

STAT3, a key regulator of cell-to-cell communication in the heart

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The signal transducer and activator of transcription 3 (STAT3) is fundamental for physiological homeostasis and stress-induced remodelling of the heart as deregulated STAT3 circuits are sufficient to induce dilated and peripartum cardiomyopathy and adverse remodelling after myocardial infarction. STAT3 activity depends on multiple post-translational modifications (phosphorylation, acetylation, and dimerization). It is regulated by multiple receptor systems, which are coupled to positive and negative feedback loops to ensure physiological and beneficial action. Its intracellular functions are diverse as it acts as a signalling protein, a transcription factor but also participates in mitochondria energy production and protection. STAT3 modulates proliferation, differentiation, survival, oxidative stress, and/or metabolism in cardiomyocytes, fibroblasts, endothelial cells, progenitor cells, and various inflammatory cells. By regulating the secretome of these cardiac cells, STAT3 influences a broad range of intercellular communication systems. It thereby impacts on the communication between cardiomyocytes, the plasticity of the cardiac microenvironment, the vasculature, the extracellular matrix, and the inflammation in response to physiological and pathophysiological stress. Here, we sum up current knowledge on STAT3-mediated intra- and intercellular communication within the heterogeneous cellular network of the myocardium to co-ordinate complex biological processes and discuss STAT3-dependent targets as novel therapeutic concepts to treat various forms of heart disease.

Keywords STAT3 • Angiogenesis • Fibrosis • Cardiomyopathy • Inflammation

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1. Introduction

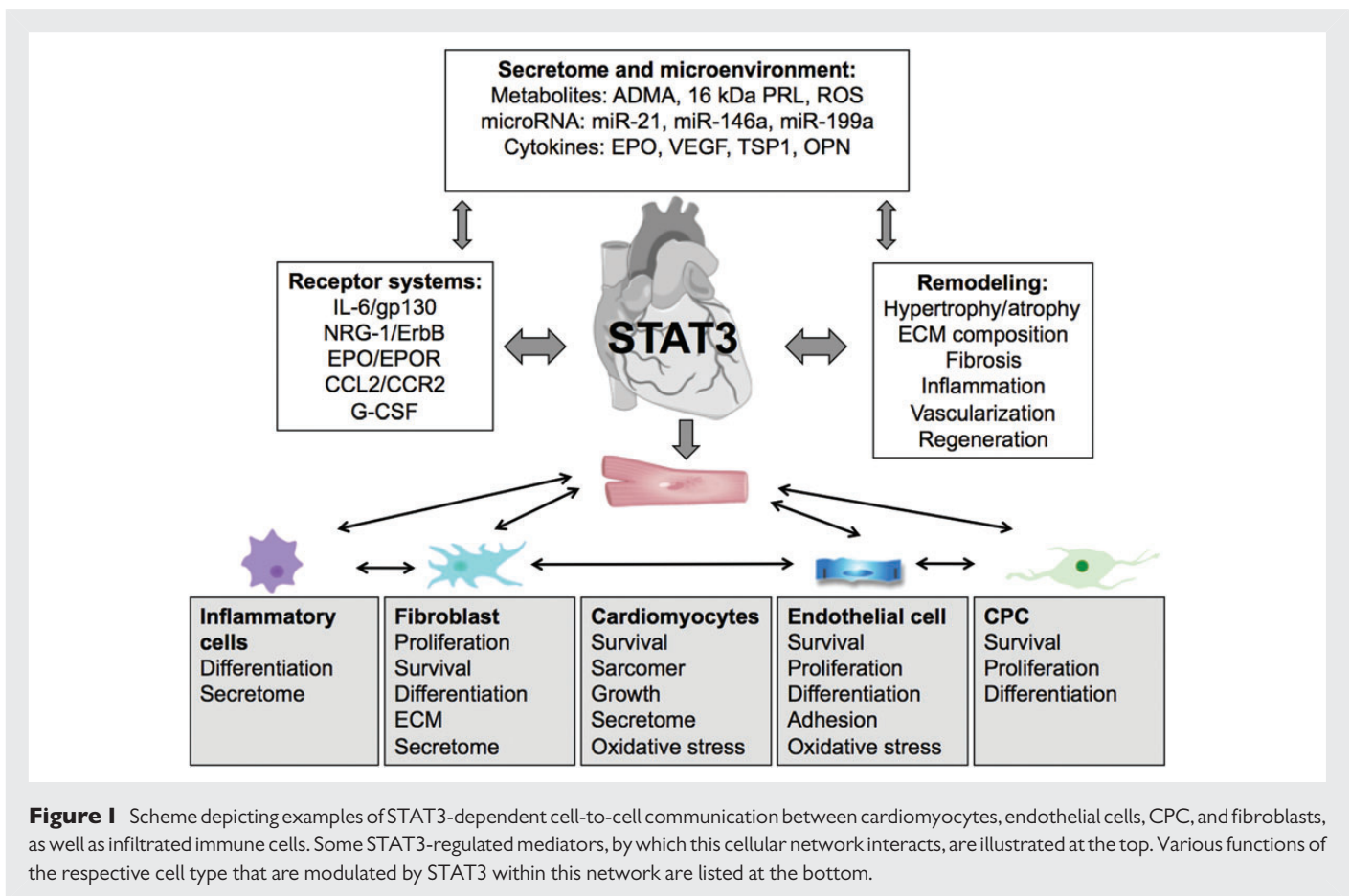
Myocardial cells undergo a multitude of alterations in response to physiological or pathophysiological stress stimuli that are described by the term ventricular remodelling. Whereas alterations caused by physiological stress, such as pregnancy and exercise, are considered adaptive and reversible, changes in pathophysiological stress, such as myocardial infarction (MI), pressure overload, infections, and cardiotoxic agents, may be maladaptive and detrimental. The ventricle consists of several different cell types, i.e. cardiomyocytes, fibroblasts, endothelial cells, progenitor cells, and various immune-competent cells, of which each one reacts in a specific form to stress and, thereby, affects the surrounding microenvironment and neighbouring cells.

