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The role of TGF β 1 and LRG1 in cardiac remodelling and heart failure

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Abstract Heart failure is a life-threatening condition that carries a considerable emotional and socio-economic burden. As a result of the global increase in the ageing population, sedentary life-style, increased prevalence of risk factors, and improved survival from cardiovascular events, the incidence of heart failure will continue to rise. Despite the advances in current cardiovascular therapies, many patients are not suitable for or may not benefit from conventional treatments. Thus, more effective therapies are required. Transforming growth factor (TGF) β family of cytokines is involved in heart development and dys-regulated TGF β signalling is commonly associated with fibrosis, aberrant angiogenesis and accelerated progression into heart failure. Therefore, a potential therapeutic pathway is to modulate TGF β signalling; however, broad blockage of TGF β signalling may cause unwanted side effects due to its pivotal role in tissue homeostasis. We found that leucine-rich α -2 glycoprotein 1 (LRG1) promotes blood vessel formation via regulating the context-dependent endothelial TGF β signalling. This review will focus on the interaction between LRG1 and TGF β signalling,

their involvement in the pathogenesis of heart failure, and the potential for LRG1 to function as a novel therapeutic target.

Keywords LRG1 · TGF β · Cardiac remodelling · Therapeutic angiogenesis · Fibrosis · Heart failure

Introduction

Heart failure is a progressive and chronic condition in which the heart is no longer able to circulate blood efficiently to meet the body's demands (Johnson 2014). A wide range of conditions such as ischemic heart disease (IHD), hypertension, valvular heart disease, myocarditis, diabetes and cardiomyopathy can lead to heart failure (Nishimura et al. 2014). In response to stress or injury, the myocardium undergoes a series of pathological changes including structural rearrangement and morphological changes of cardiomyocytes, inflammation, extracellular matrix (ECM) remodelling, microvascular rarefaction and chamber dilation (Manabe et al. 2002; Kehat and Molkentin 2010). These changes cause further deterioration in cardiac function and eventually lead to heart failure (Cohn et al. 2000).

Over the last few decades, the prevalence and incidence of heart failure continues to rise mainly due to the prolonged longevity, improved survival rate from other cardiovascular events (e.g., myocardial infarction, valvular disease, and arrhythmias), sedentary life style, and the increased prevalence of risk factors (e.g., hypertension, diabetes and obesity) (Mann DL 2012). In 2010, more than 41 million people lived with heart failure worldwide (Forouzanfar et al. 2013). Despite improved understanding of the molecular mechanisms and significant advances in treatment strategies, heart failure still carries substantial morbidity and mortality and its therapy remains a major unmet medical need. In this review, we summarise the current knowledge on the role of TGF β 1 and

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its novel modulator, LRG1, in different pathologies of cardiac remodelling and the potential of LRG1-targeted therapeutics for the treatment of heart failure.

The challenges of current treatments for heart failure

Heart failure is a heterogeneous disease with a broad spectrum of symptoms. Current treatments aim to alleviate symptoms, slow disease progression and thereby improve overall quality of life and survival. For example, IHD-induced heart failure, the most common type of the disease, is normally treated with antiplatelet drugs, anticoagulants and β -blockers. Emergent reperfusion via surgical or catheter-based revascularisation procedures is used to restore blood flow and improve survival following ischemic episodes (Heuser et al. 2000; Horvath 2000). However, a substantial portion of patients are not suitable for or do not benefit from conventional revascularisation treatments because of a poor overall health status or the presence of comorbidities (Norgren et al. 2007). Even in patients who received successful primary revascularisation, stent thrombosis and saphenous vein bypass graft disease can occur and cause recurrent myocardial ischemia and cardiac remodelling (Kaul et al. 1991). Furthermore, none of these treatments alters the natural history of heart failure and therefore offers no cure.

Both human and animal studies have shown that individuals with robust collateral circulation and microvascular perfusion are associated with delayed myocardial cell death (Antoniucci et al. 2002), reduced occurrence of myocardial infarction (MI) (Choi et al. 2013), smaller infarction size (Habib et al. 1991) and increased survival (Meier et al. 2012). However, the capacity of collateral and capillary vessel remodelling under ischemic condition is highly variable among individuals. Accelerating this innate physiological response by exogenous angiogenic factors has been considered as an attractive approach to bypass occluded vessels, revascularise ischemic tissues and restore tissue function (Carmeliet and Jain 2011). An impressive body of pre-clinical evidence has demonstrated improved myocardial perfusion and function upon therapeutic angiogenesis in animal models (Harada et al. 1994; Unger et al. 1994; Landau et al. 1995; Lazarous et al. 1996; Shou et al. 1997; Lopez et al. 1998; Lee et al. 2000; Zhang et al. 2002; Cao et al. 2005; Heintz-Green et al. 2005; Cao 2009). Initial phase I clinical trials in patients with advanced IHD, but who did not meet the criteria for standard revascularisation strategies, have also demonstrated an improved cardiac circulation and function after being treated with pro-angiogenic factors such as vascular endothelial growth factors (VEGF) and basic fibroblast growth factor (bFGF) (Losordo et al. 1998, 2002; Schumacher et al. 1998; Rosengart et al. 1999; Symes et al. 1999; Hendel et al. 2000; Udelson et al. 2000; Henry et al. 2001; Vale et al.

