CD44 molecule
HBEGF_CD82		CD82 molecule
HBEGF_CD9		CD9 molecule
HGF_CD44	Hepatocyte growth factor	CD44 molecule
HGF_SDC1		Syndecan 1
HGF_SDC2		Syndecan 2
HGF_ST14		Suppression of tumorigenicity 14
IGF1_INSR	Insulin-like growth factor 1	Insulin receptor
IGF2_IGF2R	Insulin-like growth factor 2	Insulin-like growth factor 2 receptor
IL15_IL2RB	Interleukin 15	Interleukin 2 receptor, β
IL15_IL2RG		Interleukin 2 receptor, γ
IL1RN_IL1R1	Interleukin 1 receptor antagonist	Interleukin 1 receptor, type I
INHBA_ACVR1	Inhibin, β A	Activin A receptor, type I
INHBA_ACVR1B		Activin A receptor, type IB
INHBA_ACVR2A		Activin A receptor, type IIA
INHBA_ACVR2B		Activin A receptor, type IIB
INHBA_BAMBI		BMP and activin membrane-bound inhibitor
INHBA_ENG		Endoglin
INHBA_TGFBR3		Transforming growth factor, β receptor 3
INHBB_ACVR1	Inhibin, β B	Activin A receptor, type I
INHBB_ACVR1B		Activin A receptor, type IB
INHBB_ACVR2A		Activin A receptor, type IIA
INHBB_ACVR2B		Activin A receptor, type IIB
JAG1_NOTCH1	Jagged 1	Notch 1
JAG1_NOTCH4		Notch 4
JAG2_NOTCH1	Jagged 2	Notch 1
JAG2_NOTCH4		Notch 4
KITLG_KIT	KIT ligand	Stem cell growth factor receptor/Kit
MDK_GPC2	Midkine	Glypican 2
MDK_LRP1		LDLR-related protein 1
MDK_PTPRB		Protein tyrosine phosphatase, receptor type, B
MDK_SDC1		Syndecan 1
MDK_SDC3		Syndecan 3
MDK_SDC4		Syndecan 4
NPPA_NPR1	Natriuretic peptide A	Natriuretic peptide receptor 1
NPPA_NPR3		Natriuretic peptide receptor 3
NPPB_NPR1	Natriuretic peptide B	Natriuretic peptide receptor 1

Table 1. Continued

Gene Pair	Ligand	Receptor
NPPB_NPR2		Natriuretic peptide receptor 2
NPPB_NPR3		Natriuretic peptide receptor 3
NPPC_NPR2	Natriuretic peptide C	Natriuretic peptide receptor 2
NPPC_NPR3		Natriuretic peptide receptor 3
NXPH3_NRXN2	Neurexophilin 3	Neurexin 2
PDAP1_PDGFRB	PDGFA-associated protein 1	Platelet-derived growth factor receptor, β
PDGFA_PDGFRA	Platelet-derived growth factor alpha	Platelet-derived growth factor receptor, α
PDGFA_PDGFRB		Platelet-derived growth factor receptor, β
PDGFB_LRP1	Platelet-derived growth factor β	LDLR-related protein 1
PDGFB_PDGFRA		Platelet-derived growth factor receptor, α
PDGFB_PDGFRB		Platelet-derived growth factor receptor, β
PDGFB_S1PR1		Sphingosine-1-phosphate receptor 1
PDGFD_PDGFRA	Platelet-derived growth factor D	Platelet-derived growth factor receptor, α
PDGFD_PDGFRB		Platelet-derived growth factor receptor, β
PENK_OGFR	Proenkephalin	Opioid growth factor receptor
PTN_PLXNB2	Pleiotrophin	Plexin B2
PTN_PTPRB		Protein tyrosine phosphatase, receptor type, B
PTN_SDC1		Syndecan 1
PTN_SDC3		Syndecan 3
RARRES2_CCRL2	Retinoic acid receptor responder 2	Chemokine (C-C motif) receptor-like 2
RELN_ITGA3	Reelin	Integrin, α 3
SEMA3C_PLXND1	Semaphorin 3C	Plexin D1
SEMA3F_NRP1	Semaphorin 3F	Neuropilin 1
SEMA3F_NRP2		Neuropilin 2
SEMA4C_PLXNB2	Semaphorin 4C	Plexin B2
SEMA6D_KDR	Semaphorin 6D	Kinase insert domain receptor
SFRP1_FZD6	Secreted frizzled-related protein 1	Frizzled class receptor 6
SLIT2_SDC1	Slit homolog 2	Syndecan 1
TGFB1_ACVRL1	Transforming growth factor, β 1	Activin A receptor type II–like 1
TGFB1_CD109		CD109 molecule
TGFB1_SDC2		Syndecan 2
TGFB1_TGFBR2		Transforming growth factor, β receptor 2
TGFB1_TGFBR3		Transforming growth factor, β receptor 3
TGFB2_ACVR1	Transforming growth factor, β 2	Activin A receptor, type I
TGFB2_ENG		Endoglin
TGFB2_TGFBR2		Transforming growth factor, β receptor 2
TGFB2_TGFBR3		Transforming growth factor, β receptor 3
TGFB3_TGFBR2	Transforming growth factor, β 3	Transforming growth factor, β receptor 2
TGFB3_TGFBR3		Transforming growth factor, β receptor 3
TNFSF12_TNFRSF12A	TNF superfamily, member 12	TNF receptor superfamily, member 12A
TNFSF12_TNFRSF25		TNF receptor superfamily, member 25
TNFSF13_FAS	TNF superfamily, member 13	Fas cell surface death receptor
TNFSF13_SDC2		Syndecan 2
TNFSF13_TNFRSF1A		TNF receptor superfamily, member 1A
VEGFA_SIRPA	Vascular endothelial growth factor A	Signal-regulatory protein α
VEGFA_TYRO3		TYRO3 protein tyrosine kinase
VEGFC_FLT1	Vascular endothelial growth factor C	Fms-related tyrosine kinase 1
VEGFC_FLT4		Fms-related tyrosine kinase 4
VEGFC_ITGB1		Integrin, β 1
VEGFC_KDR		Kinase insert domain receptor
VEGFC_LYVE1		Lymphatic vessel endothelial hyaluronan rec 1
VEGFC_NRP2		Neuropilin 2
WNT4_FZD6	Wingless-type MMTV integration site 4	Frizzled class receptor 6

This table is limited to signaling proteins, cytokines, growth factors, and chemokines. For other cell-surface ligand-receptor pairs, we refer to Data S1. BMP indicates bone morphogenetic protein; CD, cluster of differentiation; EGF, epidermal growth factor; LDLR, low-density lipoprotein receptor; MMTV, mouse mammary tumor virus; PDGF, platelet-derived growth factor; TEK, endothelial tyrosine kinase; TK, tyrosine kinase; TNF, tumor necrosis factor; TYRO, tyrosine-protein kinase receptor; and Wnt, wingless-type integration site family, member 1.

another 10 are expressed by both cardiomyocytes and fibroblasts (Table S3); just 4 are expressed specifically by cardiomyocytes. If one considers not only the ligands but the complete 79 ligand-receptor pairs expressed by cardiomyocytes, 31 are expressed by all 3 cell types and another 36 are expressed by both cardiomyocytes and fibroblasts; just 9 are expressed specifically by cardiomyocytes. What is also interesting is that most ligands expressed by all 3 cell

Table 2. Ligands Expressed by Cardiomyocytes, Endothelial Cells, and Fibroblasts

Gene	Ligand
ADM	Adrenomedullin
ANGPT2	Angiopoietin 2
ANXA1	Annexin A1
CTGF	Connective tissue growth factor
CXCL12	Chemokine (C-X-C motif) ligand 12
CYR61	Cysteine-rich, angiogenic inducer, 61
DKK1	Dickkopf wingless-type integration site family, member 1 signaling pathway inhibitor 1
GAS6	Growth arrest–specific 6
HGF	Hepatocyte growth factor
IGF2	Insulin-like growth factor 2
JAG1	Jagged 1
MDK	Midkine
NPPB	Natriuretic peptide B
PDAP1	PDGFA-associated protein 1
PDGFA	Platelet-derived growth factor α
PDGFD	Platelet-derived growth factor D
PSAP	Prosaposin
PTN	Pleiotrophin
SEMA3F	Semaphorin 3F
TGFB1	Transforming growth factor, β 1
TGFB2	Transforming growth factor, β 2
VEGFA	Vascular endothelial growth factor A

PDGF indicates platelet-derived growth factor.

types have been shown to play important roles in cardiac biology and in cardiac remodeling; examples are adrenomedullin, connective tissue growth factor, chemokine (C-X-C motif) ligand 12/stromal cell-derived factor-1, natriuretic peptide B or brain natriuretic peptide (BNP), TGFβ, and VEGFA. This substantial overlap in extracellular ligands and their receptors has several potential implications. First, it provides an indication that the 3 most abundant cardiac cell types can express themselves in unison in response to physiological and pathophysiological stimuli. Second, the substantial overlap in ligand expression could also function as an equalizer for heterogeneity in cellular composition within the myocardium. Third, it validates the concept that intercellular communication is crucial in cardiac biology and that different cell types in the myocardium share a common language for this. Fourth, regulation of intercellular communication will be a matter of subtle quantitative changes in ligand and receptor expression, rather than a qualitative on-off phenomenon. Unfortunately, the tools most frequently used in studying the biology of intercellular communication in vivo are transgenic and knockout mice, which replicate an on-off phenomenon. This notion does not invalidate these studies, but indicates that caution is warranted in overinterpretation of the results.