Although beneficial and adverse remodelling initially have some similar features, e.g. cardiomyocyte hypertrophy and the release of certain growth factors, a specific pattern of distinct stress signalling pathways is involved in the shift from protective to adverse and detrimental

mechanisms. The signal transducer and activator of transcription 3 (STAT3) emerged as one of the key co-ordinators to balance these mechanisms. STAT3 has multiple biological functions, with its best-described role as a transcription factor regulating the expression of numerous genes coding for proteins, but also genes encoding miRNAs. MiRNAs are short oligonucleotides that impact on post-transcriptional gene regulation. In addition, STAT3 functions as a signalling molecule, as a factor involved in cellular respiration and as a protein interacting with the mitochondrial pore.^{1–4} In cardiomyocytes, STAT3 plays an important role for survival, growth and sarcomere architecture, energetics, and metabolism (Figure 1).^{5–7} In endothelial cells, STAT3 is crucial for proliferation, survival, and generation of the vasodilators nitric oxide and prostacyclin, and is a major mediator of pro-angiogenic signalling by the vascular endothelial growth factor (VEGF) signalling and erythropoietin (EPO) (Figure 1).^{8–11} Additional roles of STAT3 in endothelial cells involve the expression of cytokines, i.e. interleukin-6 (IL-6) and chemokine (C-C motif) ligand 2 (CCL2),^{12,13} and cell adhesion molecules, such

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as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and fractalkine (Figure 1).¹⁴ STAT3 is crucial for the endothelial differentiation of endogenous cardiac progenitor cells (Figure 1).¹⁵ In cardiac fibroblasts, STAT3 regulates the synthesis of genes involved in ECM modulation, i.e. collagen, and the expression of various cytokines (Figure 1).^{16–18} By modulating the secretome of these cardiac cell types, STAT3 impacts on the cardiac microenvironment and on receptor-based intercellular communication systems, which, subsequently, induces alterations in invading inflammatory cells (Figure 1).^{19–24}

Thus, growing evidence supports the notion that STAT3 acts as a key modulator of an integrated signalling network comprising different cell types in the heart (Figure 1). We provide an overview of established STAT3-regulated biological actions that co-ordinate the cell-to-cell communication pathways in the myocardium under physiological and pathophysiological conditions. In particular, we illustrate its role in cardiomyocyte architecture, vascular homeostasis, cardiac regeneration, fibrosis, and inflammation and challenge the question whether STAT3 signalling conducts beneficial or detrimental effects with respect to the development of heart failure, and whether STAT3-dependent intercellular communication mechanisms may emerge as novel therapeutic targets.

2. The STAT family in the heart

In eukaryotic cells, seven STAT proteins have been identified so far, which are all expressed in the heart and in cardiomyocytes.²⁵ The first two members of the STAT family, STAT1 and STAT2, were discovered

by Darnell et al. in 1994, who purified factors associated with interferon-stimulated genes.²⁶ Subsequently, the other family members such as STAT3,²⁷ STAT4, STAT5a, STAT5b, and STAT6 were identified as transcription factors. These proteins share similar features such as the formation of homo- and heterodimers mediated by the Src homology-2 (SH2) domain among different family members.²⁸

Importantly, all STAT proteins are expressed in the myocardium and for each one altered expression or regulation has been reported in different types of human cardiac pathology, in particular in ischaemic and dilated cardiomyopathies.^{29,30} For example, STAT1 is activated upon ischaemia/reperfusion (I/R) injury in the rat heart where it up-regulates expression of caspase-1, Fas receptor, and its ligand FasL and thereby promotes apoptosis in cardiomyocytes.³¹ STAT2 plays an exceptional role as it can only heterodimerize with STAT1.³² STAT4 has been suggested to play a protective role in modulating inflammatory processes in autoimmune and infectious myocarditis, i.e. Coxsackievirus B3-induced myocarditis.³³

STAT5 and STAT6 participate in actions of an activated renin–angiotensin–aldosterone system, which is one of the major circuits involved in post-infarction heart failure. These STATs are rapidly phosphorylated by angiotensin II (AngII) in the remote non-ischaemic area and in turn bind to the STAT consensus sequence in the angiotensinogen promoter, and thus may cause sustained activation of AngII-mediated signalling in terms of an autocrine loop.³⁴ STAT3 plays a central role within the STAT family in cardiac physiology and pathophysiology and has distinct roles in each cardiac cell and their communication as outlined in the present review.

