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SPOTLIGHT REVIEW

Cell–cell interaction in the heart via Wnt/β-catenin pathway after cardiac injury

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Abstract The adult mammalian heart predominantly comprises myocytes, fibroblasts, endothelial cells, smooth muscle cells, and epicardial cells arranged in a precise three-dimensional framework. Following cardiac injury, the spatial arrangement of cells is disrupted as different populations of cells are recruited to the heart in a temporally regulated manner. The alteration of the cellular composition of the heart after cardiac injury thus enables different phenotypes of cells to interact with each other in a spatio-temporal-dependent manner. It can be argued that the integrated study of such cellular interactions rather than the examination of single populations of cells can provide more insights into the biology of cardiac repair especially at an organ-wide level. Many signalling systems undoubtedly mediate such cross talk between cells after cardiac injury. The Wnt/β-catenin system plays an important role during cardiac development and disease. Here, we describe how cell populations in the heart after cardiac injury mediate their interactions via the Wnt/β-catenin pathway, determine how such interactions can affect a cardiac repair response and finally suggest an integrated approach to study cardiac cellular interactions.

Keywords Interactome • Wnt • β-Catenin • Hypertrophy • Infarction

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

1. Introduction

Acute ischaemic injury and myocyte hypertrophy represent the most common acute and chronic insults to the heart. In either case, the heart responds with a characteristic injury response that brings together different populations of cells of diverse origins in a spatio-temporal manner. Different cell populations recruited to the heart following acute injury or chronic hypertrophy can interact with each other directly (cell–cell contact) or indirectly through the expression of growth factors and cytokines to affect a physiological cardiac response. Moreover, sub-populations of the same cell type may exhibit or develop heterogeneity (e.g. fibroblasts) and affect function of other sub-populations.

The word 'interactome' in biological systems usually refers to interactions of proteins or genes with one another.[1] Genes or proteins that interact with one another form a network and such networks can interact with one another to constitute an interactome.[1] Such a concept can be easily extended to populations of cells as well. Interacting populations of cell under a defined condition can form a 'cellular interactome', and different populations of cells in the heart after cardiac injury can constitute a cardiac cellular interactome (*Figure 1*). Interactions in the cardiac cellular interactome will occur in a spatio-temporal manner with different cell populations being recruited to different regions of the heart at varying time points after cardiac injury. Interactions may be isotypic (cells of the same phenotype signalling to one another, e.g. endothelial–endothelial cell signalling) or heterotypic (cells of different phenotype signalling to another, e.g. fibroblast–myocyte interactions). The dissection of how different constituents of a cardiac cellular interactome cross talk with one another may reveal a greater insight into the understanding of a cardiac injury response, especially at a holistic level than the study of a single population of cells. Understanding interactions between different cellular constituents in the cardiac interactome could also be critical for designing therapeutic strategies as pharmacological agents could exert differing, opposing or even deleterious effects on diverse cell populations.

Many signalling systems undoubtedly regulate such complex cellular interactions and it is beyond the scope of a single review to discuss all such interactions. The Wnt/β-catenin signalling system including the transmembrane Frizzled receptors and related antagonists not only plays an extremely important role in cardiac development, but also is recognized to play a critical role in orchestrating a cardiac injury

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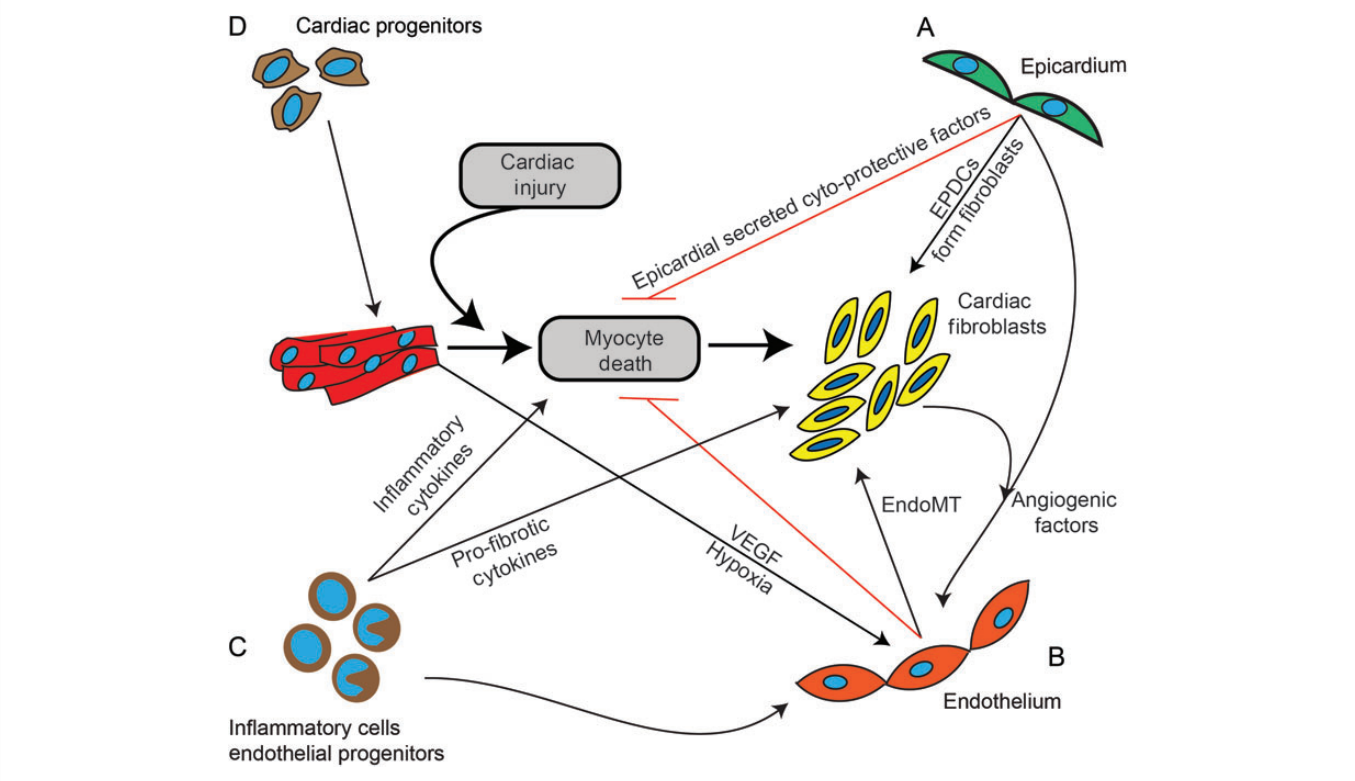