Although there exists a substantial overlap in extracellular ligand expression by the 3 main cardiac cell types, some ligands are specific to 1 cell type. For instance, apelin and its receptor, called apelin receptor, are expressed only by endothelial cells. (The role of autocrine apelin signaling in endothelial cells will be discussed later.) The same is true for growth differentiation factor 11 and its receptor (activin A receptor types IB, IIA, and IIB) and for stem cell factor (Kit ligand) and its receptor (receptor tyrosine kinase [c-Kit]).

The ligand-receptor pairs listed in Table 1 and in Table S1 and S2 are potentially involved in autocrine signaling in the heart, but we should be cautious not to overinterpret these transcriptomic data on a functional level. As discussed in Figure 3 (steps 1 and 11), transcription of the ligand and receptor is obligatory to establish an autocrine loop but not sufficient, because they are just 2 pieces of the complex autocrine puzzle. Also, transcriptomic data provide an overall view on differences and similarities between different cardiac cell types for expression of extracellular ligands and their receptors, but at the same time transcriptomic data also tend to provide variable results between different experiments if one focuses on a single gene. As an example, when comparing an RNA-sequencing experiment on freshly isolated rat cardiac endothelial cells with an experiment on cultured human cardiac endothelial cells, just 39.3% (107 of 272) of the ligand-receptor pairs is exactly the same between both experiments (Data S1). This can be explained by differences in species, in vitro cell culture, biological variations, genes that just make or miss the expression threshold, and statistical analyses of a large number of events. Therefore, as with all transcriptomic data, the tables in this review should not be viewed as definite lists of cell-specific autocrine factors in the myocardium, because for many factors, functional experiments have yet to be performed. Another note of caution for these tables is that the ligand-receptor pairs are derived from normal physiology; the results would most likely be different if they were derived from experiments involving cardiac remodeling.

In the next sections, we will expand on factors for which there is solid evidence in the literature that they are involved in autocrine signaling in the myocardium (Table 3).[31,32] An interesting fact to note is that most examples described in the literature are focused on autocrine loops in cardiomyocytes, whereas transcriptomic data indicate that endothelial cells and fibroblasts express more ligand-receptor pairs than cardiomyocytes.

Table 3. Major Autocrine Pathways Playing a Role in Cardiac Remodeling

Ligand	Cell Type	Receptor	In Vivo Evidence	Signaling Pathways	References
Adiponectin	Cardiomyocyte	ADIPOR1, ADIPOR2, T-cadherin	Antihypertrophic	AMPK	73
ANGPTL2	Cardiomyocyte	?	Decreased metabolism	AKT, SERCA2a	64
Apelin	Endothelial cell	APJ	Anti-inflammatory	PKC, PI3K	76
CCN2/CTGF	Myofibroblast	Integrins, HSPGs, LRPs, TrkA	Profibrotic	PI3K, ERK1/2	98
CGRP	Fibroblast	CRL/RAMP1/RCP	Antifibrotic	Adenylate cyclase/cAMP	100
CNP	Cardiomyocyte	NPR-C, NPR-B	Antihypertrophic	ERK1/2, PI3K, cGMP	33
CNP	Endothelial cell	NPR-C, NPR-B	Proangiogenic	ERK1/2, PI3K	33
CNP	Fibroblast	NPR-C, NPR-B	Antifibrotic	ERK1/2, cGMP	33
FGF2	Fibroblast	FGFR2	Profibrotic	PI3K, AKT, JAK-STAT	12,94,95
FGF21	Cardiomyocyte	FGFR1/β-klotho	Antihypertrophic, antioxidative	SIRT1, SOD2, UCP3	49,51
FGF23	Cardiomyocyte	FGFR4	Prohypertrophic	PLCγ, NFAT	43
FSTL1	Cardiomyocyte	?, DIP2A, TLR4, BMP receptors	Antihypertrophic	AMPK	61
HB-EGF	Cardiomyocyte	EGFR	Prohypertrophic	PI3K-AKT, ERK1/2/5, COX2, JAK-STAT	55
IL11	Fibroblast	IL11RA, GP130	Profibrotic	ERK	92
Leptin	Cardiomyocyte	LEPR	Prohypertrophic	JAK-STAT, SHP2-STAT, ERK1/2, PI3K	68
MIF	Cardiomyocyte	CD74/CD44, CXCR2, CXCR4, CXCR7	Antihypertrophic, prosurvival	cAMP, AMPK	56–59
NRG1	Endothelial cell	ERBB4	Proangiogenic	ERK1/2, AKT	31
TGFβ	Endothelial cell	ACVRL1, TGFBR1	EndoMT, proangiogenic	Smad2/3	85,86
VEGF	Endothelial cell	VEGFR2	Prosurvival	RAS, RAF, ERK1/2, MAPK	78
WISP1	Endothelial cell	?, integrin-αVβ5, integrin-αVβ3	Proangiogenic	AKT	89

ACVRL1 indicates activin A receptor type II–like 1; ADIPOR, adiponectin receptor; AKT, protein kinase B; AMPK, AMP-activated protein kinase; ANGPTL2, angiopoietin-like protein 2; APJ, apelin receptor; BMP, bone morphogenetic protein; CCN, cellular communication network factor; CD, cluster of differentiation; CGRP, calcitonin gene–related peptide; CNP, C-type natriuretic peptide; COX2, cyclooxygenase 2; CRL, calcitonin receptor-like receptor; CTGF, connective tissue growth factor; CXCR, chemokine (C-X-C motif) receptor; DIP2A, disco interacting protein 2 homolog A; EGFR, epidermal growth factor receptor; EndoMT, endothelial-mesenchymal transition; ERBB, erythroblastic leukemia viral oncogene homolog; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR, FGF receptor; FSTL1, follistatin-like 1; GP, glycoprotein; HB-EGF, heparin binding–epidermal growth factor; HSPG, heparan sulfate proteoglycan; IL11, interleukin 11; IL11RA, interleukin 11 receptor A; JAK, Janus kinase; LEPR, leptin receptor; LRP, lipoprotein lipase-related protein; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; NFAT, nuclear factor of activated T cell; NPR, natriuretic peptide receptor; NRG1, neuregulin 1; PI3K, phosphatidylinositol 3 kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; RAMP1, receptor activity modifying protein 1; RCP, receptor component protein; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium–ATPase 2a; SHP2, Src homology 2 domain containing non-transmembrane protein tyrosine phosphatase; SIRT1, sirtuin 1; SOD2, superoxide dismutase 2; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TGFBR1, TGF β receptor 1; TLR4, toll-like receptor 4; TrkA, tropomyosin receptor kinase A; UCP3, uncoupling protein 3; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; and WISP1, Wnt1-induced secreted protein-1.

C-TYPE NATRIURETIC PEPTIDE: A PANCELLULAR AUTOCRINE FACTOR IN THE HEART

As discussed above and shown in Table 2, most ligand-receptor pairs present on cardiomyocytes are also present on cardiac endothelial cells and fibroblasts. Autocrine factors present on all major cell types in the heart could be named “pancellular” autocrine factors. Obviously, it will be hard to establish whether pancellular ligand-receptor pairs are present on every single cell type in the heart and thus truly pancellular, but this is superfluous because together (cardiomyocytes, endothelial cells, and fibroblasts) represent >80% of all cells in the myocardium.[7] Nevertheless, demonstrating autocrine activity in all 3 cell types in the myocardium requires a large number of high-quality studies and, therefore, is a high bar to pass; C-type natriuretic peptide (CNP) passes that bar.