3. STAT3, a multifaceted molecule

The STAT3 protein consists of six domains that have distinct features in activating and regulating STAT3 functions.³⁵ STAT3 is activated by numerous ligand receptors, such as the IL-6-glycoprotein (gp) 130 receptor system,³⁵ the EPO-receptor (EPOR),^{36,37} the leptin-receptor,^{38,39} and the AngII type1 receptor (AT1R)⁴⁰ (Figure 2). The IL-6-gp130 receptor system was initially considered as the major regulator of STAT3. The IL-6 family of cytokines consists of a number of cytokines including IL-5, IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM), and cardiotrophin (CT)-1 and has in common that all members activate (phosphorylation at tyrosine 705, Y705 and/or serine 727, S727) STAT3 upon induction of gp130 receptor homo- or heterodimer formation. Phosphorylation of Y705 is mediated by the janus kinase (JAK) and tyrosine kinase (Tyk)-2 at the intracytoplasmic domain of the gp130 receptor.⁴¹ Importantly, the interaction of STAT3 with specific tyrosine-phosphorylated sites, such as the YXXQ sites of the gp130 receptor, requires a linker domain that is located just before an SH2 domain in the STAT3 protein. This linker domain is necessary for interaction with specific tyrosine-phosphorylated residues of activated STAT proteins to form STAT3 homo- or heterodimers (with other STAT proteins). The S727 of STAT3 is phosphorylated by a number of serine/threonine kinases, such as protein kinase C ϵ (PKC ϵ), PKC δ , ERK1/2, p38 CDK5, ZIP kinase, and mTOR.^{42,43} Notably, the S727 site is important for potentiating the transcriptional activity of STAT3 via recruitment of transcriptional cofactors, such as the histone acetyltransferase p300/CBP.⁴² STAT3 function is also altered by acetylation of lysine residues within the NH₂-terminal (K49, K87) and SH2 (K685) domains. For example, acetylation of the K49 and K87 by p300 appears to be essential for the stabilization of the STAT3-p300 interaction and the subsequent enhancement of gene transcription.⁷ STAT3 homo- and heterodimers translocate via importin- α to the nucleus, where they activate the transcription of target genes

by binding to specific DNA promoter motifs (GAS: interferon-gamma activated sequence).^{41,44}

STAT3 belongs to the so-called proto-oncogenes and needs precise regulation for physiological effects, a feature that becomes evident by the multiple negative regulatory mechanisms that have been identified.⁴⁴ For example, the suppressor of cytokine signalling [SOCS, also referred to as cytokine-inducible SH2 protein (CIS)] family represents a specific negative regulatory feedback element of STAT signalling and its upstream kinases. SOCS-3 is transcriptionally up-regulated by STAT3. SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins or the cytoplasmic phosphotyrosine residue, e.g. the Y759 of the human gp130 receptor or Y757 of the mouse gp130 receptor, resulting in the inhibition of STAT protein phosphorylation. Comparable with SOCS proteins, the Src homology domain-containing tyrosine phosphatases 1/2 (SHP-1/2) interact with the intracytoplasmic portion of cytokine receptors and dephosphorylate JAK proteins, thereby lowering the phosphorylation of STAT3.⁴⁴ The group of protein inhibitors of activated STAT (PIAS-1, -3) is capable of inhibiting the binding of dimerized phosphorylated STAT1 and STAT3 to DNA, thereby diminishing transcriptional activation of STAT target genes. The protein tyrosine phosphatase (PTP) receptor T (PTPRT) specifically dephosphorylates the Y705 residue of STAT3 and, thereby, regulates its target gene expression and its cellular localization.⁴⁴

In addition, accumulated evidence indicates that STAT3 also has gene-suppressing features.¹⁹ A mechanistic example of how STAT3 inhibits gene expression is acetylation of the K685 site of the SH2 domain that appears crucial for STAT3 interaction with DNA methyltransferase 1, resulting in methylation and silencing of distinct promoters.⁴⁵ Notably, deacetylation by histone deacetylases (in particular HDAC1 and 4) results in nuclear discharge and degradation of STAT3 and appears to be an important switch of STAT3 activity.⁴⁶

More recent studies suggest that S727 regulates STAT3 functions in the mitochondria with regard to respiration, energy production and

