REVIEW

Targeting myocardial remodelling to develop novel therapies for heart failure

A position paper from the Working Group on Myocardial Function of the European Society of Cardiology

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The failing heart is characterized by complex tissue remodelling involving increased cardiomyocyte death, and impairment of sarcomere function, metabolic activity, endothelial and vascular function, together with increased inflammation and interstitial fibrosis. For years, therapeutic approaches for heart failure (HF) relied on vasodilators and diuretics which relieve cardiac workload and HF symptoms. The introduction in the clinic of drugs interfering with beta-adrenergic and angiotensin signalling have ameliorated survival by interfering with the intimate mechanism of cardiac compensation. Current therapy, though, still has a limited capacity to restore muscle function fully, and the development of novel therapeutic targets is still an important medical need. Recent progress in understanding the molecular basis of myocardial dysfunction in HF is paving the way for development of new treatments capable of restoring muscle function and targeting specific pathological subsets of LV dysfunction. These include potentiating cardiomyocyte contractility, increasing cardiomyocyte survival and adaptive hypertrophy, increasing oxygen and nutrition supply by sustaining vessel formation, and reducing ventricular stiffness by favourable extracellular matrix remodelling. Here, we consider drugs such as omecamtiv mecarbil, nitroxyl donors, cyclosporin A, SERCA2a (sarcoplasmic/endoplasmic Ca^{2+} ATPase 2a), neuregulin, and bromocriptine, all of which are currently in clinical trials as potential HF therapies, and discuss novel molecular targets with potential therapeutic impact that are in the pre-clinical phases of investigation. Finally, we consider conceptual changes in basic science approaches to improve their translation into successful clinical applications.

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Keywords   Heart failure • Contractility • Cardiomyocyte survival • Adaptive hypertrophy • Cardiac remodelling

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Introduction

In spite of the success of current therapeutic approaches,[1] the social and economic burden of heart failure (HF) continues to increase and, with few new drugs surviving phase III trials, there is a critical need for a large pipeline of new targets.[2] To produce a more successful strategy for developing novel therapies for HF, it is still necessary to better understand the cellular and molecular basis of myocardial function to determine how this is altered in the failing heart. This requires a myocardial-centric focus. Secondly, it is time to forego ideas of a single 'magic bullet' and move towards an approach that takes into account the huge heterogeneity of different factors that result in cardiac failure. To illustrate this, we will focus on key processes that are associated with and lead to HF (cardiomyocyte contractility, cardiomyocyte death, angiogenesis, and fibrosis), highlighting aspects with therapeutic potential.

Improving cardiomyocyte function

The paradigm of providing support for the failing heart focuses on enhancing myocardial performance whilst relieving workload. Cardiomyocyte excitation−contraction coupling revolves around efficient management of intracellular Ca^{2+} and the response of the myofibrillar apparatus. There are several targets for intervention to improve myocardial performance and we provide examples to illustrate potential benefits of classical small molecule inhibitors, activators, agonists, and antagonists, in addition to gene therapy.

Modulation of beta-adrenergic signalling

Most inotropic agents in clinical practice enhance accumulation of cAMP, increasing cAMP-dependent protein kinase (PKA)-enhancing Ca^{2+} transients and cardiomyocyte contractility. These treatments are less effective in failing hearts since beta-adrenergic receptor (β-AR) signalling is down-regulated, with decreased receptor density and uncoupling from downstream signalling molecules.[3] Furthermore, long-term administration of such inotropes [i.e. beta-agonists and protein diesterase 3 (PDE3) inhibitors] is associated with a higher incidence of arrhythmias and cardiac cell death, with increased mortality. Also, the excessive catecholaminergic rise which takes place in early stages of HF to sustain function and increase heart rate is detrimental for cardiomyocytes in the long term. Beta-blockers are now well-established therapeutics for the treatment of chronic HF, since they can reverse the changes in beta-adrenergic signalling, reduce heart rate, and have potential direct antiarrhythmic effects. An alternative way to modulate β-AR signalling is to blunt the activity of G-protein-coupled receptor kinase 2 (GRK2; also known as βARK) that is elevated in HF[3] (*Figure 1*). GRK2 phosphorylates β-AR leading to recruitment of beta-arrestin, resulting in β-AR desensitization and receptor recycling/degradation. Numerous studies implicate GRK2 in β-AR control of contractile function/dysfunction, and inhibiting its activity may be beneficial. A truncated form comprising the C-terminal 194 residues or shorter 10 residue peptide inhibitors[4] of GRK2 (βARKct) competing for interaction of GRK2 with β-ARs inhibits GRK2-mediated receptor desensitization and can represent an efficient treatment for depressed cardiac function (*Table 1*).[4]
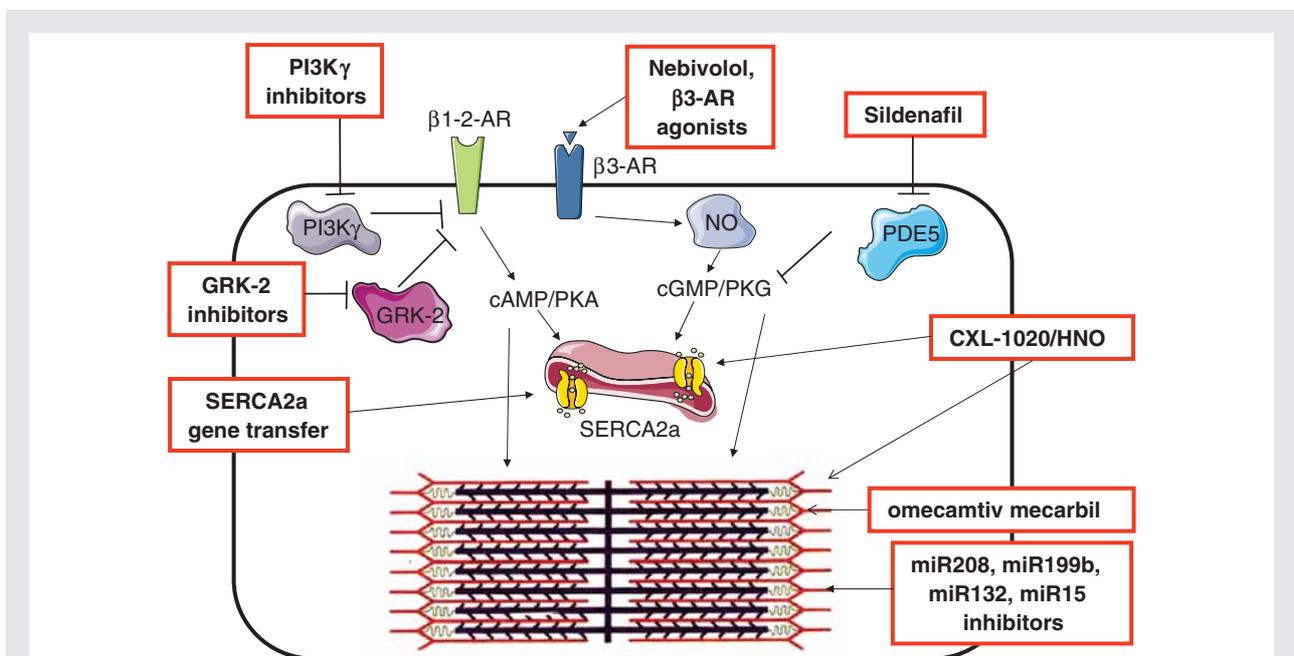

Figure 1 Therapeutic strategies to improve cardiomyocyte function. Red boxes identify molecules capable of improving cardiomyocyte function by acting on the indicated targets. GRK2, G protein-coupled receptor kinase 2; PI3K, Phosphoinositide 3 kinase gamma; SERCA2a, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a; b3-AR, beta3 adrenergic receptor.