CNP is a small 22 amino acid peptide, encoded by the *NPPC* gene, that is structurally related to atrial natriuretic peptide (ANP) and BNP.[33] CNP is produced by cardiomyocytes, endothelial cells, and fibroblasts.[33] Each of these cell types also express natriuretic peptide receptors (NPRs) B and C and, interestingly, levels

of NPR-C in endothelial cells are higher than those of NPR-B.[33] Although ANP and BNP act as hormones, CNP is quickly degraded in blood, indicating that the actions of CNP are more localized and thus paracrine and autocrine.[33] Consistently, serum levels of CNP are higher in the coronary sinus than in arterial blood, indicating the myocardium is an important production site.[34] Production of CNP can be increased by FGF2, TGFβ, and endothelin-1, at least in cultured fibroblasts.[35] CNP has antifibrotic effects in the myocardium by reducing fibroblast growth and extracellular matrix production.[35] Stimulation of cultured fibroblasts with CNP increases their cGMP levels and suppresses collagen synthesis.[35]

Cardiomyocyte- and fibroblast-specific *Nppc*-null mice have a normal cardiac structure and function, indicating that autocrine/paracrine CNP signaling plays no important role during cardiac development and in normal cardiac physiology.[36] Cardiomyocyte- and fibroblast-specific *Nppc*-null mice, however, show increased ventricular dilation and more collagen deposition, compared with wild-type mice, in response to pressure overload or sympathetic hyperactivation; cardiomyocyte-specific *Nppc*-null mice also show more hypertrophy in response to pressure overload or sympathetic hyperactivation, indicating that autocrine/paracrine CNP signaling counterbalances myocyte hypertrophy and collagen formation.[36] Mouse models with cell-specific deletion of NPR-C and NPR-B would help to better understand intramyocardial signaling of CNP, but these models are not available. However, total-body deletion of the gene coding for the receptor NPR-C, *Npr3*, resulted in comparable cardiac dysfunction, hypertrophy, and fibrosis in mice subjected to aortic banding, whereas total-body deletion of the gene coding for NPR-B, *Npr2*, did not result in comparable cardiac dysfunction.[36] Accordingly, these data suggest that NPR-C mediates the effects of CNP in myocytes and fibroblasts. Some of the effects of endogenous CNP will be paracrine in nature, but a fair conclusion is that CNP, secreted by cardiomyocytes and fibroblasts, acts as an autocrine negative feedback factor during cardiac remodeling.

With regard to the endothelium, endothelium-specific *Nppc* deletion did not change the hypertrophic and fibrotic response to aortic banding,[36] indicating that the paracrine release of CNP by endothelial cells is of little importance. In contrast, the autocrine signaling of endothelium-derived CNP seems to be more important, as it has been demonstrated that endothelium-specific *Nppc* deletion impairs bradykinin-, acetylcholine-, and flow-mediated vasodilatory responses of coronary arteries in mice.[36] The most logical conclusion that can be drawn from these data is that autocrine CNP is essential for maintenance of endothelial function in coronary circulation. CNP not only maintains endothelial function but also has proangiogenic properties. In vitro, for instance, CNP induces endothelial tube and capillary network formation, to a similar extent as VEGF.[37] In vivo, gene transfer of CNP into ischemic muscle increases capillary density and blood flow in a model of hind limb ischemia.[37] Also, de novo aortic sprouting, endothelial tubule formation, and restoration of blood flow following hind limb ischemia are diminished in mice with endothelium-specific *Nppc* deletion or total-body *Npr3* deletion, coding for NPR-C.[38] These data endorse autocrine signaling of CNP during normal endothelial function.

As indicated earlier, ANP and BNP have a hormonal function by inducing natriuresis in the kidneys, but both ANP and BNP also have autocrine functions. The autocrine/paracrine functions of ANP and BNP have been extensively reviewed previously.[39,40] In brief, both ANP and it receptor NPR-A are expressed by cardiomyocytes and ANP secretion increases during pressure or volume overload.[39] ANP induces antihypertrophic activity in cardiomyocytes by increasing intracellular cGMP levels[39]; thus, ANP/NPR-A functions as an antihypertrophic autocrine loop in cardiomyocytes. BNP interacts with both the NPR-A and the NPR-B receptor.[41] Similar to ANP, BNP expression increases in cardiomyocytes during pressure or volume overload, but the effects of BNP on cardiomyocyte hypertrophy seem to be more limited than the antihypertrophic effects of ANP. The major role of BNP in cardiac remodeling appears to be antifibrotic and thus mostly paracrine in nature.[41] Endothelial cells also express ANP and NPR-A; and ANP has been shown to induce angiogenesis in vitro.[42] Furthermore, endothelial-specific deletion of Npr1, the gene coding for NPR-A, increases capillary rarefaction after aortic banding in mice.[42]

THE 2 FACES OF FGFS AS AUTOCRINE SIGNALS IN CARDIOMYOCYTES

Many FGFs are produced in the heart, but autocrine signaling has been demonstrated in just a couple of them. Herein, we discuss 2 FGFs with an autocrine role in cardiomyocytes: FGF23 and FGF21 (Figure 5).

The classic source of FGF23 is osteocytes, and the classic targets of FGF23 are the kidneys and parathyroid glands. The main function of FGF23 is thought to be maintenance of phosphate and mineral homeostasis.[43] New data indicate that FGF23 is also produced in the heart, and that it plays a role in cardiac remodeling and heart failure.[43] Cardiac FGF23 is produced by cardiac myocytes, endothelial cells, fibroblasts, and inflammatory macrophages. Expression of cardiac *FGF23* and its receptor, *FGFR4*, positively correlates

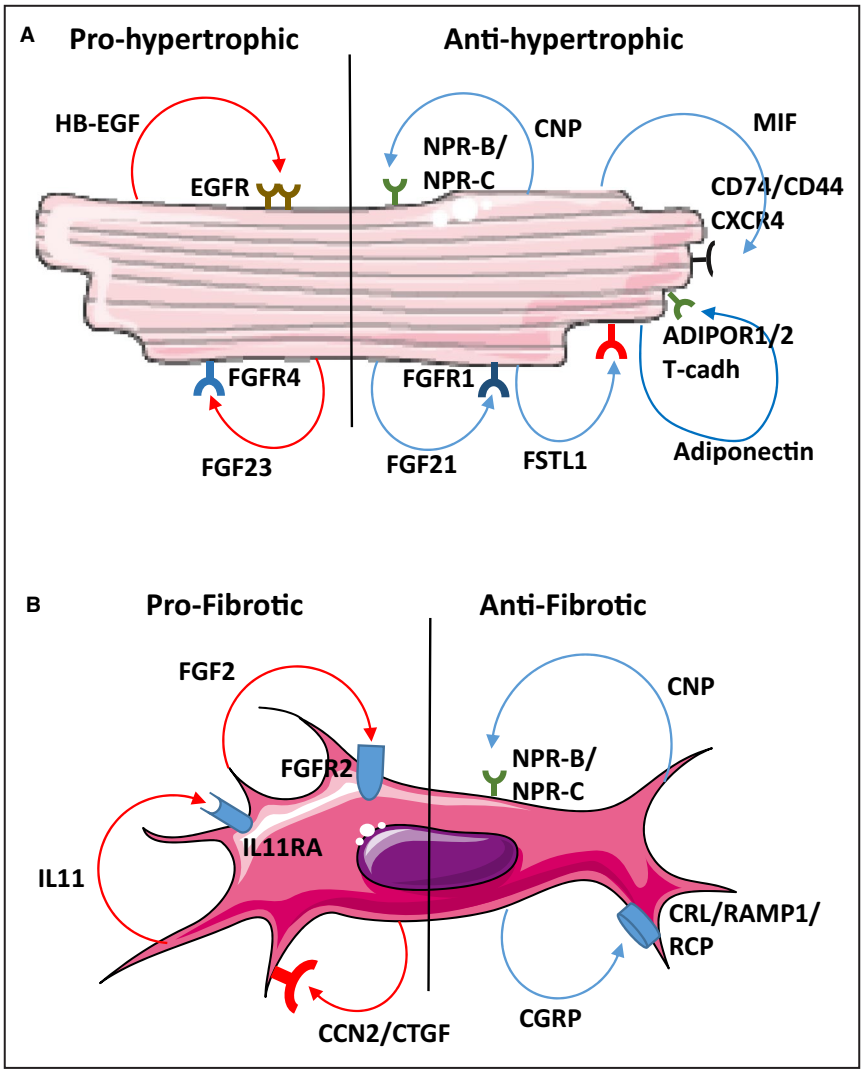

Figure 5. Overview of prohypertrophic and antihypertrophic loops in cardiomyocytes and profibrotic and antifibrotic loops in fibroblasts.
A, Heparin binding–epidermal growth factor (HB-EGF) and fibroblast growth factor (FGF) 23 are 2 examples of prohypertrophic autocrine signaling proteins in cardiomyocytes, whereas C-type natriuretic peptide (CNP), macrophage migration inhibitory factor (MIF), adiponectin, FGF21, and follistatin-like 1 (FSTL1) are examples of antihypertrophic autocrine signals. FSTL1 binds to multiple proteins; therefore, a specific receptor has not been included in this image. **B**, FGF2, interleukin (IL) 11, and cellular communication network factor (CCN) 2 are examples of profibrotic autocrine signaling proteins in fibroblasts. CCN2 binds to multiple receptors and extracellular matrix proteins. CNP and calcitonin gene–related peptide (CGRP) are examples of autocrine signaling proteins in fibroblasts with antifibrotic properties. ADIPOR indicates adiponectin receptor; CD, cluster of differentiation; CRL, calcitonin receptor-like receptor; CTGF, connective tissue growth factor; CXCR, chemokine (C-X-C motif) receptor; EGFR, epidermal growth factor receptor; FGFR, FGF receptor; IL11RA, interleukin 11 receptor A; NPR, natriuretic peptide receptor; RAMP1, receptor activity modifying protein 1; RCP, receptor component protein; and T-cadh, T-cadherin.