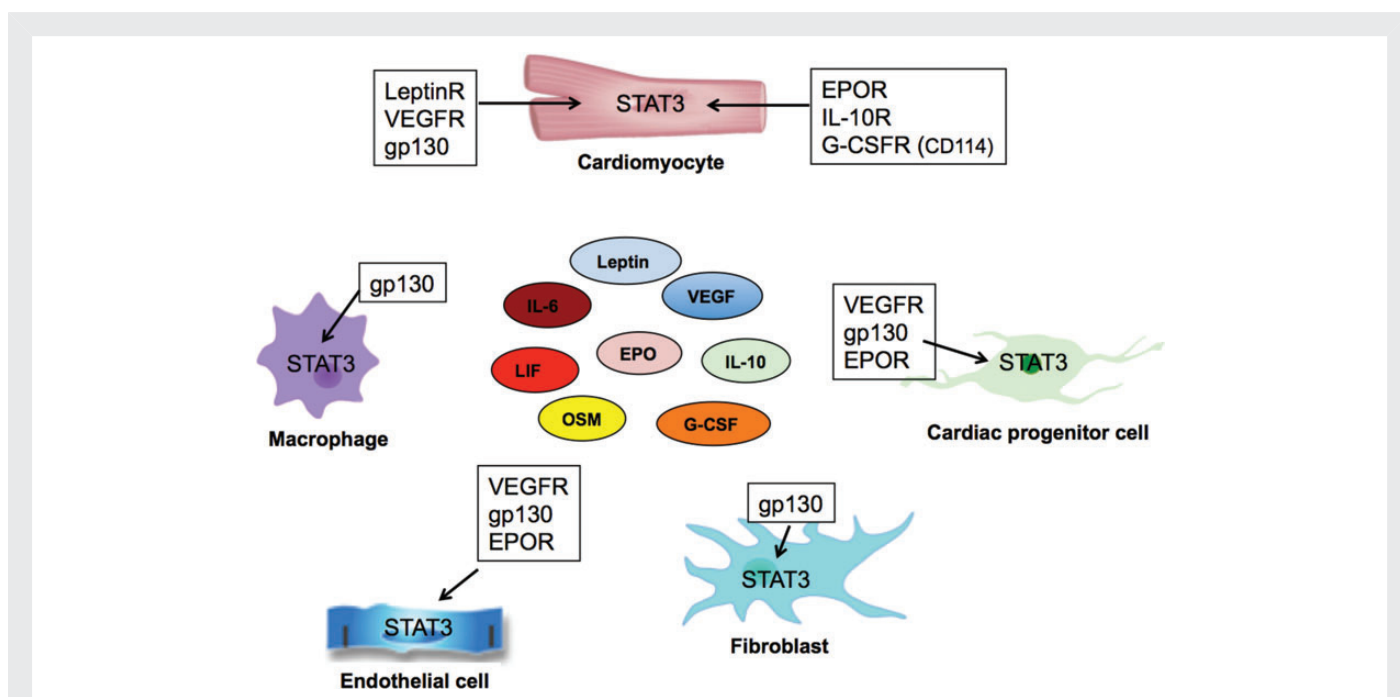


Figure 2 Receptor systems activating STAT3 on different cardiac cell types specifically discussed in the present review.

protection of the mitochondria pore.^{4,5} Furthermore, STAT3 has been suggested to possess redox-buffering capacities, particularly in the mitochondria, via redox-sensitive cysteines in the DNA-binding domain (C418, C426, and C468) and in the transcriptional activation domain (C765).^{47,48}

Although these examples represent some of the versatile aspects of STAT3 with regard to its multiple biological roles, it is likely that additional regulatory properties of distinct STAT3 domains may exist and their role for both genomic actions and mechanisms in other cellular compartments await further clarification.

4. STAT3 plays a central role in the communication between cardiomyocytes and the myocardial vasculature

Numerous experimental works have highlighted the obligatory role of endothelial cells in the heart for the maintenance and regulation of cardiac function.^{49–51} The close proximity of cardiomyocytes and endothelial cells not only secures the constant supply of oxygen and nutrients to cardiomyocytes, but also favours a direct interaction of both cell types in a paracrine manner.

A mouse model with a cardiomyocyte-restricted overexpression of active STAT3 provided the first evidence for a critical involvement of STAT3 in the communication between cardiomyocytes and endothelial cells.⁵² These mice express increased levels of VEGFA, a crucial factor for angiogenesis and for vascular permeability.^{53,54} Cell culture experiments confirmed that STAT3 induces VEGFA expression in cardiomyocytes, which is able to induce endothelial tube formation.⁵² Among factors inducing STAT3-mediated VEGF expression in cardiomyocytes are IL-6 cytokines and, especially under ischaemic conditions, EPO (Figure 1).^{55–57}

A different communication system between cardiomyocytes and endothelial cells involves the granulocyte colony-stimulating factor (G-CSF).⁵⁸ G-CSF has beneficial effects on the heart after MI by improving cardiomyocyte survival and enhancing vascularization in a STAT3-dependent manner, since beneficial effects of G-CSF on cardiomyocytes and on enhancement of capillary density were abrogated by a cardiomyocyte-restricted overexpression of a dominant-negative mutant STAT3 protein.⁵⁸ Thus, G-CSF-STAT3 signalling in cardiomyocytes in a paracrine or cell-to-cell interacting way also impacts on endothelial cells (Figure 1).