Table 1 Molecular targets and drugs with therapeutic potential on myocardial remodelling in heart failure

Drug/target	Mechanism of action	Experimental stage	References
Improving myocyte function			
GRK2	Prevent β-AR desensitization	Pre-clinical	Ciccarelli *et al.*[4]
PI3Kγ	Normalization of β-AR density and contractility	Clinical	NCT00103350
Sildenafil	Increase GMP/PKG signalling	Clinical	NCT00763867[23]
CXL-1020	HNO donor	Clinical (NCT01092325, NCT01096043)	25, 116
β_3AR agonists (e.g. nebivolol)		Pre-clinical/clinical	16–19
SERCA2a	$\uparrow$Ca^{2+} SR uptake	Clinical (NCT0045481, NCT01643330)	29
Omecamtiv mecarbil	Myosin activator	Clinical (NCT00624442)	32
miR-208, miR-199a-5p, miR-199b, miR-212/132, miR15	Inhibit maladaptive hypertrophy	Pre-clinical	34–38, 40, 117
Preventing myocyte death			
Necrostatin-1	Inhibit RIPK1 and necroptosis	Pre-clinical	44, 45
Cyclosporin A	Inhibit MPTP opening and cell death	Clinical (NCT01650662, bNCT01502774)	48, 49
Neuregulin 1	Enhance protective signalling	Clinical (NCT01251406)	52, 53
HSPB5, HSPB6, HSPB8, BAG3, Melusin	Promote protein folding and enhance protective signalling	Pre-clinical	56, 58, 59, 61, 63, 64, 118, 119
CHIP, Atrogin1, MuRF1, Telethonin	Control of protein degradation	Pre-clinical	65, 120
Boosting angiogenesis in the heart			
VEGF-A	Promote endothelial cell growth and migration	Clinical	82–85, 121
VEGF-B	Promote endothelial cell growth and migration; enhance myocyte survival	Pre-clinical	89
PLGF	Promote endothelial cell growth and migration; regulate inflammation	Pre-clinical	87, 88
miR-126, miR-210	Promote endothelial cell growth and migration	Pre-clinical	90–92
Bromocriptine	Inhibit prolactin release and protect in PPCM	Pre-clinical/clinical	94, 95
miR146, miR92a, miR-24	Protection in PPCM and MI	Pre-clinical	96–99
Erythropoietin	Protection in anthracycline-induced myopathy	Pre-clinical	100
Regulating interstitial remodelling			
Thrombospondins, SPARC, syndecans	Promote favourable matrix remodelling; reduce inflammation	Pre-clinical	40, 104, 106, 122, 123
Torasemide	Diuretic, indirectly reduces fibrosis	Clinical	108, 109
miR-21 inhibitor	Inhibit fibroblast proliferation and secretion of ECM	Pre-clinical	110, 111, 124
miR-101, miR-29	Down-regulate ECM transcripts	Pre-clinical	112

AR, adrenergic receptor; ECM, extracellular matrix; MI, myocardial infarction; MPTP, mitochondrial permeability transition pore; PI3K, phosphoinositide 3-kinase; PKG, protein kinase G; PPCM, peripartum cardiomyopathy; RIPK1, receptor-interacting serine/threonine-protein kinase 1; SERCA2a, sarcoplasmic/endoplasmic Ca^{2+} ATPase 2a; SPARC, secreted protein acidic and rich in cysteine; SR, sarcoplasmic reticulum; VEGF, vascular endothelial growth factor.

Small molecule inhibitors such as balanol and other heterocyclic compounds with GRK2 specificity have been identified[5] that appear to lock GRK2 in an inactive conformation. Although GRK2 is an attractive therapeutic target for HF, it phosphorylates other G-protein-coupled receptors (GPCRs), affects insulin signalling, and interacts with several different proteins.[6] Thus, GRK2 inhibitors should be carefully selected, avoiding potential off-target effects.

Another interesting kinase involved in beta-adrenergic contractility is the class Ib phosphoinositide 3-kinase-γ (PI3Kγ).[7,8]

This molecule functions as a stress kinase and is weakly expressed by healthy cardiomyocytes, but is dramatically up-regulated in response to mechanical and functional stress.[7,9] In cardiomyocytes, PI3Kγ catalytic activity is triggered specifically by GPCRs and contributes to their desensitization and internalization.[10] Genetic and pharmacological blockade of PI3Kγ in mouse models of HF has shown an important normalization in adrenergic receptor densities and in cardiac contractility[7] (*Figure 1*). In addition, PI3Kγ is also heavily expressed by leucocytes and plays a crucial role in the

inflammatory reaction that develops after pathologically increased cardiac workload and that leads to LV fibrotic remodelling.[11] In line with this view, genetic and pharmacological blockade of PI3Kγ in both cardiomyocytes and leucocytes significantly protects against HF (*Table 1*).[11] While a clinical trial with a putative PI3Kγ selective inhibitor has been completed (NCT00103350) in 'Heart Attack Treated With Angioplasty', the results have not fulfilled expectations. Nevertheless, it is likely that the trial involved inhibitors that were not yet optimal in terms of selectivity and potency. Better PI3Kγ selective inhibitors are currently under investigation and new attempts are foreseen in the near future.

Recently, the third isotype of β-ARs has come into focus as a new modulator of ventricular remodelling. Although initially ascribed to metabolic regulation in adipose tissue, the β_3AR has been identified in cardiac myocytes and endothelial cells from human ventricular tissue, where it couples to nitirc oxide synthase (NOS) to increase vascular and myocardial nitric oxide (NO).[12] Notably, the β_3AR is up-regulated in hypertrophic cardiac muscle[13] and, unlike β_{1-2}AR, is resistant to desensitization, which makes it an attractive therapeutic target in the context of hyperadrenergic drive during the development of heart failure (*Table 1*). Mice with cardiac-specific overexpression of the human β_3AR are protected from hypertrophic and fibrotic remodelling under neurohormonal or haemodynamic (transverse aortic constriction) stress, whereas they keep their normal contractile reserve through β_{1_2}AR stimulation. Notably, this protection is lost when NOS are inhibited; in fact β_3ARs are part of a signalosome in cardiac myocyte caveolae with both endothelial NOS (eNOS) and neuronal NOS (nNOS) acting cooperatively to sustain NO/cGMP signalling to protein kinase G (PKG). This is contingent upon nNOS activity to inhibit reactive oxygen species (ROS) production from xanthine oxidoreductase, thereby protecting eNOS from oxidative uncoupling.[14] Therefore, when both enzymes are active, β_3AR maintains its protective effect against remodelling even under oxidant stress from harsh neurohormonal or haemodynamic stress.[15]

The third-generation beta-blocker, nebivolol, combines specific β_1AR antagonism with additional agonism at β_3AR[16] (*Figure 1*), and has been proven effective (albeit not superior to other beta-blockers) to reduce the composite endpoint of all-cause mortality and cardiovascular admissions in elderly patients (>70 years) with HF (regardless of EF) (*Table 1*).[17] In animals, the additional benefit of β_3AR stimulation beyond β_1AR blockade was demonstrated on post-infarction remodelling; notably, this additional benefit was abrogated in eNOS-knockout mice, consistent with the coupling of β-AR to eNOS.[18] More recently, a similar comparison of nebivolol with metoprolol in the setting of neurohormonal stress showed that nebivolol uniquely prevented hypertrophic remodelling, while preserving β-AR functional coupling, consistent with the phenotype of β_3AR-overexpressing mice.[19]