with the development of left ventricular hypertrophy.[43–45] Activation of FGF receptor (FGFR) 4 activates phospholipase Cγ in cardiac myocytes, and induces hypertrophic cell growth using calcineurin/nuclear factor of activated T cell signaling.[43,46] Too, FGF23 increases intracellular calcium levels in cardiac myocytes and promotes contractility of isolated cardiac myocytes and ventricular muscle strips.[47] Cardiomyocyte-specific *Fgfr4*-null mice did not show hypertrophy in response to administration of FGF23 in contrast to wild-type mice.[48] These data indicate a feed-forward autocrine loop because *Fgf23* expression in

cardiomyocytes increases in response to hypertrophic stimuli and FGF23 itself can induce a hypertrophic response in cardiomyocytes. More research is needed to fully understand this feed-forward loop, however. For example, whole-body genetic deletion of *Fgf23* did not affect the hypertrophic response of murine hearts in response to aortic banding.[45] Experiments using transgenic mice with cell-specific deletion of *Fgf23* or its receptor *Fgfr4* could be more informative, and allow easier separation of paracrine and autocrine effects. In particular, do cardiomyocyte-specific *Fgfr4*-null mice develop cardiac hypertrophy when challenged with pressure overload? Another open question is whether burosumab, a monoclonal antibody against FGF23 developed for the treatment of hypophosphatemic rickets, interferes with the autocrine loop or has any effect on cardiac hypertrophy.

FGF21 is a hepatokine, a hormone produced mainly by the liver, that controls glucose, lipid, and energy metabolism.[49] FGF21 has antihypertrophic effects on the heart by its binding to FGFR1 (which is also expressed by cardiomyocytes), an interaction that is facilitated by β-klotho that serves as a Zip code for FGF21.[49,50] Expression of *Fgf21* can be induced in cardiomyocytes by lipopolysaccharide, a process that is mediated by the epigenetic regulator sirtuin-1.[51] FGF21, secreted by cardiomyocytes, can then bind to FGFR1 in an autocrine manner and activate sirtuin-1, completing the transactivation of the FGF21 autocrine loop. It has been reported that FGF21 mitigates reactive oxygen species production in cardiomyocytes by induction of superoxide dismutase 2 and mitochondrial UCP3 (uncoupling protein 3).[49,51] Therefore, it seems that FGF21 is induced in cardiomyocytes by inflammatory stimuli and acts as an antioxidative factor in the same cells. Deletion of the *Fgfr1* gene in cardiomyocytes is probably less informative in the study of FGF21 as an autocrine factor, because FGFR1 acts as receptor for many different FGFs.

HB-EGF AND SPATIAL RESTRICTION OF AUTOCRINE SIGNALING

The EGF receptor system consists of 4 receptors (EGFR, erythroblastic leukemia viral oncogene homolog [ERBB] 2, ERBB3, and ERBB4) and several ligands, including EGF and HB-EGF. HB-EGF expression in the heart is induced by mechanical overload, and the HB-EGF/EGFR autocrine signaling loop is an essential part of the hypertrophic response, as shown >2 decades ago.[52] The study of autocrine signaling in the ERBB receptor system is complicated because multiple ligands bind to multiple receptors, all expressed by multiple cell types in a single tissue. HB-EGF is a special member of the EGF family, because its heparin-binding domain increases interactions with heparan-sulfate moieties present in the cellular glycocalyx and in the extracellular matrix, thus creating a local pool of HB-EGF in the vicinity of the producing cell. It has been shown that cardiomyocytes express both HB-EGF and EGFR and that HB-EGF expression in cardiomyocytes increases with hypertrophic stimuli in vitro and that HB-EGF itself induces cardiomyocyte hypertrophy as well.[53] The main signaling pathways involved are the extracellular signal-regulated kinase–1/2/5, cyclooxygenase-2, Janus kinase/signal transducer and activator of transcription, and phosphatidylinositol 3 kinase/protein kinase B pathways.[54]

Yoshioka and coworkers have developed an ingenious in vivo method to deal with the problem of ligand and receptor promiscuity.[55] They injected an adenoviral vector encoding HB-EGF as well as GFP (green fluorescent protein), allowing visualization of transfected cardiomyocytes. Next, they studied the hypertrophic response of the transfected cardiomyocytes, as well as adjacent myocytes and remote myocytes. They showed that HB-EGF secretion by a given cardiomyocyte leads to cellular hypertrophy in the overexpressing cell and in adjacent cells but not in remote cells.[55] These findings indicate that HB-EGF acts as an autocrine and local paracrine prohypertrophic factor and that cells can coordinate growth with their immediate neighboring cells with highly localized HB-EGF signaling. Obviously, HB-EGF is not the only factor that is spatially restricted, many factors discussed in this review are spatially restricted to some extent, but it is one of the few factors for which it has been demonstrated in vivo that spatial restriction is important in mediating its physiologic effects.

NEGATIVE AND POSITIVE AUTOCRINE REGULATORS OF CARDIOMYOCYTE HYPERTROPHY

Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine and regulator of innate immunity expressed in various cell types, including epithelial cells, endothelial cells, mesenchymal cells, and cardiomyocytes.[56,57] MIF binds to several receptors, most importantly cluster of differentiation 74/cluster of differentiation 44, but also chemokine (C-X-C motif) receptors 2, 4, and 7.[56] MIF is secreted by cardiomyocytes and acts as an autocrine factor by its binding to cluster of differentiation 74.[58] MIF signaling in cardiomyocytes seems mostly mediated by AMP-activated protein kinase phosphorylation.[58] Data indicate that MIF could function as an autocrine cardioprotective

factor, because it is upregulated by cardiac ischemia and because *Mif* deletion exacerbates the ischemic injury.[58] Also, MIF is upregulated in models of pressure overload, and *Mif*-null mice show a more pronounced hypertrophic response.[59] It has been suggested that the antihypertrophic effects of MIF are in part mediated by its control of oxidation-reduction homeostasis in cardiomyocytes.[60] In summary, MIF is a cardiomyocyte-derived factor with antihypertrophic effects in the same cell type.

Another protein with autocrine antihypertrophic signaling mediated by AMP-activated protein kinase is follistatin-like 1 (FSTL1).[61] FSTL1 is a glycoprotein secreted by several cells, including endothelial cells and cardiac myocytes.[6,61] Cardiac *Fstl1* expression is induced by ischemia and pressure overload,[62] it is expressed in the human failing heart, and circulating FSTL1 levels are increased in patients with acute coronary syndrome.[63] Although a specific receptor for FSTL1 has not been assigned yet, interaction of FSTL1 with disco interacting protein 2 homolog A, toll-like receptor 4, and BMP (bone morphogenetic protein) receptors has been demonstrated. There is also convincing evidence that FSLT1 is an autocrine cardioactive factor. For example, mice with cardiomyocyte-specific deletion of *Fstl1* show decreased cardiac levels of FSTL1, with the production of FSTL1 by endothelial cells unaffected, and an increased hypertrophic response after aortic banding.[61] Consistent with this, transgenic mice overexpressing *Fstl1* show a decreased hypertrophic response.[61] Therefore, FSTL1 acts as a mostly autocrine antihypertrophic factor during pressure overload.

ANGPTL2 (angiopoietin-like protein 2) is an autocrine prohypertrophic factor playing a deleterious role in heart failure progression.[64] Cardiac expression of ANGPTL2 is found both in cardiomyocytes and noncardiomyocytes, and expression of *Angptl2* increases during pathological, but not physiological, remodeling of the myocardium.[64] In neonatal cardiomyocytes, expression of *Angptl2* can be induced by AngII or isoproterenol.[64] Transgenic cardiomyocyte-specific overexpression of the *Angptl2* gene results in progressive cardiac dilatation and decreased contractility.[64] In contrast, *Angptl2*-null mice were more resistant to cardiac remodeling after aortic banding and show upregulated protein kinase B/sarcoplasmic/endoplasmic reticulum calcium–ATPase 2a signaling.[64] Together, these data indicate that activation of ANGPTL2 in cardiomyocytes by cardiac stressors induces a maladaptive positive feed-forward autocrine loop. A better understanding of autocrine signaling of ANGPTL2 in the heart will require identification of its main receptor, which is an unresolved matter.[65]

Preferably, in vivo studies of autocrine signaling in the myocardium comprise both deletion and overexpression of the ligand gene, as has been done for both *Fstl1* and *Angptl2*. Ideally, similar studies are also performed with the receptor that is present on cardiomyocytes and other proteins involved in the autocrine signaling loop (eg, activating proteinases) (Figure 3). Some of these transgenic studies can be replaced with specific agonists, antagonists, or enzyme inhibitors if they have been developed, which in many instances is not the case.