Besides stress-induced STAT3 effects, STAT3 is also important for cardiac homeostasis under physiological conditions as a cardiomyocyte-restricted deletion of STAT3 leads to heart failure associated with disturbed sarcomere organization and a decrease in the number of capillaries in the heart of male mice under ageing conditions.^{6,19} We identified miR-199a-5p (miR-199a), a microRNA that is transcriptionally suppressed by STAT3, as being responsible for the impairment in the cardiomyocyte sarcomere organization. MiR-199a targets the ubiquitin-conjugating enzymes Ube2i and Ube2g1, thereby disturbing the protein turnover by the ubiquitin-proteasome system (UPS). This impairment of the UPS also leads to reduced expression of α - and β -sarcomeric myosin heavy-chain genes (α - and β -MHC), thereby resulting in a marked reduction in sarcomeric MHC.¹⁹ In addition, conditioned medium from cultured STAT3 knockdown cardiomyocytes contained increased levels of biologically active asymmetric

dimethylarginine (ADMA), an endogenous inhibitor of endothelial NO⁵⁹ that increased ROS production and lowered NO production in endothelial cells.¹⁹ We identified the protein arginine N-methyltransferase 1 (PRMT1) as the cause for increased ADMA synthesis and release in STAT3 knockdown conditions. PRMT1 is an enzyme that catalyses the majority of cellular arginine methylation activity and is activated in response to disturbed protein turnover and accumulation of damaged proteins.⁶⁰ Thus, loss of STAT3 in cardiomyocytes severely damages the cardiomyocyte ultra structure and function and, at the same time, impairs the endothelium in a paracrine manner—all features known to be involved in the initiation and progression of heart failure (Figure 1).

Female STAT3-KO mice are largely protected from age-related loss of capillaries, but, if exposed to pregnancy and nursing, develop heart failure with a phenotype resembling the human peripartum cardiomyopathy (PPCM).²¹ PPCM is a heart disease with systolic heart failure occurring in previously healthy women in the last month of pregnancy or the months following delivery.²¹ Postpartum heart failure in STAT3-KO mice is associated with a profound loss of capillaries.²¹ Physiological changes in the peripartum phase involve an increase in oxidative stress late in pregnancy and early postpartum.⁶¹ In wild-type females, this is associated with an up-regulation of anti-oxidative enzymes, such as manganese superoxide dismutase (MnSOD), in the heart. This up-regulation of MnSOD is absent in STAT3-KO mice or mice with a cardiomyocyte-restricted deletion of peroxisome proliferator-activated receptor c coactivator 1 α (PGC1 α), resulting in enhanced oxidative stress in the postpartum heart.^{21,62} Oxidative stress activates Cathepsin D, a protease that cleaves the circulating nursing hormone prolactin in a pro-apoptotic and anti-angiogenic N-terminal 16 kDa fragment (16kDa-PRL). Pathologically increased 16kDa-PRL levels induce endothelial cell apoptosis, reduce endothelial cell proliferation and migration, and cause impairment of endothelial function.⁶³ Blocking prolactin with the dopamine receptor 2D agonist, bromocriptine, could prevent the loss of capillaries and subsequent heart failure in postpartum STAT3-KO mice, supporting the idea that prolactin cleavage due to unbalanced oxidative stress in cardiomyocytes and the subsequent destruction of the endothelium is a key feature of PPCM.^{21,63} A first clinical trial suggests that bromocriptine treatment is beneficial in patients with PPCM.⁶⁴ 16kDa-PRL appears to have little direct effect on cardiomyocytes, while in endothelial cells most of its adverse effects are mediated by the induction miR-146a.⁶³ Moreover, 16kDa-PRL induces shedding of miR-146a-enriched endothelial exosomes, which are efficiently absorbed by cardiomyocytes, where they substantially increase miR-146a levels.⁶³ Exosomes are an important novel trafficking system in the intercellular communication of the heart, which protect miRNAs from degradation.⁶⁵ In cardiomyocytes, exosomal miR-146a reduces metabolic activity at least, in part, by down-regulating the miR-146a target gene ErbB4.⁶³ ErbB4 is constitutively expressed in cardiomyocytes, forming homo- and heterodimers with ErbB2. A functional ErbB receptor signalling system is essential to maintain the physiological status in the adult heart at baseline and during pregnancy.^{66–68} The ligand of ErbB2/4 receptors is neuregulin (NRG)-1, a factor that is mainly produced by endothelial cells and, therefore, the NRG-1/ErbB signalling system is a way through which cardiomyocytes and endothelial cells communicate in the heart.^{69–71} Thus, we discovered a novel circuit in PPCM between low cardiomyocyte STAT3, oxidative stress, generation of 16kDa-PRL, subsequent damage of the cardiac vasculature and disturbance of the intercellular communication via the NRG-1/ErbB signalling (Figure 1). This concept offers prolactin targeting and/or targeting miR-146a as novel therapy concepts for PPCM (Table 1).

