ROS signalling between endothelial cells and cardiac cells

Min Zhang and Ajay M. Shah

Cardiovascular Division, James Black Centre, King’s College London British Heart Foundation Centre of Excellence, 125 Coldharbour Lane, London SE5 9NU, UK

Received 28 January 2014; revised 24 February 2014; accepted 25 February 2014; online publish-ahead-of-print 3 March 2014

The heart contains not only cardiomyocytes but also other cell types such as endothelial cells and fibroblasts. Functional crosstalk among these cell types is important for normal cardiac function and is also involved in disease pathophysiology. Recent data indicate that redox signalling within and between endothelial cells and cardiomyocytes, both through direct and indirect mechanisms, is an important aspect of the functional communication between these cell types. Such signalling influences contractile function, cardiomyocyte growth, hypertrophy, angiogenesis, and fibrosis, and may play an important role in cardiac remodelling in disease.

Keywords
Cardiomyocyte • Endothelial cell • Paracrine • Redox signalling • NADPH oxidase • Nitric oxide

This article is part of the Spotlight Issue on: Heterocellular signalling and crosstalk in the heart in ischaemia and heart failure.

1. Introduction

Around 30% of the cells within the heart are cardiomyocytes, whereas the other 70% comprise endothelial cells (EC), fibroblasts, smooth muscle cells, and immune cells. Physical interaction and tightly regulated interplay among cardiac cells are critical for heart development and postnatal function. Effective cardiac function demands a sufficient supply of oxygen and nutrients, which is facilitated by the high capillary density of 3000–4000/mm² in the adult mammalian myocardium. This intimate anatomical arrangement of cardiac myocytes within the coronary microvascular network—no cardiomyocytes being >2–3 μm from an EC—not only allows for adequate blood supply, but also facilitates the bidirectional communication between these cell types.

The cardiac endothelium includes the endocardial EC lining the heart chambers and vascular EC lining the coronary microvasculature. They share broad common features and roles in signal transduction induced by neurotransmitters, hormones, and mechanical stimuli, but also have major differences with regard to embryological origin, developmental, morphological, and functional properties. Pioneering work by Brutsaert et al. first reported that the endocardial endothelium can directly modulate the contractile state of subjacent cardiomyocytes, an effect that was later extended to myocardial vascular EC. A similar modulatory role also appears to exist in the human heart in vivo. These acute modulatory roles of myocardial EC on cardiac contractile function can be attributed to paracrine factors released by EC including nitric oxide (NO), endothelin-1, prostanoids, natriuretic peptides, and other cytokines and agents. EC-derived NO acts to enhance myocardial relaxation, an effect that involves cyclic GMP/protein kinase G-mediated reduction in myofilament Ca²⁺ sensitivity. The importance of EC for cardiomyocyte differentiation and embryonic cardiac development is well recognized and has been reviewed elsewhere. In addition, signalling factors reciprocally secreted by cardiomyocytes that impact on EC are also required for the proper development of the heart and cardiac responses to disease.

Redox signalling (i.e. signalling involving oxidation/reduction modification of biomolecules) influences many physiological processes in the heart and plays important roles in pathological cardiac remodelling. While numerous studies have focused on redox signalling within cardiomyocytes or within EC, emerging evidence suggests that functional redox crosstalk between these cell types in the heart is also important. In general, redox crosstalk between cardiomyocytes and cardiac EC may occur in three ways (Figure 1). Firstly, there may be direct diffusion of reactive oxygen species (ROS) and NO; secondly, ROS may indirectly affect cardiomyocyte function by effects on the extracellular matrix (ECM) in the heart; thirdly, there may be ROS-dependent alteration of the paracrine release of various cytokines and growth factors from EC. In this article, we review recent work on the involvement of ROS and redox signalling in the crosstalk between cardiomyocytes and EC and its impact on (patho)physiological regulation of cardiac structure and function.