2001; Fortuin et al. 2003; Reilly et al. 2005). However, caution is needed in the interpretation of outcomes of these studies as most of them lack proper placebo controls. Not surprisingly, similar therapeutic efficacy has not yet been achieved in larger, placebo-controlled, late-stage clinical trials, which is partly due to the extent of angiogenesis observed in the placebo group (Grines et al. 2002, 2003; Simons et al. 2002; Kastrup et al. 2011).

Angiogenesis is a tightly controlled process involving multiple levels of interactions between a wide variety of molecules, cells and extracellular matrix (ECM) proteins. It is now widely accepted that a single angiogenic factor may not be sufficient to induce the formation of functional vasculatures. Indeed, the treatment of VEGF leads to the formation of leaky, chaotic and tortuous vessels that lack the normal hierarchical structure (Nagy et al. 2007; Hedlund et al. 2009; Cao et al. 2010). Furthermore, both VEGF (Thurston 2002) and bFGF (Cuevas et al. 1991) are involved in vessel dilation and their treatment is associated with severe hypotension (Hariawala et al. 1996; Horowitz et al. 1997; Unger et al. 2000; Henry et al. 2001). In addition, there is evidence that VEGF exerts detrimental pro-atherogenic effects by influencing endothelial and immune cell function (Ross 1993; Inoue et al. 1998; Kim et al. 2001). A combination treatment targeting growth factors with complementary mechanisms might be more effective and has less unwanted side effects.

ECM is essential for proper cardiac function. It provides a scaffold for different types of cells in myocardium and transmits mechanical force and signals to myocardial fibres (Banerjee et al. 2006). ECM remodelling is a critical step that allows the ordered replacement of damaged cells after injury. However, chronic inflammation and repetitive injury can cause disturbed ECM homeostasis and fibrosis, a feature shared by many conditions associated with heart failure (Weber et al. 1995). Cardiac fibrosis exaggerates mechanical stiffness of the myocardium and its vasculature, impairs myocyte contractility, disrupts electrical coupling, destroys normal tissue architecture and eventually leads to heart failure (Lopez et al. 2001; Ho et al. 2010; Karagueuzian 2011). Increasing evidence shows that fibrosis is a dynamic and reversible process (Iredale 2007). Targeting fibrosis, therefore, presents a promising strategy to prevent or slow down the deterioration of cardiac function. Despite its huge impact on cardiovascular diseases and intensive research efforts to explore new therapies, there is no approved treatment that directly targets the mechanisms of fibrosis in the heart.

TGF β 1 and heart failure

The TGF β family of cytokines plays important roles in embryogenesis, tissue homeostasis and regeneration (Massague 2012). Their secretion, activation and function are tightly

controlled by multiple mechanisms to ensure precise signal propagation (Fig. 1). TGFβs are secreted in latent form as part of a large protein complex (Khalil 1999) and their activation requires functional and physical cooperation of mannose-6-phosphate (M6P)/insulin-like growth factor II receptor (IGFIIR), urokinase-type plasminogen activator receptor (UPAR), Neuropilin 1 (NRP1) and different proteases and metalloproteases (MMPs) (Dennis and Rifkin 1991; Scott and Firth 2004; Glinka et al. 2011; Shi et al. 2011). Once released, TGFβs bind to type II receptor TGFβRII, which recruits type I receptor, activin receptor-like kinase (ALK) (Shi and Massague 2003), and activates a multitude of intracellular signalling including canonical Smad and non-canonical ERK, JNK, TAK1, P38 and Rho cascades (Derynck

and Zhang 2003). TGFβs also interact extensively with other signalling pathways leading to very different even opposite outcomes (Massague 2012).