Table 1 Therapy concepts in the context of STAT3-dependent intercellular communication systems

<i>Cardioprotection</i>
G-CSF via STAT3 promotes protection of the heart against ischaemic injury (Harada <i>et al.</i> ⁵⁸).
EPO may protect from cardiotoxic effects of anti-tumour drugs such as doxorubicin that suppresses cardioprotective STAT3 (Hoch <i>et al.</i> ²³).
IL-11 prevents cardiac fibrosis through STAT3 in cardiomyocytes (Obana <i>et al.</i> ⁷²).
<i>Treatment of cardiomyopathy</i>
Targeting miR-199a-5p may be beneficial in treatment of dilated cardiomyopathy with low STAT3 expression (Haghikia <i>et al.</i> ¹⁹).
Inhibition of the complement system by CVF reduces chronic inflammation caused by hyperactivated IL-6-gp130-STAT3 signalling after MI (Hilfiker-Kleiner <i>et al.</i> ²²).
<i>Promotion of angiogenesis</i>
Bromocriptine may protect from anti-angiogenic effects of 16 kDa prolactin in PPCM (Halkein <i>et al.</i> , ⁶³ Hilfiker-Kleiner <i>et al.</i> , ²¹ Sliwa <i>et al.</i> , ⁶⁴).
IL-10 enhances EPC survival and cardiac angiogenesis through STAT3 after MI (Krishnamurthy <i>et al.</i> ⁷³).
<i>Endothelial cell-cardiomyocyte communication</i>
Targeting miR-146a may be suited to block specifically adverse effects of 16 kDa prolactin but leaves normal prolactin signalling intact in PPCM (Halkein <i>et al.</i> ⁶³).

5. STAT3 modulates the cardiac microenvironment

Cardiomyocyte STAT3 appears to play an important role in the cardiac microenvironment, which substantially affects the phenotype of endogenous progenitor cells. This notion derives from observations that the endothelial differentiation capacity of resident cardiac Sca-1⁺ progenitor cells (CPCs) is impaired in STAT3-KO mice, even before onset of heart failure.²³ Indeed, a subpopulation of CPCs that express both the EPO receptor and the C-C chemokine receptor type 2 (CCR2) displays a high endothelial differentiation capacity, which depends on the activation of the CCR2 system. Cardiomyocyte-derived EPO hereby plays a modulatory role that affects the expression of the CCR2 ligand CCL2 (Figure 1).²³ STAT3 seems to be necessary for sufficient EPO expression and secretion from cardiomyocytes into the microenvironment.²³ Therefore, EPO levels and endothelial differentiation of CPCs are reduced in hearts of STAT3-KO mice and in wild-type mice treated with the anti-cancer drug doxorubicin (DOX), which results in reduced expression of STAT3 and, subsequently, depletion of endogenous EPO.²³ DOX is used to treat a broad range of cancers, but is limited in its therapeutic application by severe side effects, most notably heart failure.⁷⁴ Our data suggest that some of these cardiotoxic side effects of DOX derive from the impairment of the endogenous cardiac regeneration potential.²³ Interestingly, supplementation with low-dose EPO increased the activation state of the CCL2/CCR2 system in the heart and, specifically, in CPCs of both STAT3-KO mice and DOX-treated wild-type mice, thereby restoring the endothelial differentiation capacity of the CPCs.²³ Our data imply that short-term EPO administration at low-doses appear to be an attractive avenue to pursue for protecting the heart against chemotherapy-induced heart failure and may even have broader applications in cardiac regeneration (Table 1).

It is known that ischaemia as well as ischaemia/reperfusion (I/R) injury induces the secretion of inflammatory cytokines and chemokines from cardiac cells, including cardiomyocytes.⁷⁵ Some of these cytokines, i.e. IL-11, CT-1, and LIF, induce endothelial differentiation of CPCs via activation of gp130/STAT3 signalling.⁷⁶ Moreover, for LIF, it has been shown that endothelial differentiation of CPCs is induced through STAT3 activation, followed by an up-regulation of proto-oncogene serine/threonine-protein kinase (Pim)-1 *in vitro* and *in vivo*.^{15,77}

Moreover, MI induces mobilization of bone-marrow endothelial progenitor cells (EPCs) into the circulation in an IL-10-dependent manner, which signals through STAT3 activation.⁷³ IL-10 administration is associated with enhanced EPC survival and cardiac angiogenesis and improvement of cardiac function, as well as reduction in infarct size and fibrosis in the murine heart (Table 1).⁷³

It has been shown that a crosstalk between cardiomyocytes and bone-marrow-derived mesenchymal stem cells (BMSCs) affects their myogenic conversion. An important factor is the reduction of miR-124 in BMSC induced by the cardiomyocytes, which leads to increased expression of STAT3.⁷⁸ Subsequently, to increase STAT3 myocytic markers, such as atrial natriuretic peptide (ANP), troponin T (TnT) and α MHC, and the cardiac potassium channel currents became up-regulated, indicative for myogenic differentiation. These effects could be reversed by overexpression of miR-124.⁷⁸

Thus, STAT3 is a key mediator of self-renewal and differentiation processes involving different types of stem and progenitor cells in the heart, especially after cardiac injury. The detailed knowledge of communication pathways that orchestrate these processes may be of potential clinical interest for the development of therapeutics promoting cardiac repair and regeneration.