Nitric oxide/cGMP signalling and nitroxyl

Another way of enhancing cGMP/PKG signalling is inhibition of the cGMP-catabolizing PDE5, which is up-regulated in early HF, thus contributing to cardiac remodelling[20] (*Figure 1*). Inhibition of PDE5

with sildenafil reverses maladaptive remodelling in mice with HF induced by aortic banding.[21] In HF patients, sildenafil may enhance cardiac output during exercise, reducing pulmonary resistance and improving functional capacity and clinical status, impacting both systolic and diastolic function, and triggering reverse remodelling.[22] These effects are not attributable to any other vasodilatory or endothelial effects, and seem to result from a direct influence on the myocardium probably relating to increased activity of cGMP-dependent PKG that may phosphorylate a number of downstream targets.[21] Despite these positive observations, a multicentre trial in HF patients with preserved EF (RELAX, NCT00763867) failed to demonstrate any significant improvement in exercise capacity or clinical status with sildenafil compared with placebo (*Table 1*).[23] The molecular effects of alternative cGMP- or PKG-enhancing strategies on the myocardium remain to be fully determined, and understanding these may identify other therapeutic options. Concomitant activation of β_3AR may ensure sufficient activation of upstream NO/soluble guanylyl cyclase (and protection from oxidant stress) to generate enough cGMP and downstream activation of antihypertrophic mechanisms. Possibly, the combination of specific PDE inhibition with β_3AR activation may prove to be superior.

NO donors have been used for decades to relieve workload in decompensated hearts, but controversies persist.[24] The scenario is complicated because redox variants of NO or its oxidized products (i.e. nitrite/nitrate) distinctively modulate cardiac contractility and vascular function, particularly under conditions of hypoxia or increased oxidative stress. Nitroxyl (HNO) is produced by purified NOS *in vitro* under conditions of oxidative or nitrosative stress.[25] HNO donated by Angeli's salt improves cardiac function independently of β-AR signalling, with no change in cGMP levels. Instead, HNO targets excitation−contraction coupling and myofilament proteins, modifying cysteine residues to enhance Ca^{2+} handling and increase myofilament Ca^{2+} sensitivity[25] (*Figure 1*). Whereas beneficial effects of NO signalling to cGMP may be lost in conditions of increased cardiac oxidative stress (given the high reactivity of NO with ROS), this does not occur with HNO whose efficacy may be preserved in conditions with altered redox balance. These features render HNO donors attractive therapeutic alternatives to NO donors (*Table 1*).[26] Novel HNO donors (e.g. CXL-1020) are long lasting and more specific. CXL-1020 has confirmed HNO-dependent positive inotropic and lusitropic effects in animal studies, and human clinical trials are currently in progress (NCT01092325, NCT01096043).

Sarcoplasmic/endoplasmic Ca^{2+} ATPase

In HF, Ca^{2+}-handling becomes disrupted with abnormal Ca^{2+} stores in the sarcoplasmic reticulum (SR), Ca^{2+} leak from ryanodine receptors, decreased Ca^{2+} re-uptake secondary to decreased SERCA2 (sarcoplasmic/endoplasmic Ca^{2+} ATPase 2) protein, and increased Ca^{2+} extrusion.[27] Frequency-dependent up-regulation of SR Ca^{2+} load is absent, with a decline in contractility at higher heart rates. As the protein principally responsible for Ca^{2+} uptake into the SR, SERCA2a is an attractive target for HF therapy (*Table 1*).[27,28] Increased expression of SERCA2a improves cardiac

function in pre-clinical models of HF and has a beneficial impact on arrhythmias and remodelling.[28] Pharmacological approaches for manipulating SERCA2 expression have not emerged and, as a large protein (∼110 kDa), a gene therapy approach has been adopted to increase SERCA2a expression. Gene therapy is, in itself, a major challenge. For the heart, viral-based vectors are proving most successful and, for SERCA2a, adeno-associated viral vectors (AAVs) are being used. The phase I/II CUPID (Calcium Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) study (NCT00454818) brought encouraging results, with improvements in symptoms and LV remodelling in 39 severe HF patients.[29] A second phase II study is due to report in 2016 (NCT01643330) (*Figure 1*). The success of trials with SERCA2a opens the door to other gene therapy approaches for the treatment of HF.

Cardiac myosin activators

Myocyte contraction is induced by Ca^{2+} binding to troponin C. This causes a conformational change in the thin filaments, exposing the myosin-binding sites on actin and allowing the formation of acto-myosin bridges. The transition from this weak interaction to a strongly bound state, together with force generation for contraction, requires ATP hydrolysis by the myosin head and is the rate-limiting step. Compounds that enhance the transition of myosin into the force-generating state increase contractile force (*Figure 1*). These cardiac myosin activators are exemplified by omecamtiv mecarbil (CK-1827452) which binds directly to the myosin catalytic domain, stabilizing an actin-bound conformation of myosin and increasing fractional shortening in cardiomyocytes in the absence of any increase in Ca^{2+} transients.[30] In dog models of HF, it increases stroke volume and cardiac output without any increase in oxygen consumption.[31] In a phase II trial (NCT00624442), omecamtiv mecarbil improved cardiac function in HF patients,[32] raising expectations that cardiac myosin activators constitute a novel therapeutic approach for improving cardiac function without detrimental effects of indirect inotropic mechanisms (*Table 1*). However, the applicability in HF with diastolic dysfunction may be more problematic if the drug prolongs the systolic phase with a consequent reduction of relative diastolic time.