ADIPOKINES AS AUTOCRINE SIGNALS IN CARDIOMYOCYTES

Leptin, coded by *Lep*, is a 16-kDa adipokine that inhibits hunger and regulates the energy balance. When these functions of leptin were first discovered, hopes were high that it could be used as a therapy for obesity.[66,67] However, the biology of leptin turned out to be more complex than anticipated. Leptin is not only produced by adipocytes and enterocytes, but also by cardiomyocytes, which are upregulated by AngII or endothelin-1 together with the leptin receptor gene.[68] Inhibition of leptin or its receptor attenuates the hypertrophic response to endothelin-1 and AngII, which suggest an autocrine participation of leptin during the hypertrophic response.[68] The intracellular signaling pathways in cardiomyocytes that are mainly involved in the effects of leptin are Janus kinase–signal transducer and activator of transcription pathways, but also extracellular signal-regulated kinase-1/2 and phosphatidylinositol 3 kinase pathways have been implicated to mediate the effects of leptin.[69] In vivo studies show that obese mice with spontaneous mutations in either the *Lep* gene (referred to as *ob/ob* mice) or the leptin receptor gene (referred to as *db/db* mice) display an increased hypertrophic response, a finding that contradicts the in vitro findings.[70] Using *ob/ob* or *db/db* mice, it is hard to differentiate between direct effects of leptin signaling in the heart and the effects of the obese phenotype on cardiac remodeling itself. A solution to this problem is the creation of mice with cardiomyocyte-specific deletion of the leptin receptor.[71] Unexpectedly, these cardiomyocyte-specific leptin receptor gene–null mice displayed severe abnormalities in cardiomyocyte metabolism within days after leptin receptor gene deletion, a finding prohibiting the use of this model to study more subtle effects on cardiac hypertrophy.[71] However, the results indicate that autocrine leptin signaling plays an important role in cardiomyocyte metabolism.

The molecular weight of adiponectin (coded by the *ADIPOQ* gene) is almost twice as large (30 kDa) as leptin, and monomeric adiponectin easily forms trimers, hexamers, and even larger 12-mers to 18-mers,

reaching gigantic molecular weights of >500 kDa. Adiponectin is an insulin sensitizer, and its main effects are antidiabetic, anti-inflammatory, and antiatherogenic.[72] It is the most abundant protein secreted by adipocytes, but is also, to a lesser extent, secreted by other cell types, including cardiomyocytes.[72] Cardiomyocytes produce adiponectin and its receptors (adiponectin receptor [ADIPOR]1, ADIPOR2, and T-cadherin), enabling an autocrine loop.[73] Interaction of adiponectin with its receptors leads to stimulation of AMP-activated protein kinase in cardiomyocytes.[73] Moreover, heart failure and cardiac remodeling change the production of adiponectin and its receptors by cardiomyocytes. For instance, levels of adiponectin and its receptors in cardiomyocytes decrease in humans with dilated cardiomyopathy and in humans with cardiac hypertrophy.[73] Studies in mice indicate that adiponectin acts as a protective factor against cardiac remodeling, on the basis of the finding that deletion of the *Adipoq* gene or the *Cdh13* gene (coding for T-cadherin) increases the hypertrophic response.[73] It appears that downregulation of adiponectin in cardiomyocytes is an enabling factor in the pathophysiology of cardiac remodeling. As a result, pharmacological administration of adiponectin could be a therapeutic strategy in cardiac remodeling.

APELIN: FROM PARACRINE SIGNAL TO PROTECTOR OF ENDOTHELIAL CELL FUNCTION

Apelin is one of the most potent endogenous inotropic substances and is primarily expressed in endothelial cells (Table 1).[6,74] The apelin gene (*APLN*) codes for a 77 amino acid preproprotein, which results in a 55 amino acid proprotein after cleavage of the signal peptide. The proprotein can be cleaved in active apelin peptides of different sizes (ranging from 12 to 55 amino acids) that all include the C-terminal fragment.[75] The receptor for apelin is the G-protein–coupled apelin receptor (APJ) receptor.[74] APJ receptors are present on many different cell types, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells. In contrast to many other positive inotropic substances, apelin is also a cardioprotective factor that does not induce cardiomyocyte hypertrophy. Moreover, apelin induces vasodilation and as a result decreases left ventricular preload and afterload.[6]

Evidence for autocrine endothelial apelin signaling came from a study demonstrating that apelin preserves endothelial integrity in models of immune-mediated vascular injury.[76] Alloimmune-mediated vascular injury, induced by histocompatibility complex, mismatched heart transplantation in mice, which resulted in an upregulation of apelin in cardiac microvascular endothelial cells.[76] When donor hearts, derived from apelin-knockout mice, were transplanted into histocompatibility complex–matched recipient mice, a more pronounced vascular injury was observed with immune cell infiltration and blunted vascular repair.[76] Autocrine apelin signaling in endothelial cells decreases transendothelial migration of immune cells (eg, monocytes).[76] The APJ receptor activates protein kinase C and phosphatidylinositol 3 kinase in endothelial cells.[77] Overall, apelin seems to act both as a paracrine and an autocrine factor in normal cardiac physiology and in pathophysiology.

NRG1 IS CAPTURED BY ITS RECEPTOR ON ENDOTHELIAL CELLS

NRG1 is a cardioprotective protein secreted in the heart, mainly by endothelial cells.[3,4,31] The generally accepted concept is that NRG1 functions as an endothelium-derived paracrine signaling factor by activating ERBB4 receptors, which dimerize with ERBB2 receptors, in cardiomyocytes.[3] Administration of NRG1, in models of heart failure and AngII-induced cardiac remodeling, results in less cardiomyocyte apoptosis and hypertrophy.[3,25] More recent data indicate that NRG1 also affects other cell types, including fibroblasts and macrophages; NRG1 inhibits fibrosis and inflammation in the myocardium, but also in other organs, including lung, skin, and kidneys.[3,24,25]

Because NRG1 also induces angiogenic responses in endothelial cells, suggesting the existence of an autocrine loop, we studied cardiac remodeling in mice with endothelial-specific deletion of *Erbb4*.[31] Surprisingly, *Erbb4* deletion in endothelial cells did not affect myocardial capillary density during remodeling, but instead attenuated fibrosis induced by aortic banding or AngII infusion.[31] Because transcription levels of profibrotic or antifibrotic factors, secreted by endothelial cells, remained fairly unchanged by *Erbb4* deletion, one potential interpretation was that *Erbb4* deletion in endothelial cells diminishes the amount of NRG1 captured by endothelial cells, leaving more NRG1 available for antifibrotic paracrine signaling. As discussed above, capture of a ligand by its receptor has been demonstrated for EGF/EGFR,[20] a ligand-receptor pair of the same family and similar in structure to NRG1/ERBB4. Capture function of endothelial ERBB4 receptors, allowing fine-tuning of paracrine NRG1 signaling, is an exciting hypothesis that deserves further testing (eg, in mouse models with endothelium-specific overexpression of *Erbb4*).

VEGF AUTOCRINE SIGNALING PRESERVES ENDOTHELIAL FUNCTION

Autocrine secretion of VEGF by endothelial cells is required for homeostasis of blood vessels, even in

the absence of disease (Table 1).[78] Deletion of *Vegf* in the endothelial lineage leads to endothelial degeneration and premature death in over half of the mice by 25 weeks of age.[78] The autocrine nature of these effects was convincingly demonstrated by Lee and coworkers because they showed that there were no changes in the total levels of *Vegf* mRNA or VEGF protein, indicating that paracrine VEGF originating from other cell types could not compensate for the absence of endothelial VEGF, and that *Vegf*-null endothelial cells did not show phosphorylation of VEGF receptor 2, in contrast to wild-type endothelial cells.[78] Hearts from endothelial-specific *Vegf*-null mice showed multiple microinfarctions, the presence of intravascular thrombi, disrupted endothelial lining, and accumulation of both von Willebrand factor and fibrinogen.[78] These results indicate that autocrine endothelial VEGF signaling is a crucial part of the antithrombotic properties of normal endothelium.