6. STAT3 regulates the communication between cardiomyocytes and cardiac fibroblasts

Fibroblasts and cardiomyocytes express various ECM components including collagens, as well as enzymes regulating the ECM quality and turnover, i.e. matrix metalloproteinases (MMPs) and their inhibitors tissue inhibitors of metalloproteinases (TIMPs).^{79–81} Disturbance of intercellular regulatory loops that alter the ECM homeostasis may lead to excess synthesis and deposition of extracellular matrix proteins, causing enhanced cardiac fibrosis, which is a hallmark of adverse cardiac remodelling.⁸² On the contrary, exorbitant loss of ECM leads to cardiomyocyte slipping, followed by functional impairment and dilatation.⁸³

We and other laboratories observed that male STAT3-KO mice develop severe cardiac fibrosis in older age, which was associated with impaired cardiac function, ventricular remodelling, and signs of heart failure with generalized oedema and enhanced mortality.^{6,24} These STAT3-KO mice displayed up-regulation of numerous genes involved in fibrosis, such as connective tissue growth factor (CTGF), thrombospondin1 (TSP1), TIMP-1, osteopontin (OPN), tenascin C (TNC), and plasminogen activator inhibitor-1 (PAI-1) even before overt signs of heart failure, indicating an early pro-fibrotic shift that may contribute to the development of heart failure later on.⁶ Further experiments showed that supernatants of cultured STAT3-KO cardiomyocytes enhanced fibroblast proliferation indicative of a potential cardiomyocyte-driven paracrine mechanism as the underlying cause for enhanced

cardiac fibrosis in STAT3-KO mice.⁶ Beside the role of cardiomyocyte STAT3 for cardiac fibrosis under normal conditions, it also modulates cardiac fibrosis after MI. For example, activation of STAT3 in cardiomyocytes by the IL-6 family member IL-11 appears to be important to prevent extensive post infarct fibrosis and adverse ventricular remodelling.⁷²

In fibroblasts, STAT3 promotes survival and cell proliferation, as well as the synthesis of ECM components (Figure 1).⁸⁴ This is in contrast to the suppressive effect of STAT3 on pro-fibrotic circuits in cardiomyocytes as activation of STAT3 in fibroblasts, for example by IL-6, promotes collagen biosynthesis *in vitro* and in hypertrophied hearts *in vivo*,⁸⁴ suggesting that STAT3 may differently regulate the expression of collagens and ECM modulatory genes in cardiomyocytes and in fibroblasts.

Cardiac stress factors, such as ischaemia, AngII, and mechanical stress, induce the expression of IL-6 family cytokines in both cardiomyocytes and fibroblasts, providing the potential for intercellular communication between cardiomyocytes and fibroblasts via the IL6-gp130 receptor system.^{85–89} Other stress factors, such as the β -adrenergic agonist isoproterenol, induce expression of IL-6 cytokines only in cardiac fibroblasts but not in cardiomyocytes, a feature that may evoke a different reaction mediated by STAT3 in these two cardiac cell types.^{51,90,91}

These studies show that STAT3 acts as a major player in the communication between cardiomyocytes and fibroblasts with regard to the expression of paracrine mediators for STAT3 regulation in either cell type, as well as for STAT3-mediated biological effects from cardiomyocytes or fibroblasts that influence phenotypic changes in both compartments with regard to cell survival, growth, proliferation, ECM composition, and fibrosis under physiologic and pathophysiologic conditions in the heart (Figure 1).

7. Role of STAT3 in the regulation of cardiac inflammation

Most pathological conditions that injure the heart muscle involve inflammatory processes. Under physiological conditions, a variety of cells of the immune system including T cells, macrophages, and mast cells reside in the myocardium and additional recruitment of inflammatory cells is induced in response to stress stimuli such as MI, I/R and congestive heart failure. Inflammatory cells contribute to cardiac remodelling, partly by secretion of cytokines, growth factors, and MMPs, thereby impacting on various cardiac cell types via paracrine mechanisms and cell-to-cell communication.

Myocardial levels of the pro-inflammatory cytokines IL-1 β , IL-6, and tumor necrosis factor α (TNF- α) are elevated after MI.^{37,85,92} These cytokines promote the expression of chemokines that attract leucocytes to the infarcted myocardium.⁹³ Also, cardiac mast cell degranulation after MI leads to the release of preformed TNF- α and histamine.⁹⁴ TNF- α itself induces the expression of IL-6 in various cell types, such as fibroblasts,⁹⁵ endothelial cells,⁹⁶ neutrophils,⁹⁷ and mononuclear cells,⁹⁸ thereby potentiating cytokine activity in the injured heart.

Accumulated evidence points to the regulatory capacity of STAT3 on cytokine production and inflammatory activity in the injured heart. For example, lipopolysaccharide (LPS)-induced expression of TNF- α is significantly increased in STAT3-KO mice, compared with controls,²⁴ suggesting a STAT3-dependent negative control of the cytokine production in the endotoxin exposed heart. In these mice, increased cardiomyocyte apoptosis resulted from elevated inflammatory response in the absence of STAT3.²⁴ However, in ischaemic pre- and post-conditioning, TNF- α

also has cardioprotective effects by activating an alternative pathway to the pro-survival reperfusion injury salvage kinase (RISK) pathway, the JAK/STAT3 or survivor activating factor enhancement (SAFE) pathway, thereby limiting cell death during reperfusion.⁹⁹