Figure 1 Principal interactions within the cardiac cellular interactome after acute ischaemic injury. Acute cardiac injury results in myocyte death and cardiac fibroblast activation. (*A*) The epicardium is activated as well and is another source of cardiac fibroblasts. The epicardium also secretes angiogenic and cytoprotective cytokines that promote angiogenesis and cardioprotection. (*B*) Hypoxic myocytes can express VEGF that activate endothelial cells. Cardiac fibroblasts and epicardial cells by expressing angiogenic factors promote endothelial cell activation and augmented post-injury neovascularization leads to enhanced myocardial function. A subset of endothelial cells undergo EndoMT and generate cardiac fibroblasts. (*C*) Bone marrow-derived cells are recruited to the heart and inflammatory cells express cytokines that promote cell death and fibrosis, while bone marrow-derived endothelial progenitors promote angiogenesis. (*D*) Cardiac progenitors are also activated following cardiac injury and potentially contribute to cardiac regeneration. Red bars indicate inhibition; EPDCs, epicardial-derived cells; VEGF, vascular endothelial growth factor; EndoMT, endothelial–mesenchymal transition.

response.[2,3] In this review, we focus on how similar or different populations of cells in the cardiac cellular interactome following cardiac ischaemia or hypertrophy interact with each other via the Wnt/β-catenin pathway, and outline an integrated approach for studying cellular interactions, that could be useful in developing new therapeutic strategies for cardiac repair.

2. The cardiac cellular interactome after injury

Cardiomyocytes, cardiac fibroblasts, endothelial cells, smooth muscle cells, and epicardial cells are the principal cell populations residing in the uninjured adult heart. Cardiomyocytes constitute ~85% of the mass of the adult rodent and human heart, but cardiac fibroblasts residing in the cardiac interstitium are the most populous type of cells comprising approximately two-third of the total number of cardiac cells.[4,5] Cardiac fibroblasts and myocytes together comprise ~95% of the total number of cells in the adult rodent heart. The non-myocyte, non-fibroblast population of cells in the adult heart predominantly consists of endothelial cells,[6] with a smaller fraction contributed by vascular smooth muscle cells and epicardial cells.

The cellular composition of the heart is dramatically altered following ischaemic or hypertrophic injury. Following myocardial infarction, a polymorphonuclear infiltrate is seen in the injured region within the first 24 h with replacement of the polymorphonuclear infiltrate with a monocyte/macrophage infiltrate over the next 48 h.[7] Within the next 3–7 days, there is rapid cardiac fibroblast and endothelial cell proliferation and deposition of matrix proteins (mainly collagens) in the infarcted region. The epicardial cells surrounding the injured myocardium are activated within a few days of ischaemic injury; the epicardium expands and epicardial-derived cells undergo epithelial–mesenchymal transition (EMT) to adopt a fibroblast phenotype and reside in the subepicardial space.[8,9] The cellularity in the injured region subsequently decreases and over the next 6 weeks, collagen cross-linking and scar contraction lead to the formation of a mature scar.[7] The cellular population of the heart rapidly changes after ischaemic cardiac injury with neutrophils, macrophages, fibroblasts, endothelial cells, and epicardial cells interacting with each other and myocytes to set up a complex spatio-temporally dynamic cardiac interactome (*Figure 1*).

In cardiac hypertrophy, the changes in the cellular environment are more sub-acute or chronic, but myocyte hypertrophy is accompanied by similar changes of fibroblast activation, deposition of extracellular matrix, and neovascularization.[10] The different interactions within the new cellular cardiac interactome play a critical role in physiological

compensation of the heart in cardiac hypertrophy and subsequent development of heart failure.[11]

3. Wnt signalling in the cardiac interactome

The Wnts are a family of 19 lipophilic proteins that play central roles in organogenesis as well as in key cell fate decisions, such as renewal, differentiation, and apoptosis. They bind to 10 different types of the Frizzled family of transmembrane receptors and can exert their effects through canonical (β-catenin-dependent) and non-canonical (β-catenin-independent pathways).[12] In the canonical Wnt pathway, binding of Wnt to the Frizzled receptor and co-receptor lipoprotein receptor related protein (LRP)5/6 initiates a signalling cascade where glycogen synthase kinase 3β (GSK3β)-mediated phosphorylation of β-catenin is inhibited with accumulation of β-catenin within the cytoplasm. β-Catenin then subsequently translocates to the nucleus where it activates the transcription factor 4 (TCF)/Lymphoid enhancer binding factor (LEF) group of transcription factors to initiate Wnt-dependent gene transcription (*Figure 2*). A complete discussion of the molecular details of canonical and non-canonical Wnt signalling is beyond the scope of this review, but the reader can refer to recent detailed reviews on this subject.[13,14]

The Wnt signalling is modulated by two classes of antagonists. The secreted frizzled-related protein (Sfrp) comprising five members (Sfrp 1–5) has striking homology to the transmembrane Frizzled (Fz) receptors and can bind Wnts extracellularly and antagonize Wnt signalling (*Figure 2*). Sfrps can also bind to the Fz receptor thus competitively inhibiting Wnts from binding to Fz. The Dickoppf (Dkk) class of proteins (Dkk 1–3) represents another family of Wnt antagonists and primarily antagonizes canonical Wnt signalling by inhibiting the binding of Wnts to the Frizzled co-receptor LRP5/6 (*Figure 2*). A comprehensive analysis of changes in expression of Wnts, Sfrps, and Frizzled genes after ischaemic myocardial injury has been reported by several groups.[8,15–17] Several members of the Wnt family including Wnt2, Wnt5a, Wnt5b, Wnt7b, Wnt9a, Wnt11, Sfrp1, Sfrp2, and Dkk-3 are expressed in the normal heart (*Table 1*).[17] Using a TOPGAL reporter mouse (β-galactosidase expression is driven by DNA response elements of TCF4, the Wnt-driven transcription factor), mesenchymal cells in heart valves and subsets of endothelial and smooth muscle cells scattered throughout the myocardium were observed to be Wnt responsive (*Table 1*). Expression of several Wnt ligands such as Wnt1, Wnt2, Wnt4, Wnt7a, Wnt10b, and Wnt11 increased significantly in the whole heart within the first 2 weeks of ischaemic myocardial injury (*Table 1*). Regions of the heart that demonstrated Wnt responsiveness after cardiac injury included the epicardium, mesenchymal cells, and fibroblasts in the sub-epicardial region, myofibroblasts, and subsets of endothelial and smooth muscle cells in the infarcted and peri-infarct region (*Table 1*). Among the Frizzled genes examined, expression of Frizzled 1, 2, 5, and 10 were elevated after myocardial infarction. In all these studies, changes in Wnt expression after cardiac injury were reported in the whole heart, and the particular cell types expressing and responding to specific Wnts remain largely unclear. Neither is it clear whether Wnts mediate their effects through autocrine vs. paracrine mechanisms.