In mammals, there are three different isoforms: TGFβ1, TGFβ2, and TGFβ3. Each of them shows distinct expression pattern and functions. TGFβ1, the focus of this review, is the predominant and most ubiquitously expressed isoform (Millan et al. 1991). In the heart, TGFβ1 regulates the signalling and function of different types of cells, including endothelial cells (ECs), vascular mural cells (pericytes in capillaries and vascular smooth muscle cells (VSMCs) in larger vessels), myofibroblasts, macrophages and cardiomyocytes (Bujak and Frangogiannis 2007; Koitabashi et al. 2011). Aberrant TGFβ1 signalling contributes to the development of a multitude of

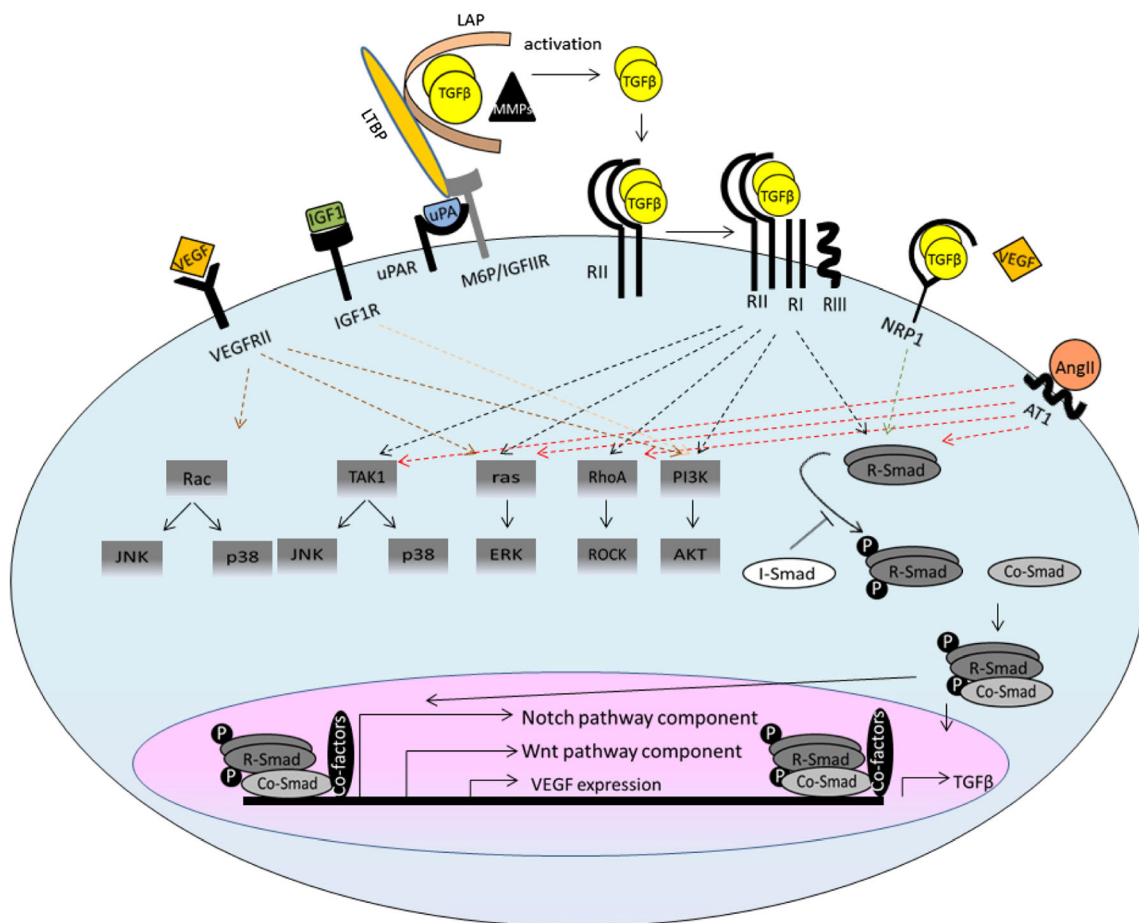


Fig. 1 Schematic representation of TGFβ signalling and crosstalk with other signalling pathways. TGFβ ligands are synthesised as a large latent complex consisting of TGFβ dimer covalently associated with a latency-associated peptide (LAP) and a latent TGFβ-binding protein (LTBP). The activation of latent TGFβ requires functional and physical cooperation of M6P/IGFIIR, UPAR, NRP1 and other proteases and MMPs. The released TGFβ dimers bind the type II TGFβ receptor (RII) first, which recruits and transphosphorylates the type I receptors (RI). RI propagates the signal into the cell by phosphorylating TGFβ receptor-regulated SMADs (R-Smads). They form heteromeric complexes with the common SMAD (co-Smad) and translocate to the nucleus. The R-Smads-co-Smad complex formation can be inhibited by