Recent data suggest that VEGF164 and VEGF188 are the isoforms with an autocrine function in endothelial cells.[79] The endothelium responds to external stimuli by altering the ratio of VEGF164/VEGF188 to improve its barrier function (more VEGF164 production) or to increase permeability (more VEGF188 production).[79] Functional analyses indicate that VEGF164 is the isoform promoting stability of endothelial monolayers, with increased adhesion to matrices and higher vascular endothelial-cadherin levels, resulting in decreased paracellular permeability and increased barrier function.[79] VEGF stimulates endothelial cell proliferation and angiogenesis through VEGF receptor 2–mediated activation of the RAS/RAF/extracellular signal-regulated kinase/mitogen-activated protein kinase pathway.[80]

As discussed earlier in the section on autocrine signaling, polarity of VEGF signaling in endothelial cells has been demonstrated in the brain. Future studies on endothelial cell polarity in the myocardium will provide crucial insight in endothelial function and cardiac remodeling.

ROLE OF AUTOCRINE SIGNALING IN ENDOTHELIAL-MESENCHYMAL TRANSITION

Endothelial-mesenchymal transition (EndoMT) is the process in which endothelial cells change their phenotype into mesenchymal cells (ie, fibroblast-like cells) and contribute to production of the extracellular matrix. EndoMT is crucial during embryological development (eg, in the development of cardiac valves), but has also been implicated in postnatal processes in the heart, in particular in maladaptive remodeling and cardiac fibrosis.[81] TGFβ is a well-known profibrotic growth factor that activates serine and threonine kinase receptors, activin A receptor type II–like 1, and TGF β receptor 1 (Table 1).[82] A large number of publications have indicated that TGFβ is crucial for the induction of EndoMT in endothelial cells.[83,84] Interestingly, recent in vitro data indicate that an autocrine TGFβ-mediated loop could be involved in EndoMT.[85] Hypoxia followed by reoxygenation in cultured microvascular endothelial cells increased *Tgfb*1 expression in these cells, which, in turn, induced their transition into myofibroblasts.[85] Others studies in cultured human primary endothelial cells, but also in zebra fish and aortic rings, indicate that an autocrine TGFβ-mediated loop is also important in proangiogenic effects of insulin on endothelial cells.[86] Thus, depending on the conditions, an autocrine TGFβ-mediated loop can be involved in EndoMT as well as angiogenesis. Future studies on the autocrine loop of TGFβ remain necessary, because EndoMT remains a controversial subject in the field of cardiac remodeling.[87]

AUTOCRINE SIGNALING IN ANGIOGENESIS FOLLOWING MYOCARDIAL INFARCTION

WISP1 (Wnt1-induced secreted protein-1)/cellular communication network factor (CCN) 4 is a member of a family of growth factors that also includes the cysteine-rich 61 (CCN1), which is part of ligand-receptor pairs in all 3 cell types (Table 2), and connective tissue growth factor (CCN2).[6,88] Although no definitive proof for the WISP1 receptor has been provided, recent evidence indicates an autocrine role in cardiac endothelial cells. Human cardiac endothelial cells not only produce WISP1, but are also responsive to it, as demonstrated by an increased angiogenic response and an increased production of VEGFA.[89] WISP1 production by cardiac endothelial cells in mice increases in the border zone of a myocardial infarct.[89] WISP1 levels are upregulated during cardiac remodeling, and expression can be stimulated by tumor necrosis factor and AngII stimulation.[90] Apart from autocrine effects, endothelium-derived WISP1 has a paracrine effect on cardiomyocytes and fibroblasts.[6] For instance, WISP1 induces cardiomyocyte hypertrophy[88] and protects against cardiomyocyte death induced by doxorubicin.[91] WISP1 also induces fibroblast proliferation and, as a result, fibrosis.[88] WISP1 interacts with many extracellular proteins, but cell-surface receptors shown to be involved in intracellular responses are integrin receptors αV and βV.[89] Although no definitive proof for the WISP1 receptor has been provided, recent evidence does indicate an autocrine role in cardiac endothelial cells. WISP1

production by cardiac endothelial cells in mice increases in the border zone of a myocardial infarct.[89] Human cardiac endothelial cells not only produce WISP1, but are also responsive to it, as demonstrated by an increased angiogenic response and an increased production of VEGFA.[89] Future studies should be tailored toward identifying the receptor for WISP1 in endothelial cells, because they will not only provide crucial information on the autocrine effects of WISP1, but also on the process of ischemia-induced angiogenesis.

TRANSACTIVATION OF FIBROBLASTS

Because fibroblasts have to be activated in times of stress, in a dramatic manner, from dormant cells to proliferating and extracellular matrix–secreting cells, transactivation using autocrine signals is more prominent in fibroblast than in endothelial cells or cardiomyocytes (Figure 5). Herein, we discuss 3 important transactivating signals (IL11, FGF2, and CCN2) and 1 negative feedback regulator (calcitonin gene–related peptide [CGRP]).

An important downstream signaling pathway of TGFβ in fibroblasts is transactivation of an autocrine loop of IL11.[92] Autocrine IL11 signaling does not primarily change mRNA levels of profibrotic factors but alters protein levels by activating translational processes and the noncanonical extracellular signal-regulated kinase pathway.[92] Blocking the autocrine IL11/interleukin 11 receptor A (IL11RA) loop limits fibrosis caused by multiple upstream stimuli (TGFβ1, AngII, FGF2, and platelet-derived growth factor) and fibrosis in preclinical models of heart and kidney disease.[92] Targeting autocrine IL11 signaling might provide new opportunities in the search for therapies that target cardiac fibrosis.[93]

FGF2 is secreted by many cell types in normal tissues and by several tumor cells, in which it functions as an autocrine growth factor.[94,95] More recent studies assign an autocrine role to FGF2 and its receptor, FGFR2, in the myocardium as well. More specifically, the FGF2-FGFR2 ligand-receptor pair has been shown to regulate release of prohypertrophic factors in fibroblasts. Germ-line genetic ablation of *Fgf2* in mice results in a blunted hypertrophic response after aortic banding. Because *Fgf2*-null cardiac myocytes responded normally to both AngII and FGF2, a direct effect on cardiomyocytes has been ruled out, but *Fgf2*-null fibroblasts display decreased production of hypertrophic factors in response to FGF2.[12,96]

CCN2, also referred to as connective tissue growth factor, is a profibrotic factor in myocardial remodeling that is secreted by cardiomyocytes, endothelial cells, and fibroblasts.[6] CCN2 binds to various surface receptors, including integrin receptors, heparan sulfate proteoglycans, LRPs (lipoprotein lipase-related proteins), and tropomyosin receptor kinase A, depending on the cell type and its phenotype.[97] Ligand-receptor pairs of connective tissue growth factor/CCN2 are present in all 3 above cell types, as determined by RNA sequencing (Table 2). Previously, the major sources of CCN2 in the myocardium were thought to be cardiomyocytes, but a recent elegant study changed this concept and points toward an autocrine loop.[98] Genetic deletion of *Ccn2* in myofibroblasts, using a Cre-recombinase activated by the periostin promotor, blunted the fibrotic response of the myocardium to AngII infusion in mice.[98] In contrast to the results obtained in myofibroblasts, deletion of *Ccn2* in cardiomyocytes did not change the fibrotic response to AngII infusion.[98] Combined, these data convincingly demonstrate that release of CCN2 by myofibroblasts is an important autocrine profibrotic loop in myocardial fibrosis.

CGRP is a neuropeptide that is coded, together with calcitonin and katacalcin, by the *CALCA* gene. The receptor for CGRP is a complex of 3 proteins: the biggest and ligand-binding part is the calcitonin receptor-like receptor that consists of 7 transmembrane domains; the RAMP1 (receptor activity modifying protein 1), which consists of a single transmembrane domain; and the RCP (receptor component protein), which is an intracellular protein.[99] In the myocardium, CGRP is mostly produced by fibroblasts, and its production can be stimulated by TGFβ.[100] CGRP, secreted by fibroblasts, induces antifibrotic effects, thus, in contrast to IL11, FGF2, and CCN2, functioning as an autocrine negative feedback loop.[100]

FUTURE PERSPECTIVES

Autocrine signaling in the heart is a neglected topic in the scientific literature. Herein, we wanted to give the reader a deeper insight into the concepts of autocrine signaling, as well as an overview of signaling proteins that have been shown to be involved in autocrine signaling in the heart. We did not attempt to provide an exhaustive list, which would be impossible, because what we know now about autocrine signaling loops is just the tip of the iceberg. In the tables in this review, we present a list of putative autocrine signaling pairs, based on expression databases. However, they will remain putative until their role as an autocrine loop in myocardial biology is confirmed by in vitro and in vivo experiments. Also, as indicated before, these tables are derived from cells isolated from healthy myocardium and therefore might not include ligands or receptors that are expressed exclusively during cardiac remodeling.