3.1 Wnt-dependent myocyte–fibroblast interactions after cardiac injury

Kobayashi *et al.*[15] observed dramatic changes in expression of Sfrp1, Sfrp2, and Sfrp4 after ischaemic cardiac injury with Sfrp2 demonstrating more than a 80- to 100-fold change in the whole heart within 7 days of myocardial infarction. Sfrp2 was primarily expressed by cardiac fibroblasts in the region of injury, enhanced pro-collagen C-proteinase activity, and promoted formation of collagen from pro-collagen.[15] Mice deficient in Sfrp2 exhibited decreased amounts of fibrosis after

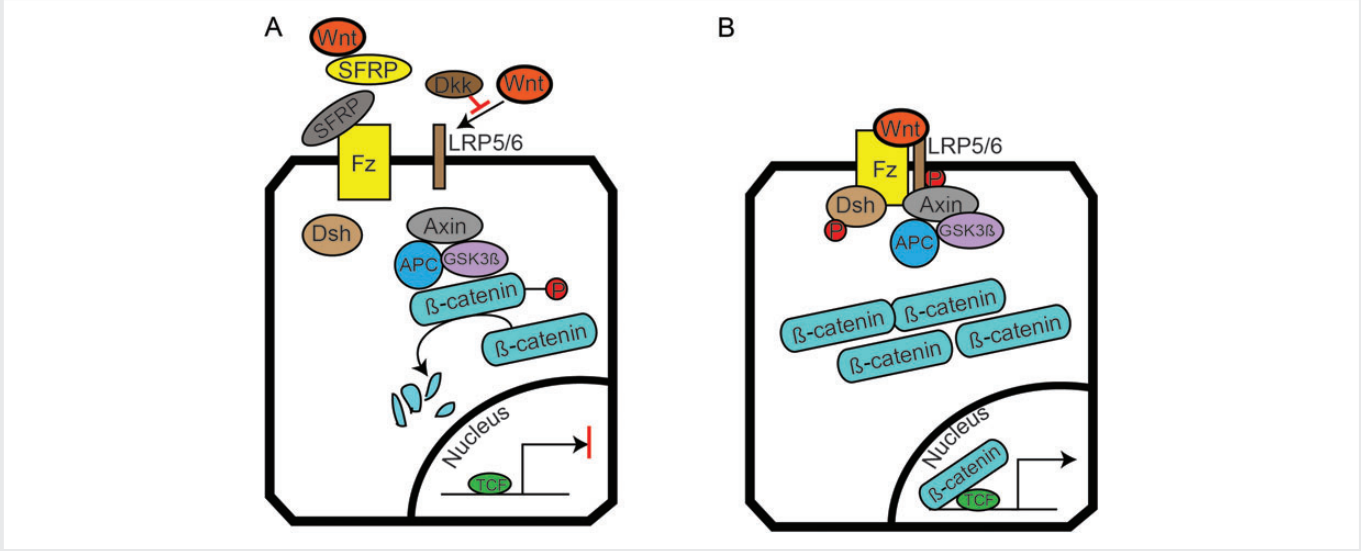

Figure 2 Canonical Wnt signalling. (*A*) In the absence of Wnt ligand, β-catenin is bound to the destruction complex of Axin, APC, and GSK3β and is phosphorylated and targeted for ubiquitin-mediated degradation. SFRP directly binds to Wnt outside the cell preventing it from binding to the frizzled (Fz) receptor, while Dkk competitively inhibits the binding of Wnt to LRP5/6 co-receptor. SFRP can also bind to Fz preventing Wnts from binding to Fzd. (*B*) Wnt binding to Fz and LRP5/6 receptor can initiate the canonical Wnt pathway. Dishevelled (Dsh) is phosphorylated and Axin relocates to the cell membrane with inhibition of the β-catenin destruction complex. β-Catenin accumulates within the cytoplasm and translocates to the nucleus where it associates with the TCF group of transcription factors to initiate Wnt-dependent target gene transcription.

ischaemic myocardial injury and had marked preservation in post-injury cardiac function[15] (*Figure 3*).

In contrast to the pro-fibrotic effect of Sfrp2, others have shown that Sfrp2, when injected into the rodent heart, exerted cardioprotective effects and led to decreased infarct sizes and amelioration of post-injury cardiac function[18] (*Figure 3*). In this report, injected Sfrp2 appeared to decrease fibrosis by inhibiting bone morphogenetic protein (BMP)-1-mediated pro-collagen processing.[18] Although the precise reasons behind the discrepant effects of Sfrp2 on myocardial infarction size are not clear, it may be related to the concentration of Sfrp2 in the infarcted heart, the degree of Wnt antagonism mediated by Sfrp2, and potential direct effects of Sfrp2. Wnt3a enhanced hypoxia–reoxygenation-induced apoptosis of rat cardiomyoblasts and Sfrp2 by binding directly binding to Wnt3a, mitigated the pro-apototic effects of Wnt3a *in vitro*,[19] suggesting that antagonism of pro-apototic Wnts *in vivo* could contribute to its cardioprotective effects.[19]

Administration of small peptides that bind to Frizzled receptors and antagonize effects of Wnt3a and Wnt5a resulted in decreased infarct expansion in mice after cardiac injury and prevented the development of post-infarction heart failure[20] (*Figure 3*). Intra-myocardial injection of pyrvinium, a small molecule antagonist of Wnts, ameliorated adverse left ventricular (LV) remodelling and was associated with better improvement of cardiac function after ischaemic cardiac injury[21] (*Figure 3*). Transgenic mice overexpressing Sfrp1 exhibited reduced infarct size and decreased rates of cardiac rupture after myocardial injury.[16] Administration of Sfrp4 after myocardial injury decreased the size of scar and ameliorated decline in post-injury heart function,[22] emphasizing the beneficial effects of antagonizing Wnt signalling in the injured region (*Figure 3*). However, cardiac myocyte-specific overexpression of Sfrp1 led to greater infarct size, worsening cardiac function, and appeared to reverse the beneficial effects of ischaemic

Table 1 Wnts and Wnt antagonists expressed in the normal heart and after ischaemic cardiac injury