micro RNA regulation of cardiomyocyte hypertrophy, function, and contractility

Non-protein-coding microRNAs (miRNAs) play important roles in regulating cardiac biology and function.[33] They generally target the 3'-untranslated region of protein-coding mRNAs, increasing mRNA degradation and/or inhibiting protein synthesis. Changes in miRNA expression profiles in cardiovascular diseases are therefore pathophysiologically important because of their impact on protein-coding mRNAs. Furthermore, the availability of stable, small molecules that mimic or down-regulate miRNAs renders them attractive therapeutic targets (*Figure 1*). Initial studies in mice indicated that miR-208a promotes cardiac hypertrophy and is required for normal cardiac conduction.[34] Antisense oligonucleotides that silence miR-208a prevent cardiac remodelling, improve cardiac
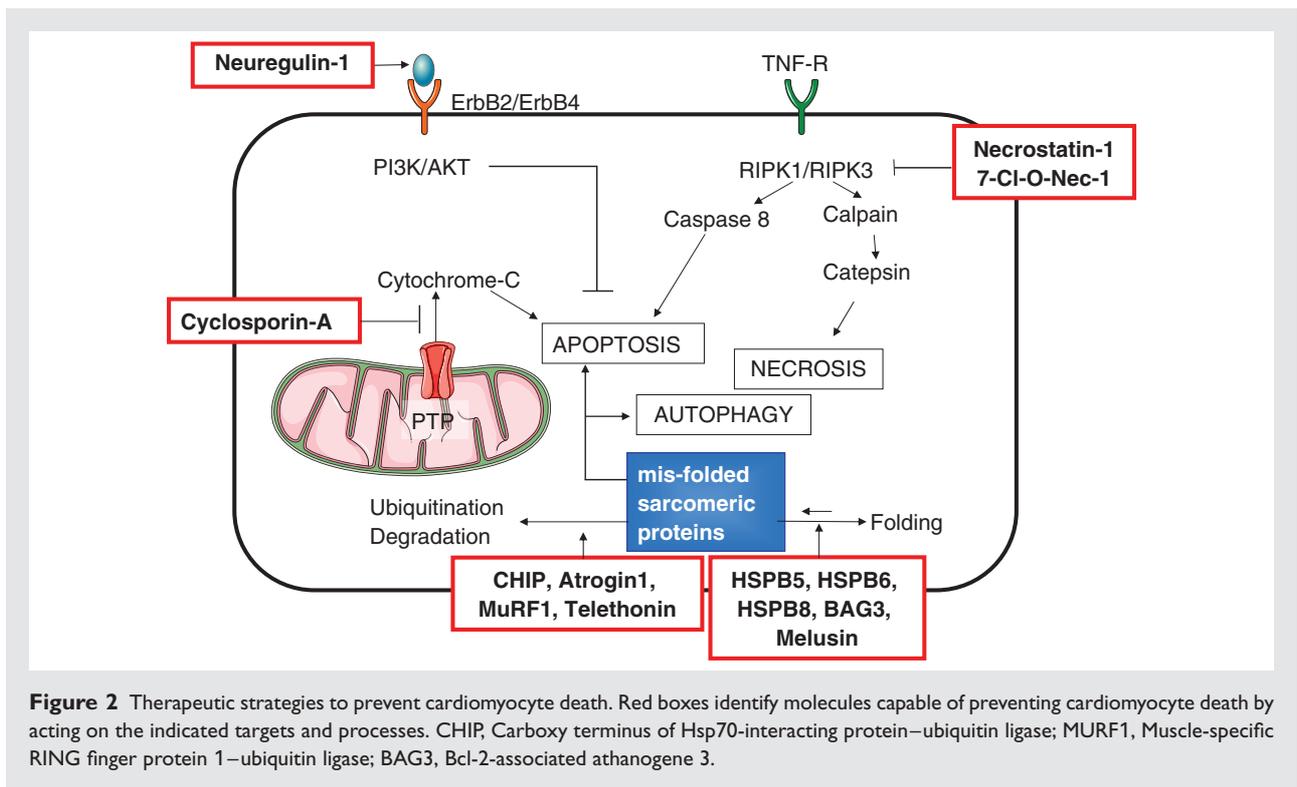
function, and enhance cardiomyocyte survival in hypertension-induced HF in rats (*Table 1*).[35] Other miRNAs regulating cardiac hypertrophy are miR-199a-5p, miR-199b, and the miR-212/132 cluster, which all increase in expression in HF.[36–38] *In vivo* inhibition of miR-199b reverses cardiac hypertrophy and fibrosis in mouse models of HF. Similarly, inhibition of miR-132 or the inflammatory miR-155[39] during LV pressure overload blocks development of cardiac hypertrophy and inhibits progression to HF. miR-15 is also up-regulated in response to ischaemia–reperfusion injury in the hearts of mice and pigs, and silencing miR-15 prevents hypoxia-induced cardiomyocyte cell death, reduces infarct size and cardiac remodelling, and enhances cardiac function in response to myocardial infarction (MI).[40] Clearly, there are many other therapeutic possibilities in this area.

Preventing cardiomyocyte death

Cardiomyocyte death is a typical feature of the failing heart, causing largely irreparable damage.[41] Cell death most commonly occurs via necrosis (passive, unregulated cell death that is energy independent and associated with inflammation) or apoptosis (regulated cell death requiring energy for cells to be destroyed in the absence of inflammation). Apoptosis is initiated by death receptors or intracellular organelles (e.g. mitochondria), leading to activation of caspases that dismantle the cellular contents for digestion by surrounding cells. Although inhibiting caspases should prevent cardiomyocyte apoptosis, this may simply trigger a switch to necrosis. Recently, a mechanism of regulated necrosis has emerged, necroptosis, for which there may be greater therapeutic potential.[42]

Receptor-interacting protein kinase 1 and necroptosis

Tumour necrosis factor α (TNFα) binding to its receptors induces formation of signalling complexes that activate survival signalling (complex I) or trigger apoptosis (complex II)[43] (*Figure 2*). In complex I, RIPK1 (receptor-interacting serine/threonine-protein kinase 1) is ubiquitinated and stimulates protective signalling. Deubiquitination of RIPK1 removes it from complex I, allowing it to integrate into complex II (the death-inducing signalling complex, DISC) that activates caspase 8, which then cleaves and inactivates RIPK1. If this pathway is blocked, RIPK1 forms a necrosome with RIPK3 and induces necroptosis via calpains with cytosolic release of lysosomal hydrolases. RIPK1 forms an adaptor in complex I/II and kinase activity is not required, but necroptosis requires protein kinase activity. Necrostatin-1 is an allosteric inhibitor of RIPK1 which reduces infarct size in *in vivo* and *ex vivo* models of ischaemia–reperfusion injury (*Table 1*).[44] It is also cardioprotective in a mouse model of ischaemia–reperfusion injury, and mice treated with necrostatin-1 have preserved cardiac function with less inflammation.[45] It remains to be determined whether these results translate into larger animal models or humans, and if other necrostatin-1 homologues (e.g. 7-Cl-O-Nec-1) with increased specificity and improved pharmacokinetics[46] may afford greater cardioprotection.

Figure 2 Therapeutic strategies to prevent cardiomyocyte death. Red boxes identify molecules capable of preventing cardiomyocyte death by acting on the indicated targets and processes. CHIP, Carboxy terminus of Hsp70-interacting protein−ubiquitin ligase; MURF1, Muscle-specific RING finger protein 1−ubiquitin ligase; BAG3, Bcl-2-associated athanogene 3.

The mitochondrial permeability transition pore and cyclosporin A

Mitochondria, the powerhouses of the cell, are key players in apoptosis. The proton gradient across the mitochondrial membrane is lost as mitochondria become depolarized, leading to apoptosis or necrosis.[47] The mitochondrial permeability transition pore (MPTP) is important in this process, and loss of cyclophilin D (a core component of the MPTP) protects against cardiomyocyte death. Cyclosporin A (CsA), a powerful immunosuppressant drug, binds to cyclophilin D, inhibiting MPTP opening and preventing cardiomyocyte apoptosis/necrosis[48] (*Figure 2*). CsA also attenuates myocardial injury in *ex vivo* and *in vivo* models of ischaemia−reperfusion injury. A pilot study of patients with acute ST-elevation MI indicates that administration of CsA at the time of PCI limits reperfusion injury and reduces infarct size (*Table 1*).[49] The results of ongoing CIRCUS (does Cyclosporine ImpRove Clinical oUtcomes in ST elevation myocardial infarction patients?) and CYCLE (CYCLosporinE A in Reperfused Acute Myocardial Infarction Prospective, Controlled, Randomized, Multicentre Trial to Examine Whether a Single i.v. Bolus of Cyclosporine A Before PCI Can Reduce Myocardial Reperfusion Injury in Patients With STEMI) trials have yet to be reported (NCT01650662; NCT01502774).