2. Reactive species and redox signalling

Reactive species involved in redox signalling include ROS such as superoxide anion (O²⁻), hydroxyl (OH), and hydrogen peroxide (H₂O₂), and

---

Corresponding author: Tel: +44 2078485189; fax: +44 2078485193, Email: ajay.shah@kcl.ac.uk

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2014. For permissions please email: journals.permissions@oup.com.
reactive nitrogen species such as NO and peroxynitrite (ONOO$^-$), the latter being formed from the reaction of O$_2^-$ with NO. The pathophysiological effects of ROS depend upon the moiety generated, its concentration, subcellular localization, and the endogenous antioxidant status. If overall ROS production is elevated sufficiently to overwhelm cellular antioxidant defences, the cells experience oxidative stress which may result in cellular damage, energetic deficit, dysfunction, and death due to irreversible modification of membrane lipids, proteins, and nucleic acids. On the other hand, the tightly regulated and spatially confined production of small amounts of ROS in response to physiological and pathological stimuli reversibly modulates the activity of molecular targets such as ion pumps, channels, protein phosphatases, kinases, and other signalling proteins, thereby causing specific changes in cellular phenotype—so-called redox signalling. In the heart, such redox-regulated effects underlie the essential roles of ROS in modulating different components of cardiac remodelling. Sources of ROS in cardiac cells include mitochondria, xanthine oxidase (XO), uncoupled NO synthases (NOS), and NADPH oxidases (Noxes). A large body of evidence indicates that ROS generated by Nox proteins and their interactions with NOS-derived NO are especially important in redox signalling during the development of heart failure (HF).

3. Nox-dependent ROS generation

Nox proteins, which are major sources of ROS in the cardiovascular system, are of particular interest since they are the only enzymes that generate ROS as their primary purpose and in a highly regulated and spatially restricted manner, apparently suited for cell signalling. Seven Nox family members have been identified so far (Nox1–5 and Duox1–2), with Nox2 and Nox4 being the predominant isoforms present in cardiomyocytes and EC. Both isoforms exit as a heterodimeric flavocytochrome with a p22phox subunit, but they exhibit distinct differences with regard to their structure, activation, function, intracellular localization, and the type of ROS generated. Nox2 activation is typically triggered by G-protein-coupled receptor agonists such as angiotensin II, endothelin-1, and α-adrenergic agonists; growth factors such as thrombin; cytokines such as tumour necrosis factor-α (TNF-α); metabolic factors such as glucose and insulin; and mechanical forces. These stimuli induce the translocation of four cytosolic regulatory subunits (p47phox, p67phox, p40phox, and Rac1) to bind to the flavocytochrome, thereby initiating the production of O$_2^-$.

In contrast, Nox4 is constitutively active at low level and does not have an essential requirement for any known cytosolic subunits but appears to be regulated mainly by its abundance. Nox4 levels increase in cardiomyocytes in response to pressure overload, and in multiple cell types (myocytes, EC, and possibly fibroblasts) in response to ischaemia/hypoxia, starvation, and transforming growth factor-β (TGF-β). Nox4 generates predominantly H$_2$O$_2$ rather than O$_2^-$, which may be important due to the higher stability and diffusibility of H$_2$O$_2$ as well as its different interaction with NO when compared with O$_2^-$(see below). Of note, Nox-derived ROS can trigger further ROS generation by other sources such as xanthine oxidoreductase, mitochondria, and NOS (as discussed in later sections). In particular, Nox-dependent stimulation of mitochondrial ROS production may be of particular importance and may explain why mitochondrial-targeted antioxidants could significantly diminish angiotensin II-induced hypertension and associated cardiac dysfunction in some studies.