Expression of Wnts and Wnt antagonists in the normal heart	Wnts and Wnt antagonists induced/up-regulated with ischaemic cardiac injury
Wnt2, Wnt 5a, Wnt 5b, Wnt7b, Wnt9a, Wnt11, Sfrp1, Sfrp2, Dkk-3	Wnt1, Wnt 2[a], Wnt4[a], Wnt7a[a], Wnt 10b, Wnt 11, Sfrp1[a], Sfrp2[a], Sfrp4, Dkk-1[a], Dkk-2
Cells/cardiac tissue responsive to canonical Wnt signalling in the normal heart	**Cells/cardiac tissue responsive to canonical Wnt signalling after ischaemic cardiac injury**
Valve mesenchymal cells, subsets of smooth muscle, and endothelial cells	Epicardium, epicardial-derived cells residing in sub-epicardial space, fibroblasts in the injury region, endothelial cells, and smooth muscle cells in injury region

Cells or tissues in the heart that are Wnt responsive were assessed by examining the TOPGAL Wnt reporter mice.
[a]Denotes increase in gene expression by at least a $\log_{10}$ fold after cardiac injury.[8,15,17]

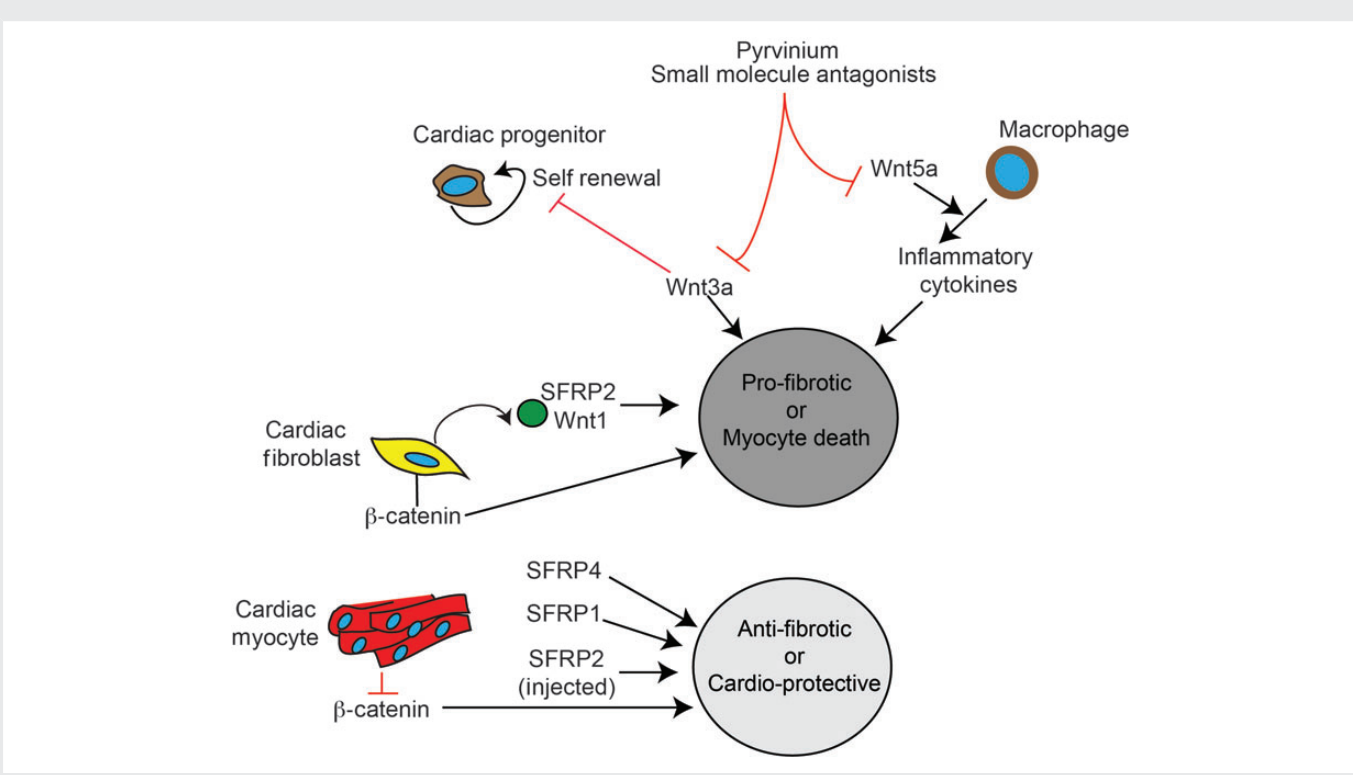

Figure 3 Wnt-dependent interactions between cardiac myocytes and cardiac fibroblasts after acute myocardial infarction. Wnt signalling regulating cardiac fibroblast and myocyte repair response. Sfrp2 and Wnt1 expressed by cardiac fibroblasts promote fibrosis. Wnt3a decreases cardiac progenitor self renewal, and Wnt5a activates expression of pro-inflammatory cytokines from macrophages. Antagonism of Wnt3a and Wnt5a by small molecule pyrvinium inhibits fibrosis. SFRPs when injected or overexpressed globally appear to be cardioprotective and anti-fibrotic. Similarly, myocyte-specific loss of β-catenin is cardioprotective. These observations support a pro-fibrotic role of Wnts in cardiac repair. Red bars indicate inhibition of pathway.

pre-conditioning on cardiac myocytes,[23] and these observations conflict with the ones made by global overexpression of Sfrp1.[16] Myocyte-specific overexpression of Sfrp1 could have preferentially affected Wnt signalling in the myocyte with less affects on other populations such as fibroblasts and may explain such discrepant observations. These findings reiterate the complexity and cellular context of Wnt signalling and emphasize the need for dissecting interactions between defined populations of cells in the cellular cardiac interactome.

Taken together, the published evidence strongly suggests that Wnt signalling system in the infarcted heart exerts effects on both myocytes and fibroblasts (*Figure 3*). Wnt/β-catenin signalling appears to be pro-fibrotic and antagonism of signalling with frizzled-related proteins or small molecules has salutary effects on fibrosis, LV remodelling, and cardiac function. It is interesting to note that Sfrp3 and Sfrp4 are elevated in hearts of patients with dilated cardiomyopathy and coronary artery disease.[24] Although the bulk of evidence suggests that Sfrps exert beneficial effects on cardiac repair following ischaemic cardiac injury, the precise mechanisms of such an effect and the specific Wnts antagonized remain unclear. Determining the cell populations expressing different Wnts in the injured heart, how Wnt/Frizzled signalling changes in different cell populations after cardiac injury and ascertaining downstream cellular mechanisms in mediating specific effects will need to be answered particularly for the development of therapeutic strategies based on manipulation of Wnt signalling in the ischaemic heart.