Neuregulin 1 and ErbB2 signalling

The importance of neuregulin 1 (NRG1) signalling via ErbB2/ErbB4 epidermal growth factor family receptors in the adult heart emerged following reports of cardiotoxicity resulting from trastuzumab, the anticancer drug targeting ErbB2.[50] In cardiomyocytes, NRG1 activates the extracellular signal-regulated kinase 1/2 (ERK1/2) and PI3K → Akt pathways,[50] two potently cardioprotective systems (*Figure 2*). Recombinant (rh) NRG1 improves cardiac function, reduces pathological changes, and extends survival in rodent models of cardiomyopathy.[51] It also improves contractility/relaxation in pacing-induced HF in dogs. Two studies in humans with chronic HF indicate that rhNRG1 is safe, and may improve cardiac dimensions and function (*Table 1*).[52,53] Phase II and phase III trials of subcutaneous administration of rhNRG1 in chronic HF are ongoing (NCT01251406). A phase I trial of the NRG1β isoform in patients with LV dysfunction and symptomatic HF is due to report (NCT01258387). All these trials focus on short-term administration of NRG1, given that a major concern for any chronic treatment is potential tumorigenic effects.

Proteostasis and cytoprotection

Management of protein turnover (proteostasis) is crucial to cell survival and requires folding of new proteins, stabilization of protein structures, protein trafficking, and refolding of damaged proteins.[54] This is managed by a large family of molecular chaperones, along with the ubiquitin proteasome system that degrades dysfunctional proteins.[54] Accumulation of misfolded proteins, whether from mutations affecting protein folding during *de novo* biosynthesis or a consequence of cellular stress, causes proteotoxicity that impairs cell function and leads to autophagy or cell death (*Figure 2*).

Chaperones and cardioprotection

Molecular chaperones [also termed heat shock proteins (HSPs)] include HSP70 and HSP90 families with ATP-dependent chaperone activity and small ATP-independent HSPs (HSPBs), the activities and specificities of which are regulated by co-chaperones. HSPBs are generally cardioprotective (*Table 1*). HSPB5 (αB-crystallin) prevents desmin aggregation, and mutations in HSPB5 cause desmin-related myopathy in humans which is partly attributable to excessive reductive stress.[55] Overexpression of HSPB5 in mice protects against cardiomyocyte apoptosis, decreases infarct size induced by ischaemia−reperfusion, and has a beneficial effect on pressure overload-induced cardiac hypertrophy.[56] HSPB6 (HSP20) controls both cell survival via the Akt pathway and contractility via the PP1−phospholamban−SERCA2 axis, and its cardioprotective function has been extensively demonstrated in several models of heart failure.[57] Other chaperones modulate survival signalling. HSPB8 (αC-crystallin or H11 kinase) has protein kinase activity and enhances PI3K/Akt signalling, promoting cardiomyocyte survival.[58] HSPB8 knockout mice exposed to pressure overload show increased mortality and more rapid transition to HF, confirming that HSPB8 is cardioprotective.[59] Melusin, a muscle-restricted 38 kDa chaperone, potentiates ERK1/2 and PI3K/Akt prosurvival pathways via the assembly of a signalosome complex.[60] Cardio-specific overexpression of melusin in mice reduces cardiomyocyte apoptosis induced by chronic pressure overload and prevents HF.[61] BAG1 and BAG3 (co-chaperones for HSPB8) regulate macroautophagy.[62] BAG1/HSPB8 inhibits apoptosis and protects cardiomyocytes, whereas BAG3/HSPB8 binds to misfolded proteins and induces autophagy.[62] BAG3 mutations are associated with dilated cardiomyopathy, probably because of impaired Z-disc assembly.[63] Overall, increasing the chaperone content of cardiomyocytes is likely to be beneficial in HF, but strategies to exploit this are currently limited (*Table 1*). Drugs that increase chaperone expression include statins and geranylgeranyl acetone; while the latter has been successfully used in a pre-clinical dog model of arrhythmia,[64] rosuvastatin has failed to prove therapeutic efficacy in a clinical trial on older patients with systolic HF (NCT00206310). Alternatively, a gene therapy approach with AAV vectors may be feasible[29] (*Figure 2*).

Ubiquitin ligases and cardiac function

Polyubiquitination of proteins targets them to the proteasome for degradation. Ubiquitin (a highly conserved 76 amino acid peptide) is linked to E1 ubiquitin-activating enzyme by one of ~40 E2 ubiquitin-conjugating enzymes. E3 ligases transfer ubiquitin from E1 to specific substrates. E3 ligases are increasingly recognized as important regulators of cardiac function.[65] For example, CHIP (a co-chaperone and ubiquitin ligase) protects against ischaemia−reperfusion injury, reducing cardiomyocyte apoptosis either by co-ordinating refolding of damaged proteins or (in the absence of repair) by targeting them to the proteasome (*Table 1*). Atrogin1 (muscle atrophy F-box) operates in a ubiquitin ligase complex that inhibits cardiac hypertrophy by promoting degradation of calcineurin, whereas MuRF1 (muscle-specific ring finger 1) and MuRF2 E3 ligases are required for myofibril turnover and sarcomeric maintenance. MuRF1 also promotes degradation of protein kinase C ϵ and suppresses hypertrophic signalling. Additionally, telethonin (titin cap or TCAP), mutations in which cause familial cardiomyopathy, binds to and increases the activity of the E3 ligase MDM2.[66] A major substrate of MDM2 is the proapoptotic transcription factor p53. Loss of or mutations in telethonin increase p53 levels, and cardiomyocyte apoptosis ensues. Thus, increasing telethonin levels or telethonin-like peptides that promote p53 degradation may be useful as myocyte-specific cytoprotective agents. Understanding of the role of E3 ligases and the ubiquitin−proteasome system in HF is clearly in its infancy (*Table 1*). With >600 potential E3 ligases in the human genome,[67] the potential for therapeutic modulation within this system is likely to be high (*Figure 2*).

Cardiomyocyte protein kinases as potential novel targets

Protein kinases regulate every aspect of cell function, including the cell fate decisions of cardiomyocytes that lead to HF. Protein kinases are readily targeted by small molecules and are therefore attractive therapeutic targets. However, with some exceptions (e.g. GRK2 and RIPK1, discussed above), few therapies targeting cardiomyocyte protein kinases are emerging. There are >500 protein kinases in the human genome,[68] but most studies of cardiac protein kinases focus on relatively few that are often ubiquitously expressed [e.g. the mitogen-activated protein kinases (MAPKs)] and which regulate key cell functions.[69] These will not necessarily constitute good therapeutic targets for HF because of side effects of such therapies in other organs. Targeting cardiac-specific components that feed into the pathways may be a viable option (e.g. upstream components of JNK and p38-MAPK signalling, such as ASK1[70]), as may targeting downstream kinases (e.g. p90 ribosomal S6 kinases or MAPK-activated kinases 2/3/5[71]) that may have more selective effects (*Table 1*). Other cardiomyocyte-specific protein kinases may also be good therapeutic targets, but remain poorly characterized.

The paucity of our knowledge of cardiomyocyte protein kinases is highlighted in a proteomics study of the most abundant protein kinases in human heart.[72] The list includes (for example) several from the 28-member mSTE20 kinase family, many of which were identified as upstream activators of JNKs and p38-MAPKs.[73] With greater structural diversity in the regulatory domains, these may be better therapeutic targets than the MAPKs themselves (*Table 1*). mSTE20 kinases do not exclusively regulate MAPKs. PAK1 is involved in cardiomyocyte excitation−contraction coupling,[74] whereas MST1 promotes cardiomyocyte apoptosis.[75] Other highly expressed cardiac mSTE20 kinases include OXSR1, MST3/STK24, and SLK.[72] In kidney, OXSR1 regulates electroneutral cation-Cl-coupled co-transporters that modulate ion and volume homeostasis,[76] potentially performing a similar function in the heart. MST3 is expressed in cardiomyocytes, and homology to OXSR1 suggests it may be similarly involved in ion channel regulation.[77,78] SLK is activated by focal adhesion kinase (FAK),[79] which is important in

cardiac hypertrophy.[80] SLK may therefore also be significant in cardiomyocyte remodelling. Other STE20 kinases expressed in cardiac cells have not been studied. Further novel kinases in human heart which have yet to be studied include NEK7, NEK9, and STK33 (*Table 1*).[72] Thus, the potential for protein kinases as therapeutic targets for HF remains to be properly investigated. It is still necessary to define the cardiac kinome, establish which protein kinases are expressed in different cardiac cell types, and define the signalling networks in which they operate.