4. NOS and nitroso/redox balance

In the heart, NO is physiologically generated by constitutive NOSs, namely (i) endothelial NOS (eNOS) which is mostly found in EC and to a lesser extent in caveolae of cardiomyocytes and (ii) neuronal NOS (nNOS) which is predominantly localized in the cardiac sarcoplasmic reticulum (SR) and possibly mitochondria. Under pathological conditions, inducible NOS (iNOS) may also be an important source. NO influences cell functions by stimulating soluble guanylate cyclase to generate guanosine monophosphate (cGMP) or through post-translational modification of effector proteins, e.g. S-nitrosylation of cysteine residues. Excess O$_2^-$, however, interacts with NO extremely rapidly to form peroxynitrite (ONOO$^-$) and disrupt physiological NO signalling; at the same time, peroxynitrite may have its own distinct signalling effects. Therefore, the balance between NO and O$_2^-$ generation is an important determinant of the effects of NO. Reduced NO production and bioavailability can also result from NOS dysfunction. NOSs can become uncoupled and generate O$_2^-$ instead of NO when their essential cofactor tetrahydrobiopterin (BH$_4$) is depleted (typically due to its oxidation to BH$_2$) or following oxidative post-translational modification of the NOS enzyme. NOS-derived O$_2^-$ generation further decreases NO bioavailability. Whereas O$_2^-$ inactivates NO, H$_2$O$_2$ does not undergo this reaction and instead may even enhance NOS activity (Figure 2). This distinction may contribute to differences between the effects of Nox2 and Nox4, for example, their effects on peripheral endothelial function and angiogenesis.

The switch from NO to O$_2^-$ production contributes crucially to the pathogenesis of many diseases. Using a hypertension model in p47phox knockout mice, Landmesser et al. showed that increased O$_2^-$ and ONOO$^-$ may act as an amplifying mechanism to further exacerbate NO uncoupling by oxidizing BH$_4$. Administration of BH$_4$ attenuated maladaptive cardiac remodelling induced by pressure-overload. Interestingly, increased BH$_4$ in EC may also reduce myocardial damage during ischaemia-reperfusion injury, suggesting the importance of endothelial BH$_4$ in protecting cardiomyocytes in this setting.
Endothelial to cardiomyocyte communication and cardiac hypertrophy

Many signalling pathways involved in the development of cardiomyocyte hypertrophy are known to be redox-sensitive, but in addition altered endothelial redox state may also impact on cardiac hypertrophy and remodelling (Table 1). Neuregulin-1 (Nrg-1), a member of epidermal growth factor (EGF) family and a ligand for receptor tyrosine kinases of the ErbB family, is produced by ECs and has an essential role in normal development of the foetal heart through interactions with ErbB2/ErbB4 receptors on cardiomyocytes. It was later reported to also modulate hypertrophy and survival of postnatal cardiomyocytes. The importance of Nrg-1 in the adult human heart was emphasized following the discovery of cardiotoxicity related to trastuzumab (Herceptin), an ErbB2-targeted antibody used in the treatment of ErbB2-positive mammary carcinomas. Nrg-1/ErbB expression increases during the compensatory stage of concentric ventricular hypertrophy and then declines when the load-stressed heart fails. Deletion of Nrg-1/ErbB2/4 leads to dilated cardiomyopathy and exacerbated HF following pressure overload or doxorubicin treatment. Interestingly, Kuramochi et al. found that H2O2 induces Nrg-1 release from ECs and that Nrg1/ErbB4 signalling had paracrine anti-apoptotic effects. These authors also found that H2O2 exerted Nrg1-independent activation of ErbB4/Akt signalling. Taken together, these results suggest that endothelial Nrg1/ErbB4 signalling may have beneficial effects although the details of such interactions remain to be better defined.

Numerous studies support an anti-hypertrophic role for NO in cardiomyocytes and in the whole heart. The antihypertrophic NO effect is reported to involve an inhibition of the calcineurin/NFAT pathway by elevated intracellular cGMP/PKG1 activity or a down-regulation of cytoskeletal muscle titin protein. Both eNOS-deficient mice and nNOS-deficient mice displayed exacerbated left ventricular hypertrophy (LVH) and dysfunction after imposition of pressure overload. NO also mediates cytoskeletal changes such as the translocation of titin from the Z-disc to the junc tion with the M-line, which is the site of increased cGMP activity. NO mediates these changes by inducing a conformational change in titin, which reduces myofilament stiffness and myocardial relaxation. Further, NO modulates changes in myofilament Ca2+ sensitivity through an increase in titin phosphorylation. The combined effects of NO on myofilament Ca2+ sensitivity and titin phosphorylation are of potential therapeutic relevance as they may contribute to the protective effects of NO on cardiac function.