Technical advances continuously change our capabilities in making new discoveries; the field of autocrine signaling will also benefit from these advances. For instance, a revolution in single-cell RNA sequencing, which started in oncology, also allows for systematic evaluation of paracrine and autocrine signaling in virtually any tissue. Single-cell RNA sequencing provides transcriptomes, including expression of proteins involved in intercellular signaling, of the different cell types present in the myocardium in vivo. This technique will vastly increase our understanding of cell-cell signaling in different phases of cardiac remodeling. Recently, a general characterization of intercellular communication networks of nonmyocytes has been performed using single-cell RNA sequencing, indicating a prominent role for fibroblasts.[8] Analyzing and interpreting these data and expanding on these data in terms of physiology and pathophysiology will be an enormous, but rewarding, task.

Knowledge on autocrine signaling loops in the myocardium is relevant to gain a better understanding of the physiology of the heart, but in the long-term will be of translational value as well. As indicated throughout this review, several of these autocrine loops are viewed as therapeutic targets, not just to treat heart failure, but for various disorders. Surface receptors have been great therapeutic targets in the past, resulting in many marketed therapies. They will remain so in the future.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Data S1
Tables S1–S3

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SUPPLEMENTAL MATERIAL

Data S1. All ligand-receptor pairs identified in different cardiac cell types (see Excel file). Cell types include human cardiomyocytes (CMs), cultured human cardiac endothelial cells (ECs), freshly isolated rat cardiac ECs, and human cultured fibroblasts (fibro). Gene expression levels in all cell-types were determined with RNA sequencing. Minimum expression levels for inclusion in CM and fibro is 10 TPM; minimum expression levels for inclusion in ECs is 1.5 times the median expression level of all genes. Expression levels in ECs has been normalized to median expression level. Only ligand-receptor pairs are shown of which the expression ratio of ligand to receptor is between 0.05 and 20. When this ratio was outside the 0.05 to 20 interval, we assumed that paracrine signaling is probably more important than autocrine signaling. BP indicates binding protein; ECM, extracellular matrix; LDLR, low density liprotein receptor; TM, transmembrane.

Table S1. Autocrine ligand-receptor pairs expressed by isolated human cardiac myocytes.

Gene Pair	Ligand	Receptor
ADM_CALCRL	adrenomedullin	calcitonin receptor-like
ANGPT1_ITGA5	angiopoietin 1	integrin, alpha 5
ANGPT1_TEK		TEK tyrosine kinase, endothelial
ANGPT2_TEK	angiopoietin 2	TEK tyrosine kinase, endothelial
ANGPTL1_TEK	angiopoietin-like 1	TEK tyrosine kinase, endothelial
ANXA1_EGFR	annexin A1	epidermal growth factor receptor
CTGF_LRP1	connective tissue growth factor	LDLR-related protein 1
CTGF_ITGA5		integrin, alpha 5
CXCL12_ITGB1	chemokine (C-X-C motif) ligand 12	integrin, beta 1
CXCL12_SDC4		syndecan 4
CYR61_CAV1	cysteine-rich, angiogenic inducer, 61	caveolin 1
CYR61_ITGA5		integrin, alpha 5
CYR61_ITGB5		integrin, beta 5
DKK1_KREMEN1	dickkopf WNT signaling pathway inhibitor 1	kringle containing transmembrane protein 1
EDN1_ELTD1	endothelin 1	EGF, latrophilin and 7 TM domain containing 1
FGF7_FGFR1	fibroblast growth factor 7	fibroblast growth factor receptor 1
FGF7_NRP1		neuropilin 1
GAS6_AXL	growth arrest-specific 6	AXL receptor tyrosine kinase
GAS6_TYRO3		TYRO3 protein tyrosine kinase
HGF_CD44	hepatocyte growth factor	CD44 molecule
HGF_SDC2		syndecan 2
IGF2_IGF2R	insulin-like growth factor 2 (somatomedin A)	insulin-like growth factor 2 receptor
IGF2_INSR		insulin receptor
IL1B_IL1R1	interleukin 1, beta	interleukin 1 receptor, type I
IL1B_IL1RAP		interleukin 1 receptor accessory protein
IL6_IL6ST	interleukin 6 (interferon, beta 2)	interleukin 6 signal transducer (gp130)
IL8_SDC2	interleukin 8	syndecan 2
IL8_SDC3		syndecan 3
JAG1_NOTCH1	jagged 1	notch 1
JAG1_NOTCH3		notch 3
KAL1_FGFR1	Kallmann syndrome 1 sequence	fibroblast growth factor receptor 1
KAL1_SDC2		syndecan 2
LIF_LIFR	leukemia inhibitory factor	leukemia inhibitory factor receptor alpha
MDK_ITGB1	midkine (neurite growth-promoting factor 2)	integrin, beta 1
MDK_LRP1		LDLR-related protein 1
MDK_SDC4		syndecan 4
MDK_ITGA4		integrin, alpha 4
MDK_SDC3		syndecan 3
NPPB_NPR1	natriuretic peptide B	natriuretic peptide receptor 1
NPPB_NPR3		natriuretic peptide receptor 3
PDAP1_PDGFRB	PDGFA associated protein 1	platelet-derived growth factor receptor, beta
PDGFA_PDGFRA	platelet-derived growth factor alpha	platelet-derived growth factor receptor, alpha
PDGFA_PDGFRB		platelet-derived growth factor receptor, beta

PDGFC_PDGFRA	platelet derived growth factor C	platelet-derived growth factor receptor, alpha
PDGFC_PDGFRB		platelet-derived growth factor receptor, beta
PDGFD_PDGFRA	platelet derived growth factor D	platelet-derived growth factor receptor, alpha
PDGFD_PDGFRB		platelet-derived growth factor receptor, beta
PSAP_LRP1	prosaposin	LDLR-related protein 1
PTN_PLXNB2	pleiotrophin	plexin B2
PTN_PTPRS		protein tyrosine phosphatase, receptor type, S
PTN_SDC3		syndecan 3
RTN4_CNTNAP1	reticulon 4	contactin associated protein 1
SEMA3F_NRP1	semaphorin 3F	neuropilin 1
SEMA3F_NRP2		neuropilin 2
SEMA4B_DCBLD2	semaphorin 4B	discoidin, CUB and LCCL domain containing 2
TGFB1_ENG	transforming growth factor, beta 1	Endoglin
TGFB1_ACVRL1		activin A receptor type II-like 1
TGFB1_CAV1		caveolin 1, caveolae protein, 22kDa
TGFB1_CD109		CD109 molecule
TGFB1_ITGAV		integrin, alpha V
TGFB1_SDC2		syndecan 2
TGFB1_TGFBR1		transforming growth factor, beta receptor 1
TGFB1_TGFBR2		transforming growth factor, beta receptor 2
TGFB2_ACVR1	transforming growth factor, beta 2	activin A receptor, type I
TGFB2_ENG		Endoglin
TGFB2_TGFBR1		transforming growth factor, beta receptor 1
TGFB2_TGFBR2		transforming growth factor, beta receptor 2
TNFSF12_TNFRSF12A	tumor necrosis factor family, member 12	TNF receptor superfamily, member 12A
VEGFA_EGFR	vascular endothelial growth factor A	epidermal growth factor receptor
VEGFA_EPHB2		EPH receptor B2
VEGFA_GPC1		glypican 1
VEGFA_ITGAV		integrin, alpha V
VEGFA_ITGB1		integrin, beta 1
VEGFA_NRP1		neuropilin 1
VEGFA_NRP2		neuropilin 2
VEGFA_SIRPA		signal-regulatory protein alpha
VEGFA_TYRO3		TYRO3 protein tyrosine kinase
VEGFB_NRP1	vascular endothelial growth factor B	neuropilin 1
VEGFB_TYRO3		TYRO3 protein tyrosine kinase

This table is limited to signaling proteins, cytokines, growth factors, and chemokines. For other cell-surface ligand-receptor pairs, we refer to Data S1.

Table S2. Autocrine ligand-receptor pairs expressed by cultured human cardiac fibroblasts.