inhibitory Smad (I-Smad). Once in the nucleus, the R-SMAD-co-SMAD complex associates with other DNA-binding transcription factors to modulate the expression of target genes. In the non-canonical pathways, the activated transforming growth factor-β (TGFβ) receptor complex transmits a signal through other factors, such as TGFβ-activated kinase 1 (TAK1), p38 mitogen-activated protein kinase (p38 MAPK), RHO, phosphoinositide 3-kinase (PI3K)–AKT, extracellular signal-regulated kinase (ERK), Rho-associated protein kinase (ROCK), or JUN N-terminal kinase (JNK). TGFβ signalling interacts extensively with other pathways, such as the WNT, Notch, AngII, IGF and VEGF pathways, which defines the context-dependent TGFβ signalling

conditions associated with heart failure such as dilated and hypertrophic cardiomyopathies, post-infarction myocardial remodelling, valvular diseases and arrhythmia in both mice and humans (Cambien et al. 1996; Schultz Jel et al. 2002; Euler-Taimor and Heger 2006; Khan and Sheppard 2006; Kapur et al. 2013).

TGF β 1 and cardiac fibrosis

TGF β 1 is a potent fibrogenic factor that mediates ECM homeostasis through different mechanisms, for example, by inducing ECM (such as collagens and fibronectin) synthesis via both canonical and non-canonical signalling cascades (Chen et al. 2000; Qiao et al. 2005; Leask 2007), decreasing the production of proteinase regulating ECM degradation (such as MMPs), promoting the production of inhibitors of these proteases (such as TIMPs) (Biernacka et al. 2011) and promoting integrin expression to increase the adhesion of cells to matrix (Thannickal et al. 2003). Studies have shown that TGF β 1 signalling pathway components, including TGF β 1, ENG and Smads, are markedly up-regulated at the site of injury after MI (Hao et al. 1999; Krum et al. 2002; Dean et al. 2005; Kapur et al. 2012), in patients suffering from hypertrophic cardiomyopathy (Villarreal and Dillmann 1992, Li et al. 1998) and dilated cardiomyopathy (Pauschinger et al. 1999; Sanderson et al. 2001), and all these conditions are characterised by excessive fibrosis in the heart. Consistently, TGF β 1 overexpression in transgenic mice leads to myocardial fibrosis (Rosenkranz et al. 2002; Seeland et al. 2002). Studies have shown that TGF β 1-mediated endothelial-to-mesenchymal transition (EndoMT) also contributes to myocardial fibrosis (Zeisberg et al. 2007; van Meeteren and ten Dijke 2012). Recently, ENG, a previously considered EC specific TGF β 1 receptor, has been found to be expressed in cardiac fibroblasts and to mediate the pro-fibrotic effect of angiotensin II (AngII) via angiotensin II receptor type 1 (AT1) (Chen et al. 2004). Reduced ENG activity led to attenuated cardiac fibrosis and increased survival in an in vivo model of heart failure (Kapur et al. 2012). This study demonstrated that the expression of ENG is significantly up-regulated in human failing left ventricles. Inhibition of the activity of ENG-attenuated TGF β 1 induced Smad2/3 phosphorylation, ECM deposition and cardiac fibrosis. *Eng*^{+/-} mice with pressure overload-induced heart failure showed an increased capillary density in the heart, preserved cardiac function and improved survival. Thus, targeting the TGF β 1 signalling pathway might provide an attractive strategy to limit structural deterioration of myocardium and ultimately lead to improved cardiac function, and survival of heart failure patients.

TGF β 1 and cardiomyocyte hypertrophy

In addition to fibrosis, hypertrophic growth of cardiomyocytes occurs in response to haemodynamic overload and represents the heart's effort to maintain cardiac output sufficient to meet the body's demands (Glennon et al. 1995). There is compelling evidence that TGF β 1 plays a critical role in this process. Increased TGF β 1 expression is observed in the myocardium of human patients with idiopathic hypertrophic cardiomyopathy (Li et al. 1998). Consistently, TGF β 1 overexpression in transgenic mice results in hypertrophic growth of cardiomyocytes (Rosenkranz et al. 2002). Interestingly, this TGF β 1-induced cardiac hypertrophy is associated with increased myocardial β -adrenergic receptors (ARs) density (Rosenkranz et al. 2002) and β -AR blockade treatment in TGF β 1 transgenic mice prevents cardiac hypertrophy. On the other hand, angiotensin II was shown to induce TGF β 1 expression in myocardium and TGF β 1 (Wenzel et al. 2001) is required for AngII-mediated cardiomyocyte hypertrophy (Gray et al. 1998). However, the AT1 receptor blockade is insufficient to prevent the hypertrophic response in TGF β 1 transgenic mice (Rosenkranz et al. 2003), and TGF β 1 knockout mice are resistant to AngII-induced cardiac hypertrophy (Schultz Jel et al. 2002). Together, these data show that TGF β 1 plays a key role in AngII-mediated growth responses of cardiomyocytes via β -AR.