### Table 1: Paracrine factors involved in redox-sensitive signalling between ECs and cardiomyocytes

<table>
<thead>
<tr>
<th>Factor</th>
<th>Source cells</th>
<th>ROS-sensitive signalling pathway within the source cells</th>
<th>Signalling triggered within the target cells</th>
<th>Resulting biological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nrg1</td>
<td>EC</td>
<td>Release stimulated by ROS</td>
<td>ErbB4/Akt activation</td>
<td>Myocyte hypertrophy, survival</td>
</tr>
<tr>
<td>NO</td>
<td>EC</td>
<td>Activation of eNOS (e.g. by angiogenic factors)</td>
<td>RGS4 degradation</td>
<td>Myocyte hypertrophy</td>
</tr>
<tr>
<td>VEGF</td>
<td>Myocyte</td>
<td>Nox4-mediated Hif1 stabilization</td>
<td>Angiogenic signalling</td>
<td>Increased perfusion</td>
</tr>
<tr>
<td>VEGF</td>
<td>Myocyte</td>
<td>ROS-mediated GATA4 activation</td>
<td>Angiogenic signalling</td>
<td>Increased perfusion</td>
</tr>
<tr>
<td>H2O2</td>
<td>Myocyte</td>
<td>Increased Nox4 or other sources</td>
<td>eNOS activation/angiogenic signalling</td>
<td>Increased perfusion</td>
</tr>
<tr>
<td>NO</td>
<td>EC</td>
<td>Superoxide-mediated decrease in bioactivity</td>
<td>cGMP/PKG-mediated change in myofilament Ca2+ sensitivity; change in titin phosphorylation</td>
<td>Altered myocyte stiffness and myocardial relaxation</td>
</tr>
</tbody>
</table>

See text for full details of interactions.
overload or myocardial infarction (MI), while cardiomyocyte-specific overexpression of eNOS\textsuperscript{73} or nNOS\textsuperscript{74} attenuated LVH and contractile dysfunction after pressure overload. However, if NOSs are uncoupled and generate ROS instead of NO, this may promote hypertrophy as found in global eNOS knockout mice subjected to severe pressure overload.\textsuperscript{75} Since both cardiomyocytes and EC express NOS, it is of interest to know whether there are paracrine-specific effects of endothelial NO on cardiac hypertrophy. Interestingly, recent work suggests that NO from ECs may promote cardiac hypertrophy.\textsuperscript{76} These authors found that enhancement of angiogenesis in the murine heart through endothelial-specific expression of a pro-angiogenic peptide resulted in cardiac hypertrophy. An NOS inhibitor L-NAME was able to reduce the angiogenesis-driven myocardial hypertrophy despite no effect on EC mass and capillary density, suggesting that NO generated from endothelium might directly promote cardiomyocyte growth. The same group recently identified the NO-dependent mechanism responsible for these effects, which couples cardiac vessel growth with myocyte growth and heart size.\textsuperscript{77} In a series of studies in mouse models, Jaba et al.\textsuperscript{77} found that increased NO production subsequent to angiogenic stimulation in the heart led to the degradation of regulator 4 of G protein signalling (RGS4), followed by induction of cardiomyocyte hypertrophy through the GBγ/PI3K/PI3K/AKT/mTORC1 pathway. RGS4, a GTPase-activating protein for specific Gα subunits, negatively modulates G protein-mediated cardiomyocyte hypertrophy. RGS4 bears an N-terminal cysteine (Cys) residue that is degraded by arginylation through the N-end rule pathway,\textsuperscript{78,79} an ubiquitin-dependent proteolytic degradation of intracellular proteins. Importantly, NO controls the rate of N-terminal Cys oxidation prior to its arginylation, and arginylation may serve as a sensor of nitrosative/oxidative stress.\textsuperscript{80} Therefore, high NO levels from EC, e.g. during increased angiogenesis, may promote nearby cardiomyocyte hypertrophy by favouring RGS4 degradation (Figure 3 and Table 1).