In STAT3-deficient macrophages, the expression of pro-inflammatory cytokines upon LPS stimulation is augmented because of the blocked inhibitory activity of the anti-inflammatory cytokine IL-10^{100,101} which is mainly produced by T cells and macrophages.¹⁰² The IL-10R-induced STAT3 activates genes, which, in turn, regulate the expression of pro-inflammatory cytokines.¹⁰³ In addition, under systemic inflammatory conditions after cardiac surgery, STAT3 appears to selectively suppress TNF- α (but not IL-6) expression in human peripheral blood monocytes (PBMCs).¹⁰⁴ Pre-treatment of PBMCs with a STAT3 inhibitor peptide or neutralization of IL-10 with an antibody in post-surgery plasma samples restored TNF- α expression in activated monocytes.¹⁰⁴

While these findings highlight the regulatory potential of STAT3 to control cytokine production and release in inflammatory cells with subsequent impact on resident cardiac cells, recent studies from our group suggest a substantial regulation of inflammatory responses by the gp130-STAT3 signalling pathway in cardiomyocytes.²² For example, in mice with a cardiomyocyte-specific mutation at the gp130 receptor that replaces Y757 by a phenylalanine (α MHC-Cre^{tg/-};gp130^{fl/Y757F} mice), STAT3 is constantly activated after MI because the negative feedback loop via SOCS-3 that normally limits STAT3 activation is interrupted. These mice experience increased recruitment of leucocytes and macrophages, increased left ventricular rupture rates, scar dilatation, heart failure and high mortality after MI.²² Hyperactivated STAT3 in cardiomyocytes of infarcted α MHC-Cre^{tg/-};gp130^{fl/Y757F} mice was mainly responsible for these adverse effects since genetic reduction of STAT3 by deletion of one allele (α MHC-Cre^{tg/-};gp130^{fl/Y757F};STAT3^{fl/+}) diminished the pro-inflammatory effects and reduced mortality after MI.²² The mannose-binding lectin (MBL)/lectin complement activation seemed to play a key role, since the C3 antagonist cobra venom factor (CVF) attenuated cardiac inflammation after MI and improved post MI survival in α MHC-Cre^{tg/-};gp130^{fl/Y757F} mice.²² The results of these experiments support the hypothesis that a balanced STAT3 activity in cardiomyocytes is required to prevent excessive and harmful inflammatory responses to cardiac injury.

The vascular endothelial cells and cellular compartment are critically involved in the induction of inflammatory events in the myocardium as they regulate the transmigration of immune-competent cells. For example, a JAK3/STAT3-mediated up-regulation of adhesion molecules in the endothelium is mediated by TNF- α as shown in endotoxemic mice, which displayed an increase in phosphorylated STAT3 levels in the heart and subsequent up-regulation of ICAM-1 expression in the arteriolar and capillary endothelium after LPS injection.¹⁴ In cardiomyocytes, IL-6 enhances the expression of ICAM-1 via activation of STAT3.¹⁰⁵ Elevated levels of adhesion molecules at the cell membrane promote migration of inflammatory cells into the tissue and activation at the site of infection or injury. Thus, the endothelial adhesion molecules, which appear partly regulated by STAT3, serve as mediators of the cellular interplay between the vasculature and inflammatory cells in the injured heart.

Overall, STAT3 seems crucial for a balanced recruitment of inflammatory cells to the injured myocardium in response to various stress stimuli (Figure 1). This process requires the STAT3-dependent intercellular communication between cardiomyocytes and endothelial cells, as well as invading inflammatory cells (Figure 1).

8. Conclusion

Recent findings imply multiple biological functions of STAT3 in different resident cardiac cell types, as well as in invading cells, which are far from being understood. We begin to understand how, in the various cardiac cell types, STAT3 critically impacts on environmental changes that occur with cardiac remodelling on the vasculature, the extracellular matrix, the inflammation, and the regeneration further add to the complexity of STAT3-mediated regulatory processes in the heart. This way STAT3 participates in the co-ordination of cardiac physiological function, as well as in the responses to physiological and pathological stress stimuli and emerges as a central player, not only in intracellular regulatory mechanisms but also in the intercellular communication system of the heart. Albeit, STAT3 may not be suited as a direct therapeutic target since it regulates a multitude of different biological processes in almost all human cells and organs. As pointed out in the present review, if STAT3 is constantly activated it induces hypertrophy and inflammation in the heart and also in other organs like the gut and it may induce cellular de-differentiation processes in specific cell types thereby promoting transformation towards cancerous cells. In turn, reducing STAT3 or blocking it under a certain threshold leads to diminished cardiac vasculature and increased fibrosis and to the development of heart failure. It therefore needs a precise and often cell-type-specific regulation to act beneficial, a task that is probably difficult to be reached by pharmacological interventions directly targeting STAT3. However, as discussed in this review and shown in *Table 1*, experimental modulation of STAT3 in cell-type- and organ-type-specific manners may identify downstream targets in STAT3-mediated processes that are much more cell-type-specific. To develop such STAT3 downstream mediators as novel therapeutic targets may therefore address pathomechanisms in various cardiovascular disease types more specifically with fewer unwanted side effects.

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