TGF β 1 and post-infarction inflammatory response

Cardiomyocyte death and hypoxia following infarction initiate inflammatory response, which leads to the infiltration of immune cells into the infarcted area, an important process for clearing debris from the wound and tissue repair (Mehta and Li 1999). In the meantime, a timely repression of inflammatory mediator synthesis is important for scar maturation. TGF β 1 plays a highly important and complex role in inflammatory response following cardiac injury (Celada and Maki 1992). It acts as a direct chemoattractant to monocytes (Wahl et al. 1987) and neutrophils (Fava et al. 1991) to recruit them to the infarct site. However, its effects on macrophages are primarily inhibitory (Frangogiannis et al. 2001). There is a need for a better understanding of the TGF β 1-modulated post-infarction inflammatory response for specific intervention that could attenuate inflammatory injury without interfering with myocardial healing.

TGF β 1 and cardiac neovascularisation

Genetic studies in mouse and human revealed that proper TGF β 1 signalling is essential for blood vessel formation (Chang et al. 2001; Harradine and Akhurst 2006). The vascular response to TGF β 1 is highly context-dependent and is shaped by factors such as ligand bioavailability and

concentration, receptor availability and internalization, cross-talk with other signalling pathways, micro/marcovessel origin of vascular cells, and cellular density (Massague 2012). Although not fully understood, it is generally considered that TGF β 1 signalling in ECs occurs through TGF β RII recruiting either the ubiquitously expressed ALK5 or the EC-specific ALK1 (possibly with ALK5). Signalling via ALK5 leads to the activation of downstream transcription factors Smad 2 and 3, and thus plays an essential role in maintaining the vasculature at quiescent state. The TGF β 1/ALK1 signalling activates Smad 1, 5 and 8, resulting in increased EC migration, proliferation and angiogenesis (Goumans et al. 2003). The two pathways interact with each other at both receptor and the Smad level (Goumans et al. 2003), with ENG as a key player in switching TGF β signalling toward the pro-angiogenic pathway (Lebrin et al. 2004). This paper showed that ENG regulates the balance of TGF β signalling in endothelial cells by promoting TGF β /ALK1 and inhibiting TGF β /ALK5 signalling, and the subsequent endothelial proliferation.

Besides ECs, blood vessels comprise another essential component. The vascular mural cells support endothelium and are involved in blood vessel maturation and homeostasis. Similar to that in ECs, TGF β 1 mediates the proliferation of VSMC in a dose-dependent manner with high-dose being inhibitory and low-dose being stimulatory (Seay et al. 2005; Tsai et al. 2009). TGF β 1 induces the contractile phenotype of VSMCs by promoting the expression of α -smooth muscle actin and smooth muscle myosin (Hautmann et al. 1997; Seay et al. 2005). Perturbed TGF β 1 signalling results in failure of VSMC recruitment (Pardali et al. 2010) and the formation of aneurysm (Choudhary et al. 2009). TGF β 1 also stimulates the expression of plasminogen activator inhibitor (PAI)-1, a potent inhibitor of matrix MMPs, and therefore promotes blood vessel maturation by preventing the degradation of provisional matrix surrounding the nascent vessel (Jain 2003). On the other hand, the juxtaposition and collaboration between mural cells and ECs are important for local activation of latent TGF β 1, which further defines its context-dependent signalling in vascular cells (Antonelli-Orlidge et al. 1989; Sato et al. 1990).

Together, extensive investigations have shown that TGF β 1 modulates the signalling and function of different vascular cells and participates in multiple stages of blood vessel development, which makes it an attractive target for therapeutic angiogenesis. Indeed, it has been reported that exogenous application of TGF β 1 stimulates blood vessel formation in peripheral circulation (van Royen et al. 2002). This study showed that exogenous TGF β 1 promotes peripheral collateral artery formation and collateral circulation in rabbit hind limb model of femoral artery occlusion,

partly by increasing monocyte adhesion and transmigration and enhancing the expression of growth factors and cytokines. Further studies are required to evaluate the impact of TGF β treatment on functional blood vessel formation in the heart.