6. Cardiomyocyte to endothelial communication and angiogenesis

Myocardial angiogenesis is tightly coupled to cardiomyocyte growth during heart development.\textsuperscript{81} Angiogenesis and/or other mechanisms to preserve myocardial capillary density are also induced in response to disease-causing stresses such as pressure overload that promote hypertrophy.\textsuperscript{82–84} It is now recognized that an increase in capillary density is essential for physiological cardiac hypertrophy whereas in pathological hypertrophy, the transition from a compensated to decompensated state is accompanied by a significant reduction in capillary density. Therefore, a failure to maintain appropriate matching between the vasculature and myocyte growth is believed to promote decompensation of hypertrophy. In the heart, a complex bi-directional crosstalk between cardiomyocytes and ECs influences both angiogenesis and hypertrophy (Table 1). On the one hand, myocardial angiogenesis is able to promote cardiomyocyte growth and hypertrophy, as discussed in the previous section. On the other hand, cardiomyocytes in the heart under chronic stress send ‘signals’ to surrounding EC to enhance angiogenesis and/or preserve existing vessels, thereby maintaining an adequate blood and oxygen delivery. Both NO and ROS play critical autocrine and paracrine roles in these processes.

Cardiomyocytes produce and release multiple paracrine signals to dynamically regulate the coronary vasculature. The best characteristic of the angiogenic factors released by cardiomyocytes is a vascular endothelial growth factor (VEGF), which can signal to adjacent EC to enhance capillary formation.\textsuperscript{5,81} Mice with cardiomyocyte-targeted deletion of VEGF-A exhibit defects in vasculogenesis/angiogenesis and a thinned ventricular wall.\textsuperscript{81} Interestingly, cardiomyocyte-specific knockout of VEGF-A results in >85% decrease in the VEGF mRNA level in the heart compared with normal controls, indicating that cardiomyocytes are a major source of this angiogenic factor in the heart.\textsuperscript{81}
The redox-sensitive transcription factor, hypoxia-inducible factor 1 (Hif1), is a major regulator of myocardial VEGF levels. Hif1 itself is subject to posttranslational regulation by oxygen-requiring prolyl hydroxylase enzymes (PHD), which promote the pro teaseal degradation of Hif1 following its hydroxylation at specific proline residues. Therefore, hypoxia is a strong stimulus for an increase in Hif1 levels.85 It was shown that in the heart hypertrophying in response to pressure overload, Hif1/VEGF signalling was critical for the maintenance of capillary density and that the inhibition of this pathway by p53 led to decompensation.86 Recently, with the use of complementary loss-of-function and gain-of-function mouse models, we found that cardiomyocyte Nox4 enhances Hif1/VEGF signalling and paracrine preservation of myocardial capillary density during pressure overload.22 Nox4 appears to enhance Hif1 signalling by inhibiting PHD enzymes, in line with a previous study in tumour cells.86 As a result, Nox4 knockout mice developed worse overload-induced LVH, contractile dysfunction, and dilatation than wild-type littermates, whereas Nox4-overexpressing mice exhibited the opposite phenotype.22 These findings reveal a novel protective role of ROS generated by cardiomyocyte Nox4 during the cardiac response to load-induced stress, involving a paracrine crosstalk with ECs and an increase in myocardial capillary density (Figure 3). Nox4-dependent enhancement of Hif1 signalling may also exert protective effects through other mechanisms, as suggested by a recent study in a model of ischaemia-reperfusion,87 although earlier work from the same group reported that Nox4-dependent ROS production may be detrimental in a model of severe pressure overload.88

In the peripheral vasculature, recent studies also found a pro-angiogenic effect of Nox4, but in this case this was attributable to an H2O2-dependent increase in eNOS expression and NO generation,49,50 consistent with the knowledge that NO is potentially pro-angiogenic during hypoxia.89 NO can S-nitrosylate Hif1 and increase its stability90 as well as directly inhibit PHD,91,92 thereby enhancing the expression of pro-angiogenic genes such as VEGF. As discussed earlier, EC-derived NO can degrade RGS4 in cardiomyocytes. RGS4 is an in fact also a physiological inhibitor of angiogenesis through selective antagonism of G protein and VEGF signalling,93 providing another mechanism for the pro-angiogenic effects of NO. Whether such NO-dependent mechanisms contribute to enhanced Hif1/VEGF signalling in the heart, and whether this may involve Nox4-dependent increase in eNOS in the EC, remains to be studied.