Regulating cardiac angiogenesis

Cardiomyocytes constitute only ~30% of the cells in the cardiac system and these rely on an efficient blood supply delivered by the vasculature. Hence, increasing angiogenesis is a vital element in HF therapy. The challenges differ markedly from therapies that target cardiomyocytes. The aim is to increase the endothelial cell population through proliferation of existing endothelial cells or proliferation/differentiation of progenitor cells (neovascularization). Most efforts focus on extracellular growth factors that promote cell proliferation/migration via endothelial cell surface receptors, with particular emphasis on the vascular endothelial growth factor (VEGF) system (*Figure 3*). However, more recent research also emphasizes important roles of antiangiogenic factors.

Proangiogenic strategies

Vascular endothelial growth factor family ligands

Vascular endothelial growth factors derive from five genes [VEGF-A (producing alternatively spliced isoforms including $VEGF_{121}$ and $VEGF_{165}$), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PLGF)] and they bind to receptor tyrosine kinases encoded by three genes [VEGFR-1 (Flt-1), VEGFR-2, and VEGFR-3].[81] Ligand binding induces receptor dimerization and activation. VEGF-A, VEGF-B, and PLGF bind to VEGFR-1. VEGFR-1 has limited signalling ability, and its main function may be to trap VEGF-A, preventing interaction with VEGFR-2. VEGF-A, VEGF-C, and VEGF-D bind to VEGFR-2, whereas VEGFR-3 is only bound by VEGF-C and VEGF-D.

Successful therapeutic angiogenesis was reported in pre-clinical models with rhVEGF-A or genes encoding VEGF-A[82] (*Figure 3*). Phase I trials of recombinant $rhVEGF_{165}$ in patients with myocardial ischaemia and peripheral vascular disease were also encouraging (*Table 1*).[83] However, significantly positive effects of VEGF therapy were not detected in either the VIVA (178 patients with stable ischaemic heart disease receiving $rhVEGF_{165}$ or placebo)[84] or RAVE (105 patients with peripheral artery disease receiving intramuscular injection of adenoviruses for $VEGF_{121}$ expression or placebo) trials (*Table 1*).[85] The reasons for the disparity are not clear. One problem may be that the pharmacokinetics of peptide growth factors such as VEGF differ in pre-clinical models compared with humans. Here, greater understanding of VEGF signalling in humans may be useful. Another factor might be the mode of delivery. High plasma concentrations required for adequate myocardial uptake using systemic delivery increase the risk of adverse effects (e.g. hypotension, local oedema, anaemia, or thrombocytopenia) that may offset beneficial effects of the therapy. Targeting VEGF to the heart may overcome this,[86] and transfer of genes encoding angiogenic proteins to target cells (i.e. angiogenesis by gene therapy) may be a more viable option.
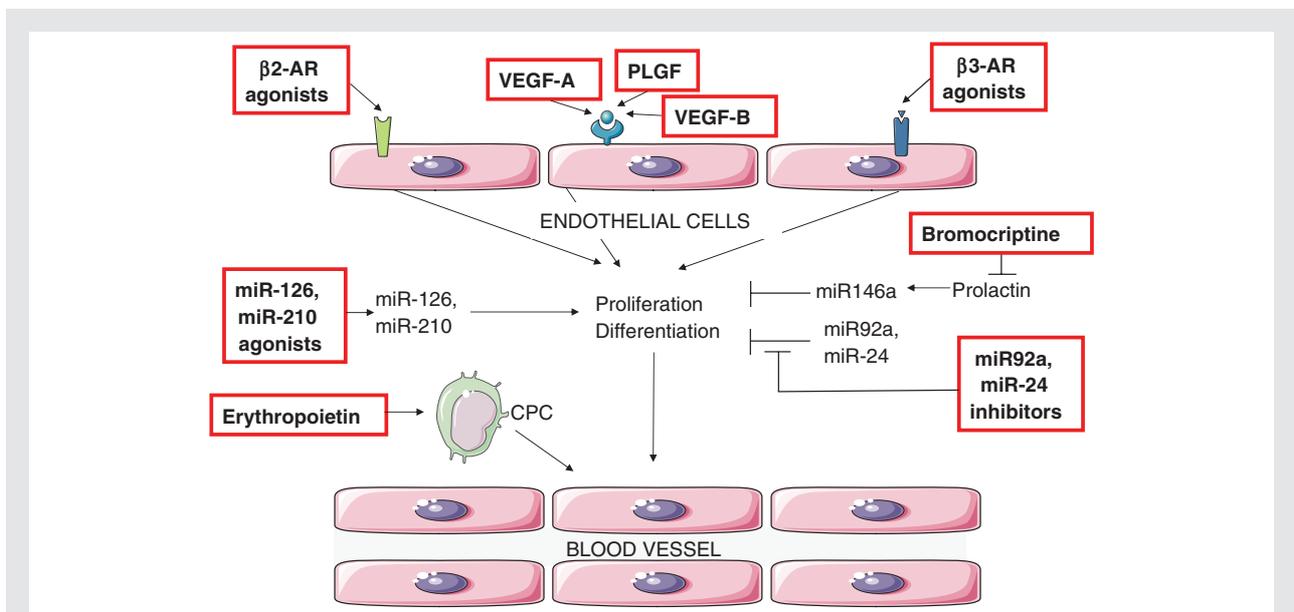

Figure 3 Therapeutic strategies to promote growth of blood vessels. Red boxes identify molecules capable of boosting growth of novel blood vessels by acting on the indicated targets and processes. b3-AR, beta3 adrenergic receptor; b2-AR, beta2 adrenergic receptor; VEGF-A, vascular endothelial growth factor A; VEGF-B, vascular endothelial growth factor B; PLGF, placenta growth factor.

Other ligands for VEGFR-1 may promote angiogenesis. PLGF is not essential during development or for vascular maintenance, but acts as a cytokine or proangiogenic factor in pathological conditions (*Figure 3*). In pre-clinical models, PLGF, delivered by systemic gene therapy with adenoviral vectors, increases angiogenesis in the heart and improves cardiac performance following MI,[87] suggesting that it could provide an alternative to VEGF-A. PLGF also regulates cardiac inflammation (*Table 1*).[88] AAV delivery of VEGF-B to mouse skeletal or heart muscle induces little angiogenesis.[89] However, cardio-specific overexpression of VEGF-B in transgenic mice or rats promotes cardiac hypertrophy whilst maintaining systolic function. This may result from effects on large vessels rather than the microvasculature, given that, in transgenic rats (though not mice), there is increased growth of epicardial coronary vessels and large arteries deep inside the subendocardial myocardium. Because cardiomyocyte damage in myocardial ischaemia begins in the subendocardial myocardium, VEGF-B-induced increased arterial supply to this area has particular therapeutic potential in ischaemic heart disease.