TGF β 1 signalling as a therapeutic target for heart failure?

TGF β 1 coordinates a broad spectrum of cellular processes that contributes to cardiac remodelling after MI and subsequent progression to heart failure (Bujak and Frangogiannis 2007). It can be beneficial or deleterious depending on the stage of disease development. For example, TGF β 1 plays a pivotal role in wound repair after infarction by suppressing inflammation, promoting the myofibroblast transition, and inducing blood vessel remodelling (Dobaczewski et al. 2011). However, prolonged TGF β activation leads to excessive ECM deposition remote from the infarct site causing further damage to normal tissue architecture and cardiac function (Bujak et al. 2007). Indeed, inhibition of TGF β before or immediately following MI led to further deterioration on cardiac function and increase mortality (Ikeuchi et al. 2004; Frantz et al. 2008), whereas its inhibition at 24 h post-MI attenuated remodelling with improved cardiac function in animal models of ischemic heart failure (Ikeuchi et al. 2004; Okada et al. 2005; Ellmers et al. 2008). In addition, due to its multifunctional and context dependent actions, a complete blockage of TGF β signalling may cause undesirable side effects on immune regulation (Sasaki et al. 1992), angiogenesis (Bertolino et al. 2005), cancer surveillance (Salomon 2014) and wound healing (Faler et al. 2006). Taken together, TGF β -targeted treatment must be carefully designed. A strategy that selectively attenuates the fibrogenic effect but stimulates the pro-angiogenic aspect of TGF β 1 may serve as an ideal treatment option for heart failure.

The interaction between LRG1 and TGF β signalling

Leucine-rich α -2 glycoprotein 1 (LRG1) is a member of leucine-rich repeat (LRR) family of proteins, many of which are involved in protein–protein interactions, signalling and cell adhesion (Ng et al. 2011). Studies have shown that differential expression of LRG1 is associated with different types of cancer (Kawakami et al. 2005; Kakisaka et al. 2007; Ferrero et al. 2009; Andersen et al. 2010; Guergova-Kuras et al. 2011; Li et al. 2011; Sandanayake et al. 2011; Ladd et al. 2012; Linden et al. 2012, 2013; Liu et al. 2012; Wu et al. 2013; He et al. 2014; Wen et al. 2014), neurodegenerative disease (Miyajima et al. 2013), inflammatory diseases (Kentsis et al. 2012; Kharbanda et al. 2012; Serada et al. 2012),

hydrocephalus (Li et al. 2006, 2007; Nakajima et al. 2010, 2011), heart failure (Watson et al. 2011), autoimmune disease (Serada et al. 2010), and ageing (Nakajima et al. 2012)

We found recently that LRG1 is expressed in quiescent vasculature at low levels but is significantly up-regulated together with TGF β 1 in remodelled and neovascular vessels in the eye (Wang et al. 2013). We showed that LRG1 interacts with multiple TGF β receptors, especially ENG, which together with TGF β 1 further promotes the ability of LRG1 to bind angiogenic ALK1 but inhibits the association between LRG1 and angiostatic ALK5. The recruitment of LRG1 into the pro-angiogenic TGF β receptor complex leads to enhanced Smad 1, 5 phosphorylation, EC proliferation, tube formation and blood vessel outgrowth. LRG1 inhibition by genetic knockout, siRNA knockdown or neutralizing antibodies led to reduced angiogenesis. In summary, our study showed that LRG1 plays a critical role in defining the context-dependent TGF β signalling in ECs (Wang et al. 2013).

There is evidence that LRG1 is involved in other TGF β -regulated processes. TGF β is known to stimulate the expression of endothelin (ET1) (Ahmedat et al. 2012), an important molecule involved in myocardial hypertrophy and fibrosis. ET1 has been shown to inhibit LRG1 expression in dermal fibroblasts suggesting a potential role of LRG1 in TGF β -mediated fibrosis (GEO accession GDS1980 / 1417290_at / Lrg1) (Vallender and Lahn 2006).