Pro-angiogenic signalling with cardiomyocytes may also be induced by the transcription factor GATA4,94 which functions as a critical regulator of cardiac differentiation as well as adult heart hypertrophy.95 Conditional transgenic overexpression of GATA4 in adult cardiomyocytes resulted in an augmentation of capillary density and increased coronary flow reserve due to a Hif1-independent increase in release of pro-angiogenic factors including VEGF-A.96 Mechanical stressors such as pressure overload or stretch may activate GATA4.96,97 Interestingly, it was recently found that Nox4-derived ROS activate GATA4 in pluripotent embryonal carcinoma cells through the redox-sensitive transcription factor c-Jun.98 These results raise the possibility that cardiomyocyte Nox4-mediated VEGF release and pro-angiogenic effects in the adult heart might also involve GATA4.

### 7. ROS signalling affecting the ECM and fibrosis

Myocardial fibrosis is an important feature of the adversely remodelling heart and contributes to impaired cardiac function, particularly diastolic dysfunction due to increased myocardial stiffness. Fibrosis may be triggered by local tissue injury, increased mechanical load, and activation of the renin–angiotensin–aldosterone system (RAAS). Complex functional interplay among multiple cell types, i.e. fibroblasts, EC, cardiomyocytes, and immune/inflammatory cells, is involved in the development of fibrosis. Accumulating evidence indicates that redox regulation within these individual cell types as well as the effects of altered redox state on cellular crosstalk influences fibrosis in the heart.

The role of Nox proteins in cardiac fibrosis has been extensively studied in various gene-modified models and indicates an important profibrotic role for Nox2. Interstitial cardiac fibrosis was significantly attenuated in global Nox2 knockout mice subjected to subpressor or pressor angiotensin II infusion,99,100 chronic genetic RAAS activation,101 aldosterone infusion,100 pressure overload induced by aortic banding,102 or permanent coronary artery ligation.103,104 A similar antifibrotic effect was reported in cardiomyocyte-specific Rac1 knockout mice.105 Levels of active TGF-β and connective tissue growth factor, which promote the transformation of fibroblasts into myofibroblasts, are potentiy increased by ROS106,107 and TGF-β in particular may mediate crucial crosstalk between cardiomyocytes and fibroblasts.108 While Nox4 has also been implicated in myofibroblast differentiation in vitro,2,109 Nox4-deficient mice actually developed more fibrosis than wild-type controls after pressure overload.22

The activation of matrix metalloproteinases (MMPs) is a key driver of ECM remodelling, e.g. after MI.110 ROS enhance MMP activity both by increasing gene transcription and by promoting posttranslational cleavage and activation of pro-MMPs.111 ONOO- is also a potent activator of MMPs.112 Nox2 has been shown to increase in vivo MMP activity and to contribute to adverse remodelling in the post-MI setting.103 Recent studies demonstrated that Nox2/ROS-mediated Ca2+-/calmodulin-dependent protein kinase II activation and an increase in MMP9 activity contributes to early cardiac rupture after MI.113 This is likely to involve MMP production by cardiomyocytes although more work is needed to definitively dissect out the cell-specific contributions to such redox-regulated effects in vivo.

Activation and dysfunction of the microvascular EC may also drive cardiac fibrosis. The healthy endothelium has anti-inflammatory properties but in disease settings, e.g. during RAAS activation, it becomes activated and pro-inflammatory due to the expression of surface adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1).114 Previous studies have suggested an important role for Nox2-derived ROS in EC activation and VCAM-1 expression115–117 as well as transendothelial migration of leukocytes.118 We recently directly investigated the effects of altered EC redox on the development of chronic angiotensin II-induced cardiac fibrosis using an in vivo mouse model of endothelium-targeted overexpression of Nox2.21 This study showed that the extent of angiotensin II-induced cardiac fibrosis in vivo was markedly increased in the mice with EC-specific Nox2 overexpression, and was accompanied by significant left ventricular diastolic dysfunction with preserved systolic function as assessed by pressure–volume relationships. Investigation of the underlying mechanisms revealed that increased EC Nox2 activity promoted leucocyte infiltration in the myocardium by enhancing VCAM-1 expression and leucocyte-EC interaction, and enhanced the process of endothelial-to-mesenchymal transition (EndMT). EndMT, a process whereby ECs transform into fibroblasts and which is important in formation of the atrioventricular cushion during heart development,119,120 has recently been shown to be an important contributor to cardiac fibrosis in mouse models of pressure overload121 and diabetes.122 The results of this recent study
8. Endothelial dysfunction and HFPEF