In contrast to myocardial infarction, cardiac hypertrophy is characterized by an increase in cardiomyocyte cell size, increased fibrosis, and expression of a foetal gene programme in affected myocytes. Less is known about the specific Wnts up-regulated after cardiac hypertrophy. Horst and colleagues[25] reported that they did not observe any up-regulation of gene expression of Wnts after cardiac hypertrophy. Wnt signalling is depressed in hearts of patients who have developed heart failure,[24] and whether changes in Wnt signalling are causally related or represent compensatory changes after the onset of heart failure remains to be delineated.

GSK3β is a key molecule regulating canonical Wnt transduction.[12] In the presence of Wnt ligand, GSK3β is inhibited and leads to accumulation of β-catenin (*Figure 2*). Overexpression of GSK3β leads to attenuation of cardiac hypertrophy[26] and inhibition of GSK3β leads to augmented hypertrophy in response to a hypertrophic stimuli.[27] β-Catenin levels are increased in cardiomyocytes subjected to hypertrophic stimuli.[28] β-Catenin loss-of-function mutations affecting the myocyte was associated with enhanced cardiac function after chronic angiotensin infusion, and conversely, gain-of-function mutations were associated with reduced cardiac pump function and cardiac dilatation resembling a phenotype of dilated cardiomyopathy.[29,30] Mice with myocyte-specific deletion of β-catenin subjected to aortic constriction had significantly decreased hypertrophy and enhanced cardiac function compared with mice with preserved β-catenin.[31] These observations thus support the view that canonical Wnt signalling at least in cardiac myocytes promotes cardiac hypertrophy and suggests that antagonism of canonical Wnt signalling may represent a pharmacological target for attenuating cardiac hypertrophy.

3.2 Wnt-mediated fibroblast –other non-myocyte cell interactions after cardiac injury

In the previous section, we described how Wnt signalling can affect myocytes and myocyte–fibroblast cross talk after cardiac ischaemia or hypertrophy. Fibroblasts are the most abundant cell type in the adult heart, and their ability to migrate as well as their location in the cardiac interstitium enables them to interact with other cell populations in the heart. In this section, we will mainly focus on how Wnts regulate interactions between fibroblasts and other non-myocyte cells in the heart. We have recently demonstrated that a Wnt1/β-catenin pro-fibrotic repair response is activated in cardiac fibroblasts after myocardial infarction.[8] Cardiac fibroblasts within the first few days of myocardial infarction expressed Wnt1. Cardiac fibroblasts were also Wnt responsive, and it is likely that Wnt1 acts via autocrine and paracrine mechanisms to induce cardiac fibroblast activation, proliferation, and expression of fibrogenic genes, such as collagen and endothelin. The Wnt1/β-catenin was critically required for this fibroblast-mediated repair response, as deletion of β-catenin specifically in cardiac fibroblasts led to decreased fibroblast proliferation and impaired wound healing. The granulation tissue in the infarcted region was loosely organized with little deposition of collagen and animals developed cardiac dilatation and heart failure.[8]

Fibroblasts also interact with endothelial cells to regulate angiogenesis (*Figure 4*). Fibroblasts are known to express both angiogenic [vascular endothelial growth factor (VEGF)] and anti-angiogenic molecules (e.g. connective tissue growth factor, CTGF).[32] Wnts are expressed by cardiac fibroblasts after ischaemic cardiac injury, and Wnt1 and Wnt3 can induce VEGF expression during development.[33,34] It is currently not clear whether specific Wnts expressed by fibroblasts regulate endothelial cell mobilization and proliferation in the infarct border zone. Fibroblasts after cardiac injury express tissue metalloproteinases (MMPs) that break down extracellular matrix and help in endothelial cell migration and sprouting, and Wnts are known to induce several types of MMPs.[35] Tissue inhibitor of metalloproteinases (TIMPs) are also expressed by cardiac fibroblasts and are known to regulate downstream effects via Wnt/β-catenin signalling.[36] TIMPs can exert both pro- and anti-angiogenic effects[37], and one group of investigators observed that direct fibroblast–endothelial contact appeared to be required for fibroblast-mediated angiogenesis via expression of TIMP (*Figure 4*). Macrophages also limit aberrant or excessive angiogenesis by expressing thre potent anti-angiogenic molecule Flt-1 [FMS-related tyrosine kinase 1 (VEGF receptor 1)] via a non-canonical Wnt-dependent pathway (*Figure 4*). These observations demonstrate that both indirect and direct fibroblast-mediated cell–cell interactions in the heart regulate angiogenesis.

3.3 Wnt-mediated endothelial interactions after cardiac injury

Endothelial cells express most types of Frizzled receptors (Fzd 1,2,4,5,6,7,9, and 10), and Wnt1, Wnt3a, and Wnt5a have been shown to regulate endothelial cell proliferation and migration, both being critical processes for neovascularization.[38–41] β-Catenin has been observed to accumulate in endothelial cells of the rat heart after myocardial infarction suggesting potential activation of the canonical Wnt signalling pathway in endothelial cells after ischaemic cardiac injury.[42] Sfrps exert both pro- and anti-angiogenic effects. Sfrp1 enhanced migration and tube formation of endothelial cells *in vitro*, and the pro-angiogenic effect of Sfrp1 was independent of VEGF, FGF, or angiopoietin-1 induction.[43] However, Sfrp1 has also been shown to decrease endothelial cell proliferation,[44] and it is currently not clear how Sfrp1 influences angiogenesis in the infarcted heart. Wnts and Sfrps expressed by other cells including myocytes and fibroblasts likely influence the endothelial