The involvement of LRG1 in cardiac remodelling and heart failure

LRG1 and ageing heart

As an unavoidable process of life, different systems of the body undergo progressive structural and functional alterations, and the heart is not an exception. With ageing, there is increased plaque formation in coronary arteries, cardiac wall thickness and interstitial fibrosis (Mendes et al. 2012; Dayal et al. 2013). These structural changes of myocardium are accompanied with concurrent vascular abnormalities, such as reduced diameter and density of collateral vessels, decreased vasodilation, and increased stiffness of vessel walls (Heil and Schaper 2004; Faber et al. 2011; Wang et al. 2011). In addition, there is a decreased expression and availability of growth factors such as hypoxia-inducible factor 1 α and VEGF in response to hypoxic stress (Rivard et al. 2000), and ECs become less responsive to the stimulation of angiogenic growth factors with ageing (Lahtenvuo and Rosenzweig 2012). These alterations contribute to compromised cardiac function, increased susceptibility to damage and reduced ability to repair, which

subsequently lead to increased incidence of heart failure in ageing population. Two separate studies have reported an decreased expression of LRG1 in the heart of aged mice compared to that in young mice (GDS2996 / 1417290_at / Lrg1 and GDS2972 / 97420_at / Lrg1) (Reiter et al. 2007). However, it is not clear whether the reduced LRG1 expression is the cause or the consequence of ageing-related structural and functional changes in the heart. Further studies are required to explore the role of LRG1 in specific patho-physiologies of the ageing heart and to discover whether it is possible to prevent or reverse age-dependent deterioration of the heart by overexpressing LRG1.

LRG1 and cardiac hypertrophy

Cardiac hypertrophy is the thickening of the heart wall in response to increased pressure or volume stress. Under certain conditions, such as during pregnancy or after sustained exercise, the enlargement of heart muscle is beneficial and is normally associated with a proportional increase in chamber dimensions and neovascularization (Catalucci et al. 2008). There is no concurrent fibrosis or reactivation of a foetal gene program in this physiological adaptation process (Beisvag et al. 2009). In addition, physiological hypertrophy does not cause increased risk of arrhythmia, impairment in cardiac function or future heart failure. Instead, exercise training has been shown to protect the heart against ageing-induced up-regulation of collagen deposition, collagen cross-linking, TIMP synthesis and down-regulation of active MMPs (Thomas et al. 2000, 2001; Kwak et al. 2008). The ageing-associated increase in extra-myocyte space was also significantly attenuated in rats which underwent exercise training (Kwak et al. 2008, 2011). Pathological cardiac hypertrophy, on the other hand, occurs as a consequence of hypertension, aortic stenosis, or other disease-causing stimuli. It is associated with significant structural abnormalities, which can lead to contractile dysfunction, arrhythmias and eventually heart failure (Scheuer et al. 1982; Breisch et al. 1986).

The activation of insulin-like growth factor-1 (IGF-1)/phosphoinositide 3-kinase (PI3K)/Akt pathway has been implicated in adaptive cardiac hypertrophy with endurance exercise (Neri Serneri et al. 2001). Studies have shown that transient activation of Akt1 leads to reversible cardiac hypertrophy, which is associated with reduced expression of Lrg1 (GDS2304 / 1417290_at / Lrg1) (Schiekofer et al. 2006). Consistent with this observation, a dominant negative form of PI3K significantly attenuates cardiac hypertrophy in the heart of transgenic mice overexpressing IGF1 receptor, which is also associated with a concurrent up-regulation of Lrg1 (GDS648 / 97420_at / Lrg1) (McMullen et al. 2004). However, a separate study

showed no change in *Lrg1* expression in transgenic mice with either constitutive active PI3K or dominant negative PI3K (GDS446 / 97420_at / *Lrg1*). Also, no change in *Lrg1* expression was detected in the heart of rats following moderate physical training (GDS3134/1374626_at / *Lrg1*) (Giusti et al. 2009). These seemingly contradictory observations might be due to differences in the design of transgenic strategies, spatial and temporal expression pattern of transgenes, and the use of different animal species. Further studies are required to elucidate the role of LRG1 in physiological cardiac hypertrophy.

A reduced expression of LRG1 is observed in mouse models of pathological cardiac hypertrophy. LRG1 expression is significantly attenuated in the heart of mice with compensated pressure overload hypertrophy induced by transverse aortic constriction (TAC) (GDS794 / 97420_at / *Lrg1*, GDS3465 / 1417290_at / *Lrg1*) (Zhao et al. 2004; Smeets et al. 2008). A missense E180G mutation in α -tropomyosin (TM), an important contractile protein involved in sarcomeric function, is associated with familial hypertrophic cardiomyopathy (Chang et al. 2005). Transgenic mice overexpressing α -TM E180G exhibit severe cardiac hypertrophy characterized by myocyte disarray, asymmetric ventricular enlargement, fibrosis, cardiac arrhythmia and eventually die of heart failure (Michele et al. 2002). The expression of *Lrg1* is significantly down-regulated in the ventricle of α -TM E180G transgenic mice (GDS2134 / 1417290_at / *Lrg1*) (Rajan et al. 2006).