It has long been appreciated that the endothelium is a central regulator of cardiovascular homoeostasis. Endothelial dysfunction contributes to reduced myocardial perfusion and hence impaired cardiac function, and is a common feature in patients with chronic HF or in animal models of cardiac dysfunction. Using a non-invasive measure of endothelial function, Akiyama et al. recently reported that endothelial dysfunction was independently associated with prognosis in patients HFPEF. Studies on myocardial biopsy tissue obtained from HFPEF patients have reported morphological alterations such as increased interstitial fibrosis, increased cardiomyocyte stiffness, increased inflammation, and increased expression of adhesion molecules. These findings along with the results from animal studies reviewed in previous sections of this article suggest that altered endothelial redox state and endothelial dysfunction could be an important pathogenic mechanism in human HFPEF. Consistent with this idea, evidence of increased ROS generation and reduced myocardial protein kinase G activity was reported in heart tissue from HFPEF patients, although the precise role of EC is difficult to establish with certainty in such studies. If endothelial dysfunction does indeed pre-dispose or contribute to HFPEF, it may in part explain the increased incidence of this condition in patients with hypertension or diabetes, who are known to exhibit impaired endothelial function due to nitro-redox imbalance.

In addition to chronic changes in myocardial fibrosis and stiffness, EC-derived factors—in particular, NO—may also directly affect myocardial relaxation as mentioned in Section 1. EC-derived NO and its downstream effects were also confirmed in the human heart. NO produced by EC is suggested to regulate vascular tone and reduce contraction of smooth muscle cells, potentially through the reversible inhibition of cytochrome oxidase, whereas OONO− may irreversibly suppress respiration. In a recent study in a mouse model of hypertension, diastolic LV dysfunction was related to uncoupling of nNOS, with increased oxidative stress and reduced NO production. Taken together, these clinical and experimental studies support an important role for endothelial dysfunction in promoting HFPEF, probably through multiple NO- and ROS-dependent mechanisms that affect coronary perfusion, myocardial relaxation, cardiomyocyte stiffness, oxygen consumption, inflammation, and interstitial fibrosis.

9. Conclusions

There are complex functional interactions between cardiomyocytes and EC within the heart. Both cell types sense/send signals and their bidirectional crosstalk is an important determinant of structural and functional characteristics in both the healthy and diseased heart. The conversations between these cell types need to be precisely co-ordinated in order to ensure an appropriate and integrated biological response to different stimuli and physiological conditions. Conceptually, a proportional and balanced growth of cardiomyocytes and the microvasculature is critical for normal cardiac function. There also need to be functional alterations within these cell types in the heart that is responding to disease stresses. Recent studies indicate that nitro-redox balance and redox signalling between cardiomyocytes and EC play significant roles in these processes. These interactions are important in several conditions including pressure overload hypertrophy, the remodelling heart after MI, and in HFPEF. While the importance of redox signalling in cardiovascular physiopathology has received extensive attention, its role in the bidirectional crosstalk between myocytes and EC is still emerging. An improved understanding of the mechanisms and functional consequences of such signalling may allow the formulation of new therapeutic strategies for cardiac diseases.

Acknowledgements

We thank all current and past members of our laboratory for their contributions to the primary data reviewed herein.

Conflict of interest: none declared.

Funding

This work was supported by the British Heart Foundation; a Foundation Leducq Transatlantic Network of Excellence Award; and the Department of Health via a National Institute for Health Research (NIHR) Biomedical Research Centre award to Guy’s & St Thomas’ NHS Foundation Trust in partnership with King’s College London and King’s College Hospital NHS Foundation Trust.

References

ROS signalling between endothelial cells and cardiac cells


256

M. Zhang and A.M. Shah