Proangiogenic micro RNAs

Certain miRNAs (e.g. miR-126 and miR-210) are enriched in endothelial cells and appear to regulate entire angiogenic gene programmes. Thus, miR-126 null mice die prematurely because of impaired angiogenesis,[90] and silencing of miR-126 reduces angiogenesis in a model of hindlimb ischaemia.[91] miR-210 improves angiogenesis, inhibits apoptosis, and improves cardiac function in a mouse model of MI (*Table 1*).[92] In these cases, miRNA mimics may be useful in promoting vascularization in ischaemic tissues (*Figure 3*).

Antagonizing antiangiogenic factors

Specific pathological conditions, such as peripartum cardiomyopathy (PPCM) and anthracycline-induced cardiotoxicity, are associated with loss of existing microvessels. In these cases, maintaining capillary density may prevent HF progression.

sFlt-1

A soluble, truncated form of VEGF1-R (sFlt-1; equivalent to the extracellular, ligand-binding domain) binds VEGF proteins, sequestering them from transmembrane receptors and inhibiting VEGF signalling.[81] To prevent extensive bleeding following release of the placenta, large quantities of sFlt-1 are released at the end of pregnancy. This produces an antiangiogenic environment, potentially compromising organs such as the heart that rely on a high capillary density. Usually, protective systems are enacted, including up-regulation of VEGF expression in cardiac cells.[93] Failure in this process seems to promote HF in pre-eclampsia and PPCM. Thus, application of rhVEGF may be a useful therapy for women at risk of PPCM (*Table 1*).

16 kDa prolactin

The nursing hormone prolactin is proangiogenic but, under conditions of oxidative stress, it is cleaved to generate a potently antiangiogenic N-terminal 16 kDa fragment (16 kDa prolactin) that is implicated in PPCM.[93,94] Inhibiting prolactin release with bromocriptine in experimental models attenuates PPCM,[94] and studies in humans indicate that bromocriptine is beneficial in acute PPCM[95] (*Figure 3*). 16 kDa prolactin up-regulates miR-146a in endothelial cells, where it suppresses proliferation and survival signalling.[96] miR-146a is also released into the circulation (indeed, serum levels of miR-146a are a specific biomarker for human PPCM) that are taken up by cardiomyocytes. Here, miR-146a down-regulates ErbB4, and, with this, epidermal growth factor receptor signalling, and compromises cardiomyocyte function.[96] Indeed, miR-146a antagomirs rescue PPCM in a pre-clinical model without affecting normal prolactin signalling.[96] Thus, 16 kDa prolactin destroys the vasculature and impairs cardiomyocyte function via miR-146a. A combination of bromocriptine (to prevent generation of 16 kDa prolactin) together with miR-146a antagomirs and/or rhVEGF may be a viable approach for preventing HF or improving recovery in women at risk of PPCM. The first experimental studies of this combination therapy are promising (*Table 1*).[93]

micro RNAs in the ischaemic heart

Other antiangiogenic miRNAs include miR92a and miR-24. Antagomirs of miR-92a increase neovascularization in mice following MI, with functional improvements, reduction of infarct size, and an increase in capillaries, particularly in the infarct border zone,[97] while miR-92a inhibition reduces infarct size and post-ischaemic loss of function in a porcine model of ischaemia−reperfusion (*Table 1*).[98] miR-24 is up-regulated in cardiac endothelial cells by ischaemia, and triggers endothelial cell apoptosis resulting in impaired vascularization.[99] Consistent with this, miR-24 antagonism after MI increases capillary density, reduces infarct size, and improves cardiac function (*Figure 3*). In such cases, antagomirs offer a promising therapeutic approach for myocardial ischaemia.

Anthracyclines and the cardiac vasculature

Cardiac complications develop in ∼10% of cancer patients treated with anthracyclines such as doxorubicin.[50] A contributing factor is probably doxorubicin-induced loss of endothelial cells and blood vessels in the heart.[100] The effect may involve up-regulation of miR-146a in the heart[101] and/or blocking the chemokine CCL2/CCR2 receptor signalling.[100] CCL2 increases production of regenerating endothelial cells from resident cardiac progenitors in a VEGF-independent manner. This system is down-regulated by doxorubicin, potentially via depletion of erythropoietin within the cardiac microenvironment. Provision of a synthetic erythropoietin derivative (in low non-haematocrit-active doses) restores the CCL2/CCR2 system in mouse models of doxorubicin-induced cardiomyopathy, overcoming the detrimental effects on endothelial cell regeneration. Thus, low-dose erythropoietin therapy and/or miR-146a antagomirs may provide a means of ameliorating at least some of the cardiotoxic effects of anticancer drugs such as doxorubicin (*Table 1*).

Regulating interstitial remodelling

The cellular components of the heart are embedded in fibrous extracellular matrix (ECM). In addition to collagen fibres forming the basis of the ECM, the ECM also contains proteoglycans, glycoproteins, glycosaminoglycans, and matricellular proteins.[102] Cardiac cells interact with this matrix via transmembrane proteins (e.g. integrins and syndecans) that, intracellularly, interact with signalling complexes. Increased cardiac stress produces a requirement for increased tensile strength and, whilst subsequent remodelling is a necessary adaptation, increasing interstitial fibrosis can increase myocardial stiffness with associated cardiac dysfunction.[102] Cardiomyocyte death also results in recruitment of myofibroblasts for cardiac repair.[103] Myofibroblasts produce a scaffold for collagen deposition and secrete autocrine/paracrine factors (e.g. angiotensin II, transforming growth factor β, and connective tissue growth factor) that sustain matrix production. In normal wound healing, the process is terminated by myofibroblast apoptosis. In the damaged heart, myofibroblasts persist and perpetuate ECM deposition. Targeting myofibroblast survival signalling may therefore provide a means of modulating interstitial remodelling in HF (*Figure 4*).

Matricellular proteins are structurally unrelated extracellular molecules that modulate cell−cell and cell−matrix interactions.[102] They include thrombospondin 1 (TSP1), SPARC (secreted protein acidic and rich in cysteine), tenascin C, TSP2, TSP4, tenascin-X, osteopontin, periostin, CCN1/Cyr61, and CCN2/CTGF. Affiliated proteins include syndecans, transmembrane proteins that can shed their extracellular domains and produce soluble molecules with autocrine/paracrine effects. These molecules are up-regulated in response to cardiac stress and have a significant impact on inflammation and fibrosis.[102] Thrombospondins, SPARC, and syndecans prevent systolic dysfunction by inhibiting infarct dilatation after MI, reducing the death rate of hypertrophic cardiomyocytes, and reducing collagen degradation (*Table 1*).[104−106] However, persistent SPARC and syndecan expression increases diastolic dysfunction through increased collagen production or cross-linking, causing enhanced cardiac stiffness. Nevertheless, these matricellular proteins may provide important protection following MI (*Figure 4*).

Currently, this area of cardiac interstitial remodelling is severely underinvestigated with, consequently, few potential therapies. However, modulating the ECM to attenuate myocardial stiffness may be particularly important in HF with preserved EF, a frequent cause of HF with no proven evidence-based therapies to date. The challenge is to manage the remodelling whilst maintaining tensile strength and sustaining cardiomyocyte function. Here, we highlight two areas with particular promise.