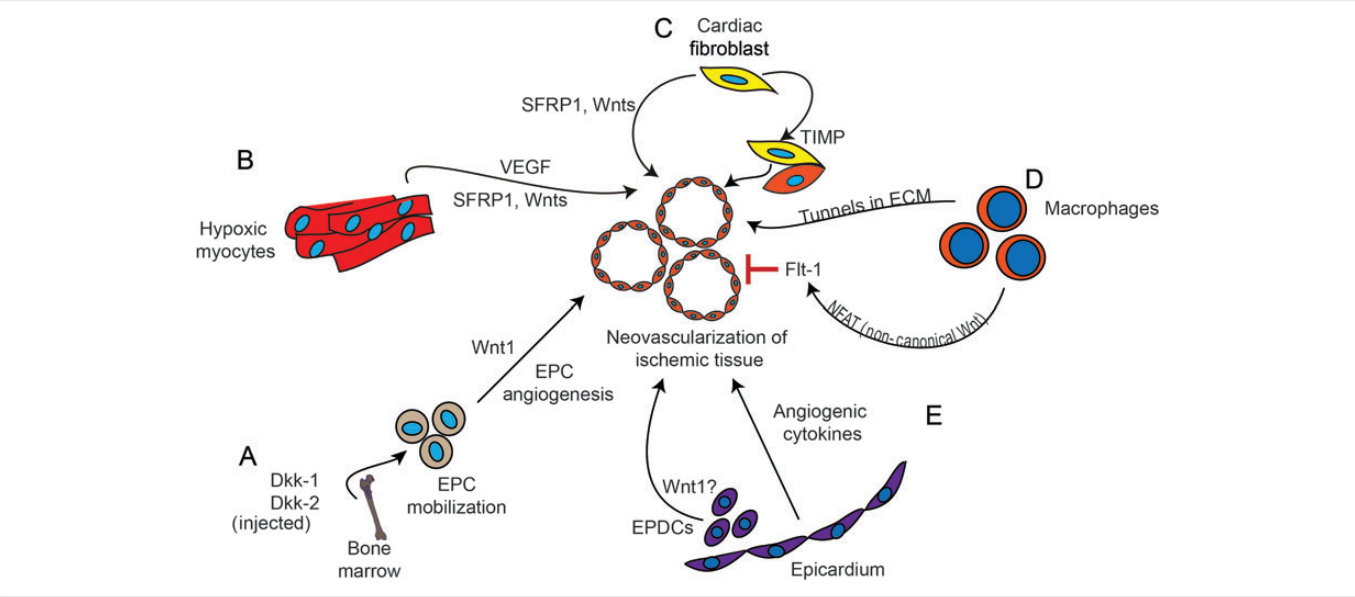

Figure 4 A model of Wnt-dependent endothelial interactions regulating neovascularization in the infarcted heart. (*A*) Dkk-1 and -2 promote mobilization of EPCs from the bone marrow, while Wnt1 promotes angiogenic ability of EPCs. (*B*) Hypoxic myocytes release VEGF and potentially Wnts and SFRPs that promote angiogenesis. (*C*) Cardiac fibroblast secretes angiogenic factors, Wnts and SFRPs, that can affect angiogenesis; in addition, they may express TIMP that promotes angiogenesis, but requires direct fibroblast–endothelial cell contact. (*D*) Macrophages and myeloid cells recruited to the wound area may create tunnels in the ECM that potentiate endothelial cell migration and angiogenesis. Macrophages may also inhibit excessive angiogenesis by expressing Flt via a NFAT/non-canonical Wnt pathway. (*E*) The epicardium releases angiogenic cytokines that promote neovascularization and EPDCs may adopt an endothelial cell fate. EPC, endothelial progenitor cell; TIMP, tissue inhibitor of metalloproteinase; EPDCs, epicardial-derived cells; figures not drawn to scale.

response depending on the type and local concentration of Wnts, the presence of other antagonists, and the relative affinity of Wnts to the repertoire of Fzd receptors expressed by endothelial cells (*Figure 4*).

Endothelial progenitor cells (EPCs) are recruited to the heart following ischaemic cardiac injury, and endothelial progenitors are being administered to patients following myocardial infarction to enhance neovascularization and cardiac function.[45] However, patients with coronary artery disease have reduced numbers and dysfunctional EPCs. Our group has observed that Wnt1 enhances the function of EPCs and injection of Wnt1 overexpressing human EPCs led to enhanced blood flow in a murine model of hind limb ischaemia compared with injection of green fluorescent protein overexpressing human EPCs.[46] This suggests a potential role of Wnt signalling in correcting functional deficits of EPCs in patients with vascular disease (*Figure 4*). Wnts have also been shown to regulate potency and differentiation of EPCs. Wnt5a induced transdifferentiation of EPCs with induction of a cardiac gene expression programme in EPCs.[47] The Wnt antagonist (Dkk-2 promotes pro-angiogenic abilities of EPCs[48] and enhances angiogenesis of rodent and human endothelial cells[49] (*Figure 4*). Antagonism of canonical Wnt signalling with Dkk-1 also augmented mobilization of vascular progenitors from the bone marrow (*Figure 4*).[34,50] The effects of Dkk-1 in mobilizing vasculogenic progenitors appeared to be secondary to its effects in inhibiting canonical Wnt signalling in endosteal cells in the bone marrow niche. These observations suggest that various members of the Wnt family may exert pro- and anti-angiogenic effects on endothelial cells and progenitors to regulate post-injury angiogenesis.

3.4 Wnt-dependent interactions with the epicardium

The epicardium is a single layer of modified epithelial cells that surround the heart and develop from the pro-epicardium.[51] The epicardium plays a critical role in heart development and gives rise to the majority of cardiac fibroblasts as well as smooth muscle cells of the coronary arteries.[52] Less is known about the role of the epicardium in the adult heart and whether it contributes to cardiac homoeostasis. Following ischaemic injury, the epicardium expands and we and others have shown that the epicardium can robustly undergo EMT after ischaemic myocardial injury and generate cardiac fibroblasts and myofibroblasts (*Figure 5*).[8,53] The epicardium expressed Wnt1 after myocardial ischaemia and Wnt1 drove epicardial cells to adopt a pro-fibrotic fate *in vivo* (*Figure 5*).[8] Deletion of β-catenin in epicardial cells resulted in an inability of the epicardium to expand after ischaemic cardiac injury. Epicardial EMT was decreased and associated with impaired cardiac repair. The epicardium also expresses angiogenic factors after injury that promote neovascularization, and a subset of epicardial cells with more progenitor properties are capable of adopting an endothelial cell fate (*Figure 4*).[53,54] Wnt1 is pro-angiogenic and increases endothelial cell proliferation and tube formation *in vitro*,[46] and Wnt1 may thus affect neovascularization mediated by the epicardium (*Figure 4*). Taken together, these observations suggest that the Wnt/β-catenin pathway may be important for regulating the fate of epicardial-derived cells (EPDCs) after ischaemic injury. It is interesting to note that a recent clinical report has described decreased Wnt1 levels and increased Dkk-1 levels in patients with pre-mature myocardial infarction, suggesting that a certain degree of basal Wnt signalling may be required for cardiac or vascular homoeostasis.[55]