Gene Pair	Ligand	Receptor
ADM_CALCRL	adrenomedullin	calcitonin receptor-like
ADM2_CALCRL	adrenomedullin 2	calcitonin receptor-like
ANGPT1_ITGA5	angiopoietin 1	integrin, alpha 5
ANGPT1_TEK		TEK tyrosine kinase, endothelial
ANGPT1_TIE1		TK with Ig-like and EGF-like domains 1
ANGPT2_TEK	angiopoietin 2	TEK tyrosine kinase, endothelial
ANGPT2_TIE1		TK with Ig-like and EGF-like domains 1
ANGPTL2_TIE1	angiopoietin-like 2	TK with Ig-like and EGF-like domains 1
ANGPTL4_TIE1	angiopoietin-like 4	TK with Ig-like and EGF-like domains 1
ANXA1_DYSF	annexin A1	dysferlin
ANXA1_EGFR		epidermal growth factor receptor
BDNF_DDR1	brain-derived neurotrophic factor	discoidin domain receptor tyrosine kinase 1
BDNF_NGFRAP1		NFG receptor associated protein 1
BDNF_SORT1		sortilin 1
BMP4_ACVR1	bone morphogenetic protein 4	activin A receptor, type I
BMP4_ACVR2A		activin A receptor, type IIA
BMP4_BMPR2		bone morphogenetic protein receptor, type II
BMP6_ACVR1	bone morphogenetic protein 6	activin A receptor, type I
BMP6_ACVR2A		activin A receptor, type IIA
BMP6_BMPR2		bone morphogenetic protein receptor, type II
CTGF_ITGA5	connective tissue growth factor	integrin, alpha 5
CXCL12_ACKR3	chemokine (C-X-C motif) ligand 12	atypical chemokine receptor 3
CXCL12_ITGB1		integrin, beta 1
CXCL12_SDC4		syndecan 4
CYR61_CAV1	cysteine-rich, angiogenic inducer, 61	caveolin 1
CYR61_ITGA5		integrin, alpha 5
CYR61_ITGAV		integrin, alpha V
CYR61_ITGB5		integrin, beta 5
DKK1_LRP5	dickkopf WNT signaling pathway inhibitor 1	LDLR-related protein 5
DKK1_LRP6		LDLR-related protein 6
EDN1_ELTD1	endothelin 1	EGF, latrophilin and 7 TM domain containing 1
FGF7_FGFR1	fibroblast growth factor 7	fibroblast growth factor receptor 1
FGF7_NRP1		neuropilin 1
FST_BMPR2	follistatin	bone morphogenetic protein receptor, type II
GAS6_AXL	growth arrest-specific 6	AXL receptor tyrosine kinase
GDF6_BMPR2	growth differentiation factor 6	bone morphogenetic protein receptor, type II
GREM1_KDR	gremlin 1, DAN family BMP antagonist	kinase insert domain receptor (a type III RTK)
HBEGF_CD44	heparin-binding EGF-like growth factor	CD44 molecule
HBEGF_CD82		CD82 molecule
HBEGF_CD9		CD9 molecule
HBEGF_EGFR		epidermal growth factor receptor
HBEGF_ERBB2		ERBB2
HGF_CD44	hepatocyte growth factor	CD44 molecule

HGF_SDC2		syndecan 2
HMGB1_THBD	high mobility group box 1	thrombomodulin
IGF2_IGF1R	insulin-like growth factor 2	insulin-like growth factor 1 receptor
IGF2_IGF2R		insulin-like growth factor 2 receptor
IL1A_IL1R1	interleukin 1, alpha	interleukin 1 receptor, type I
IL1A_IL1RAP		interleukin 1 receptor accessory protein
IL1B_IL1R1	interleukin 1, beta	interleukin 1 receptor, type I
IL6_F3	interleukin 6 (interferon, beta 2)	coagulation factor III (thromboplastin)
IL6_IL6ST		interleukin 6 signal transducer
IL8_KDR	interleukin 8	kinase insert domain receptor (a type III RTK)
IL8_SDC2		syndecan 2
IL8_SDC3		syndecan 3
INHBA_ACVR1	inhibin, beta A	activin A receptor, type I
INHBA_ACVR1B		activin A receptor, type IB
INHBA_ACVR2A		activin A receptor, type IIA
INHBA_BAMBI		BMP and activin membrane-bound inhibitor
INHBA_ENG		endoglin
JAG1_NOTCH1	jagged 1	notch 1
JAG1_NOTCH3		notch 3
LIF_IL6ST	leukemia inhibitory factor	interleukin 6 signal transducer
LIF_LIFR		leukemia inhibitory factor receptor alpha
MDK_ITGA4	midkine	integrin, alpha 4
MDK_ITGA6		integrin, alpha 6
MDK_ITGB1		integrin, beta 1
MDK_LRP1		LDLR-related protein 1
MDK_SDC3		syndecan 3
MDK_SDC4		syndecan 4
NGF_KIDINS220	nerve growth factor (beta polypeptide)	kinase D-interacting substrate, 220kDa
NGF_NGFRAP1		NFG receptor associated protein 1
NGF_SORT1		sortilin 1
NPPB_NPR1	natriuretic peptide B	natriuretic peptide receptor 1
NPPB_NPR3		natriuretic peptide receptor 3
PDAP1_PDGFRB	PDGFA associated protein 1	platelet-derived growth factor receptor, beta
PDGFA_PDGFRA	platelet-derived growth factor alpha	platelet-derived growth factor receptor, alpha
PDGFA_PDGFRB		platelet-derived growth factor receptor, beta
PDGFB_ITGAV	platelet-derived growth factor beta	integrin, alpha V
PDGFB_LRP1		LDLR-related protein 1
PDGFB_PDGFRA		platelet-derived growth factor receptor, alpha
PDGFB_PDGFRB		platelet-derived growth factor receptor, beta
PDGFB_S1PR1		sphingosine-1-phosphate receptor 1
PDGFC_KDR	platelet derived growth factor C	kinase insert domain receptor (a type III RTK)
PDGFC_PDGFRA		platelet-derived growth factor receptor, alpha
PDGFC_PDGFRB		platelet-derived growth factor receptor, beta
PDGFD_PDGFRA	platelet derived growth factor D	platelet-derived growth factor receptor, alpha
PDGFD_PDGFRB		platelet-derived growth factor receptor, beta
PENK_OGFR	proenkephalin	opioid growth factor receptor

PGF_NRP1	placental growth factor	neuropilin 1
PGF_NRP2		neuropilin 2
PSAP_LRP1	prosaposin	LDLR-related protein 1
PTN_PLXNB2	pleiotrophin	plexin B2
PTN_PTPRS		protein tyrosine phosphatase, receptor type, S
PTN_SDC3		syndecan 3
SEMA3C_NRP1	semaphorin 3C	neuropilin 1
SEMA3C_NRP2		neuropilin 2
SEMA3C_PLXND1		plexin D1
SEMA3D_NRP1	semaphorin 3D	neuropilin 1
SEMA3F_NRP1	semaphorin 3F	neuropilin 1
SEMA3F_NRP2		neuropilin 2
SEMA3F_PLXNA3		plexin A3
SEMA4B_DCBLD2	semaphorin 4B	discoidin, CUB and LCCL domain containing 2
SEMA7A_ITGA1	semaphorin 7A	integrin, alpha 1
SFRP1_FZD2	secreted frizzled-related protein 1	frizzled class receptor 2
SFRP1_FZD6		frizzled class receptor 6
TGFB1_ACVRL1	transforming growth factor, beta 1	activin A receptor type II-like 1
TGFB1_CAV1		caveolin 1
TGFB1_CD109		CD109 molecule
TGFB1_CXCR4		chemokine (C-X-C motif) receptor 4
TGFB1_ENG		endoglin
TGFB1_ITGAV		integrin, alpha V
TGFB1_SDC2		syndecan 2
TGFB1_TGFBR1		transforming growth factor, beta receptor 1
TGFB1_TGFBR2		transforming growth factor, beta receptor II
TGFB2_ACVR1	transforming growth factor, beta 2	activin A receptor, type I
TGFB2_ENG		endoglin
TGFB2_TGFBR1		transforming growth factor, beta receptor 1
TGFB2_TGFBR2		transforming growth factor, beta receptor II
VEGFA_EGFR	vascular endothelial growth factor A	epidermal growth factor receptor
VEGFA_EPHB2		EPH receptor B2
VEGFA_GPC1		glypican 1
VEGFA_ITGAV		integrin, alpha V
VEGFA_ITGB1		integrin, beta 1
VEGFA_KDR		kinase insert domain receptor (a type III RTK)
VEGFA_NRP1		neuropilin 1
VEGFA_NRP2		neuropilin 2
VEGFA_SIRPA		signal-regulatory protein alpha
VEGFB_NRP1	vascular endothelial growth factor B	neuropilin 1
VEGFC_KDR	vascular endothelial growth factor C	kinase insert domain receptor (a type III RTK)
VEGFC_NRP2		neuropilin 2

This table is limited to signaling proteins, cytokines, growth factors, and chemokines. For other cell-surface ligand-receptor pairs, we refer to Data S1.

Table S3. Ligands expressed by both cardiomyocytes and fibroblasts.

Gene	Protein
ANGPT1	angiopoietin 1
EDN1	endothelin 1
FGF7	fibroblast growth factor 7
IL1B	interleukin 1, beta
IL6	interleukin 6
IL8	interleukin 8
LIF	leukemia inhibitory factor
PDGFC	platelet derived growth factor C
SEMA4B	semaphorin 4B
VEGFB	vascular endothelial growth factor B