Collagen cross-linking and myocardial stiffness

Procollagen is secreted from cells (mostly fibroblasts) and processed extracellularly by specific proteases to produce collagen fibrils. These are cross-linked to generate fibres with high tensile strength, a reaction catalysed by lysyl oxidase (LOX).[107] Collagen cross-linking is influenced by non-structural matrix proteins.[102] The myocardial collagen network mainly comprises type I and type III fibres. Large diameter type I fibres have a high degree of cross-linking and are stiffer than type III fibres. Although the ratio of type I to type III fibres may influence myocardial stiffness, overall concentrations of extracellular collagen and/or the degree of
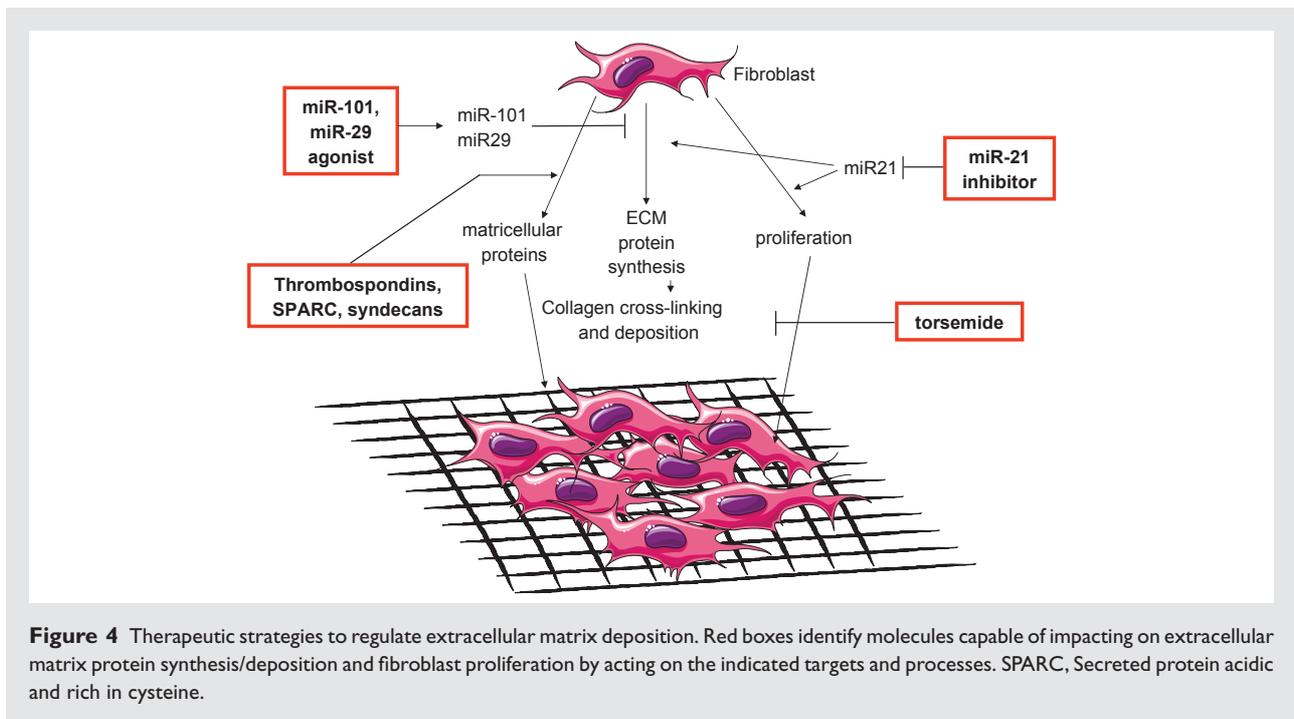

Figure 4 Therapeutic strategies to regulate extracellular matrix deposition. Red boxes identify molecules capable of impacting on extracellular matrix protein synthesis/deposition and fibroblast proliferation by acting on the indicated targets and processes. SPARC, Secreted protein acidic and rich in cysteine.

cross-linking have a greater impact. Thus, inhibiting the extracellular proteases that process collagens or inhibiting LOX may be beneficial (*Figure 4*). Both LOX and myocardial stiffness are increased in animal models of HF and in the myocardium of patients with hypertensive heart disease or HF.[107] Systemic administration of LOX inhibitors is associated with a high degree of toxicity *in vivo*. This is unsurprising given the importance of collagen cross-linking throughout the body, and targeting such therapy to the heart may be more fruitful. The loop diuretic torasemide may be effective in conditions with increased LOX expression (*Table 1*). Initial studies of HF patients indicated that torasemide (but not an alternative loop diuretic, furosemide) decreases LOX expression, reduces collagen cross-linking, and improves LV function[108] (*Figure 4*). However, the phase IV TORAFIC trial failed to show any significant decrease in myocardial fibrosis in hypertensive patients with clinically stable HF with either drug.[109] Further studies are clearly required.

micro RNAs to target fibrosis

Cardiac fibroblasts are enriched with specific miRNAs. Of these, miR-21 promotes ERK1/2 signalling in cardiac fibroblasts, increasing survival and growth factor secretion, thus inducing interstitial fibrosis.[110] In mice, miR-21 antagomirs reduce cardiac ERK1/2 signalling, inhibit interstitial fibrosis, and attenuate cardiac dysfunction induced by pressure overload. This is also observed in models of atrial fibrosis[111] (*Figure 4*). Validation in large animal models of cardiac fibrosis is clearly needed for future development of clinical applications. Other miRNAs implicated in cardiac fibrosis include miR-101 and miR-29.[112] miR-29 targets mRNAs encoding ECM proteins so its down-regulation allows up-regulation of matrix protein mRNAs (*Table 1*). Despite the promising data for miRNA therapeutics focusing on interstitial fibrosis, many miRNAs remain to be studied in this context.

The future for heart failure research?

So, how do we generate a more successful strategy for identifying therapeutic targets? A realistic evaluation of actual therapeutic options for any proposed target is necessary, accounting for the way in which a particular gene/protein may be targeted and potential off-target effects. For example, the oncogenic potential of proangiogenic or cardioprotective strategies must be considered. Targeting therapies to the heart and/or identifying cardiac-selective targets will almost certainly be required for these and other therapies. We must place greater value on fundamental cardiac-focused research to understand better the molecular basis of the 'healthy' cardiac system. Such 'foundation science' is important not only in blue skies research required to identify novel potential therapeutic areas, but also in answering questions of how current therapeutic approaches may be better targeted to individuals and applied in the clinical setting. Another key aspect requiring careful consideration, particularly with the ethical and financial costs, is the disparity between pre-clinical data from different laboratories and between pre-clinical (usually mouse) models and clinical trials.

Here, we suggest that our research community would benefit from standardization in reporting, if not application, of at least some experimental models. We need to develop criteria for minimum reporting information for pre-clinical models, and we must consider not only species and strain, but also aspects such as gender, age, gene or drug doses, and reproducibility between animal models.

Finally, it is worth considering who should be treated with which drugs. The concept of personalized medicine is highly attractive, given that we can now define the genome of any individual and identify polymorphisms or gene mutations.[113] Genome-wide association studies (GWAS) can be useful in identifying candidate genes/proteins that contribute directly to a disease and for establishing genetic predisposition (e.g. BAG3[62]). There is also the potential for correlating genetic profiles with drug metabolism (pharmacogenomics) to identify patients who will benefit most from existing drug therapies (e.g. ACE inhibitors or β-AR antagonists[114]) and for risk stratification (e.g. co-morbidity, cardiotoxicity of cancer drugs[115]). Current emphasis is placed on the genetic component, but epigenetic profiling (e.g. DNA methylation or histone acetylation/phosphorylation) may be at least as informative. Such studies are long-term research investments, but potential benefits are extremely high.

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