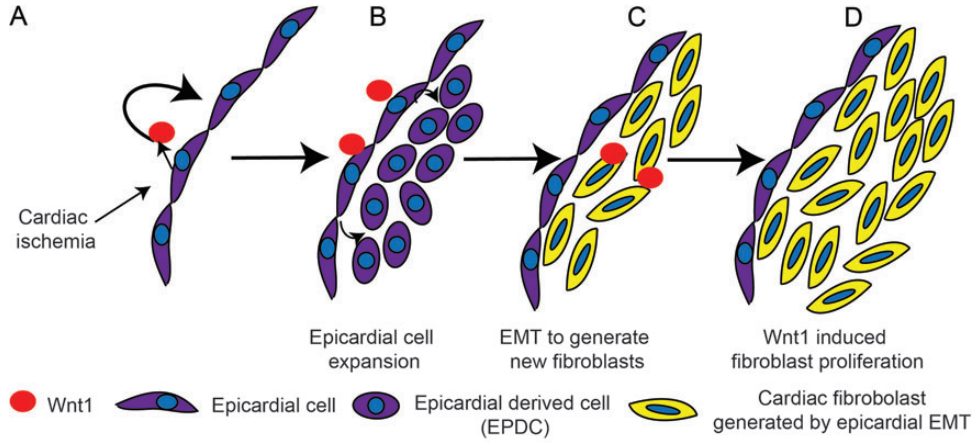

Figure 5 A model of Wnt-dependent epicardial interactions. (*A*) The epicardium expresses Wnt1 following ischaemic injury. (*B*) Wnt1 drives epicardial cells to expand and form EPDCs. (*C*) Subsequently, EPDCs undergo EMT to generate fibroblasts in the sub-epicardial region. (*D*) Wnt1 also promotes proliferation of cardiac fibroblasts. This model demonstrates that how Wnt1 can exert effects on two different cell populations in a temporal manner to mediate a pro-fibrotic repair response.

3.5 Wnt-dependent interactions with inflammatory cells

There is an increasing body of evidence to suggest that Wnts may modulate inflammatory responses of the body. Stimulation of Toll-like receptors in macrophages induces Wnt5a expression, which in turn up-regulates expression of pro-inflammatory genes such as interleukin (IL)-6, IL-1β, and IL-8.[56] Acute cardiac injury is associated with increased expression of IL-6 and IL-1β and as alluded earlier, blockade of Wnt5a with small molecules confers cardioprotective benefits after acute ischaemic cardiac injury (*Figure 3*). Wnt5a was observed to be present in greater amounts in blood of patients with sepsis, and an intact Wnt5a/Fzd/calmodulin kinase pathway was required for macrophage activation.[57] SFRP1 and SFRP5 can inhibit Wnt5-mediated macrophage activation.[57,58] These observations suggest that Wnts and the SFRP family could contribute to the regulation of the inflammatory response after acute ischaemic cardiac injury.

3.6 Wnt-mediated regulation of cardiac progenitor interactions

During cardiac development, Wnts are thought to inhibit cardiogenesis and antagonism of Wnt3A and Wnt8 by Dkk-1 can initiate cardiogenesis.[59] Wnt3, 3a, and 8a can induce cardiomyogenic differentiation of embryonic stem cells in a concentration dependent bi-phasic manner,[60,61] and canonical Wnt/β-catenin signalling promotes expansion of cardiac progenitor cells.[62] Less is known about the role of Wnts in regulating function of cardiac progenitors in the adult heart after injury or hypertrophy. Bergmann's group noted that down-regulation of β-catenin specifically in cardiac myocytes improved cardiac repair after myocardial infarction (*Figure 3*) and was in part secondary to enhanced cardiomyogenic differentiation of GATA4/stem cell-related antigen 1 (Sca-1) expressing resident cardiac precursor cells.[30] Injection of recombinant Wnt3a suppressed proliferation of cardiac side population cells (represents a population of cardiac progenitors) and when injected into animals after myocardial injury limited renewal of side population progenitors, blocked endogenous cardiac regeneration, and led to decreased cardiac performance.[63]

In this review, we have focused on Wnt-mediated interactions, but after injury, may other signalling pathways such as the Notch, transforming growth factor β (TGFβ), and BMP pathways are activated in the heart. The Wnt signalling system potentially interacts with other such pathways to modulate a physiological response. For instance, the Notch signalling system is known to exert a negative feedback effect on the Wnt/β-catenin pathway and the intra-cellular domain of Notch receptor can bind to downstream Wnt signalling components such as Dishevelled, inhibiting Wnt signalling.[64] signalling are activated in the epicardium and appear to drive the formation of fibroblasts from epicardial-derived cells and the two signalling systems potentially could regulate the degree of EMT and epicardial contribution to fibrosis.[8,9] The Wnt antagonist Sfrp2 when injected in high concentration affects fibrosis by inhibiting the proteolytic activity of BMP-1.[18] These observations highlight the complexity of many signalling systems crosstalking with one another in the cellular cardiac interactome to affect a physiological response.

4. Discussion

4.1 Cardiac cell–cell interactions—an approach to study them in an integrated manner

The above sections have provided a brief overview of how a signalling system can mediate interactions between different cell populations in the heart following injury or hypertrophy. Undoubtedly, many different signalling systems play a role in mediating cross talk between different cell populations to affect the cardiac repair response. Identification of cellular interactions that have the most effect on a cardiac repair response and ejection function may help in prioritizing and designing pharmacological strategies affecting this interaction. Moreover, the process is complex with many interacting components that can affect various aspects of the repair response (e.g. fibrosis and angiogenesis). Therefore, the question becomes: what would be the most rational

way to study these interactions to understand better the regulation of the cardiac repair response? We propose that a system biology approach could be adopted for a rational approach to cell–cell interactions (*Figure 6*).

Over the last decade, enormous strides have been made in integrating genomic information with the transcriptome and proteome to determine how components (genes, transcripts, or proteins) interact with each other to affect a cellular response. It can be argued that a similar system biology-based approach can be used to study interactions between different cell populations to predict an organ-wide physiological response. Such computational approaches form the broad principles of the Physiome Project (a worldwide collaboration built to model the integration of structure and function of an organism from the cell to the tissue to the organ and whole organism). Interactions between different components of an organ system can be modelled to predict to predict a physiological response.[65]

The heart can be thought of as a system with 'maintenance of ejection fraction' as the principal property of this system.[66] It can be argued that such an essential function of this system is an inherent property of the system and is not governed by the properties of only one component of the system (e.g. muscle), but is dependent on proper functioning of different components (e.g. muscle, fibroblasts, and blood vessels). Each such cellular component can be thought of as a module; modules can function independent of each other, but changes in modules can change the environment (which links all the modules) and affect the principal property of the system[65] (*Figure 6*). For instance, cardiac hypertrophy results in increases in mass of the muscle module, but the 'blood vessel' module also exhibits an increase in the number of blood vessels. Failure to increase the number of blood vessels after onset of cardiac hypertrophy results in a rapid drop of ejection function and development of heart failure. Thus, the changes in two modules can be modelled (increase in cardiac muscle and increase in blood vessels) to determine how they regulate cardiac function following a hypertrophic insult. Subsequently, a signalling pathway that is known to increase or decrease the activity of one module (e.g. Wnts affecting hypertrophy) can be included as a parameter that can be altered in this model. Thus, cellular modules can be thus thought of as computational units and mathematical models using multiscalar approaches can be utilized for predicting organ-wide physiological effects for changes affecting one or more cellular modules (*Figure 6*).[66] A critical assumption in such

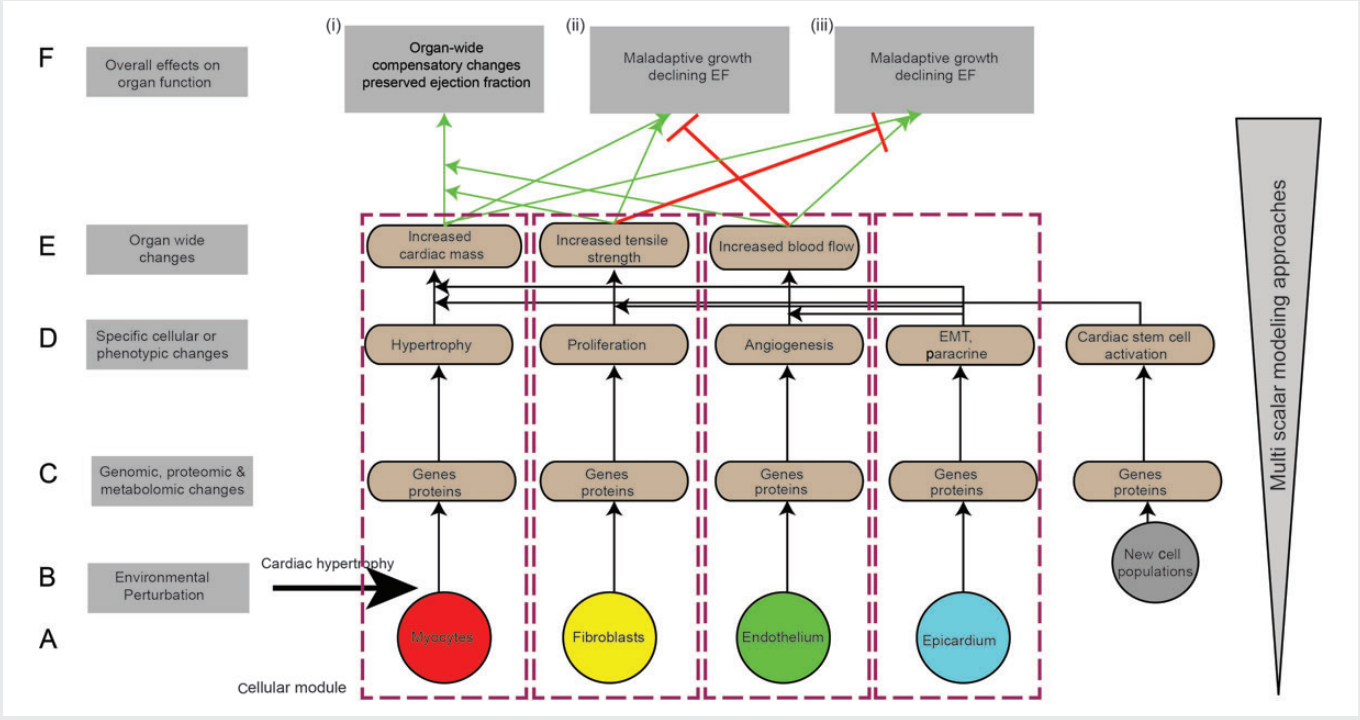

Figure 6 A system biology approach for the study of cell–cell interactions. (*A*) Cell populations in the uninjured heart, each cell population can be thought of as a module (dashed boxes). (*B*) Following an environmental perturbation, each cell population exhibits (*C*) changes in genomic, proteomic, and metabolomic profiles, and new populations are also recruited such as inflammatory cells and cardiac progenitor cells (illustrated here as an example). (*D*) Changes in each cell population result in specific functional changes such as myocyte hypertrophy or fibroblast proliferation (thus each module is associated with specific functional changes with a defined environmental perturbation). (*E*) Functional changes in each cell population (module) result in organ-wide changes (such as increased cardiac mass and increased blood flow to enhance angiogenesis). The functional effects occurring in each module are critical for supporting organ function (e.g. increased blood flow supporting increased muscle mass). (*F*) (i) Appropriate and proportionate changes (green arrows) lead to organ-wide compensation and preservation of ejection fraction (EF), but (ii and iii) modular changes not commensurate with the demand (red bars) result in maladaptation and declining EF. (ii) Appropriate hypertrophy and appropriate fibrotic response but inappropriate decrease in blood flow cannot sustain organ compensation (thus, changes exclusively occurring in the endothelial cell module can be modelled against appropriate changes in the muscle and fibroblast module for predicting how a vascular response affects cardiac function after hypertrophy). (iii) Similarly, inappropriate changes in the fibroblast module cannot maintain cardiac function even with appropriate degrees of compensation from the muscle and endothelium modules. Green arrows indicate appropriate degree of compensation, while red bars indicate an inappropriate degree of compensation.

organ-wide mathematical modelling is the isolation of modules as functioning independent of each other and this is rarely the case especially in the diseased heart, but a system-wide approach may be useful for determining the physiological relevance of specific cell–cell interactions in the heart[1,66] (*Figure 6*).

Such the system-wide approach to study cellular interactions in the heart following ischaemia or hypertrophy could be potentially used for identifying targets that play critical roles in mediating interactions between defined cell populations at specific times to affect a physiological outcome, while not interacting between other cell components, thus having minimal effect on other cardiac physiological variables. However, a system-wide approach cannot identify new cellular interactions or novel genes affecting a specific cellular interaction. Future biological studies will be required to obtain new knowledge in identifying the cardiac cell populations exhibiting changes in specific Wnts or related members and the physiological effect on other possible interacting cell populations in a temporally defined manner. It is only through such a rigorous and detailed approach combining basic biological investigation and a system biology analysis that we can hope to unravel the complexity and significance of cell–cell interactions in the diseased heart.

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