Together, the literature supports lower LRG1 expression being associated with cardiac hypertrophy. Further studies are required to investigate the underlying molecular mechanism. Analysis of cardiac phenotypes in *Lrg1*^{-/-} with exercise or pressure overload-induced hypertrophy will provide valuable information regarding the role of LRG1 in physiological and pathological cardiac hypertrophy. LRG1 overexpression might be able to reverse cardiac hypertrophy induced by the activation of IGF1/PI3K/Akt1 pathway, exercise, pressure overload, and in α -TM E180G transgenic mice. The activation of latent TGF β requires functional and physical cooperation of mannose-6-phosphate (M6P)/IGF II receptor (IGFIIR) and the urokinase-type plasminogen activator receptor (uPAR) (Leksa et al. 2005). Furthermore, both TGF β 1 and IGF1 signal through the PI3K/Akt pathway and there is an extensive crosstalk between the two signalling pathways during cardiac fibrosis (Butt et al. 1995), cardiomyocyte apoptosis (Hynes et al. 2009), cardiac remodelling following myocardial infarction (Stavropoulou et al. 2010) and cardiac hypertrophy (Lisa et al. 2011). Understanding the role of LRG1 in TGF β 1 and IGF1 interactions may shed new light on the molecular mechanism of cardiac hypertrophy.

LRG1 and dilated cardiomyopathy

Muscle LIM protein (MLP) is a muscle-restricted cytoskeletal binding protein. The down-regulation of MLP protein is observed in human patients with idiopathic dilated cardiomyopathy (Zolk et al. 2000). Consistent with this observation, MLP^{-/-} mice develop dilated cardiomyopathy and eventually heart failure (Arber et al. 1997). Calsequestrin (CSQ) is a high-capacity sarcoplasmic reticulum Ca²⁺ binding protein. The myocardial-targeted overexpression of CSQ also leads to heart failure associated with dilated cardiomyopathy and left ventricular dysfunction (Jones et al. 1998). Both mouse models exhibited many key features present in the failing heart in human, such as functional β -AR uncoupling (Rockman et al. 1998). Advanced heart failure is normally developed in 6-month old MLP^{-/-} mice and 14-week old CSQ transgenic mice. Despite different aetiologies, a decreased *Lrg1* expression is associated with deterioration of cardiac function with lowest *Lrg1* expression observed at the advanced stage of heart failure in both mouse models (GDS411 / aa172851_s_at / *Lrg1*) (Blaxall et al. 2003). However, the molecular basis of LRG1 down-regulation in both mouse models remains to be resolved, which is vital for dissecting the mechanism of dilated cardiomyopathy pathogenesis. LRG1 overexpression in myocardium might restore cardiac function and slow down the progression of heart failure in both models. Information extracted from this study will assist the designing effective treatment for dilated cardiomyopathy associated heart failure.

LRG1 and hypertension

Hypertension is a major risk factor for heart failure. To work against the high pressure, the heart must pump harder, which may lead to left ventricular hypertrophy and heart failure over time. The S.LWEx12x2x3x5 congenic rat is a model for hypertension and exhibits concentric cardiac hypertrophy. The expression of LRG1 is significantly down-regulated in the left ventricle of hypertensive S.LWEx12x2x3x5 rat exhibiting concentric cardiac hypertrophy with augmented contractile function (GDS3873_1374626_at / *Lrg1*) (Gopalakrishnan et al. 2011). However, it is not clear whether hypertension has a direct impact on the expression of *Lrg1* or mediates LRG1 expression indirectly via hypertension-induced compensation. To study the expression of *Lrg1* in other hypertension animal models with or without myocardial abnormalities will enrich our understanding of direct association between LRG1 and hypertension aetiology and development. LRG1 overexpression at the right dosage and right timing might

prevent or reverse hypertension and hypertension-induced cardiomyopathy.

Perspectives

There is compelling evidence to suggest that TGF β 1 is associated with various cardiac pathologies involved in heart failure. Targeting TGF β 1 therefore represents an attractive strategy in managing progression of the disease. A number of therapeutic approaches for blocking the actions of TGF β 1 have been suggested, such as TGF β 1 neutralizing antibody (Kuwahara et al. 2002), soluble TGF β receptor II (Okada et al. 2005), and small molecule inhibitors (Engebretsen et al. 2014). Some of these successfully attenuated cardiac fibrosis, decreased ventricular chamber dilation, improved cardiac function, and reduced mortality after infarction in preclinical studies (Kuwahara

et al. 2002; Ellmers et al. 2008; Lian et al. 2010). However, given its role in angiogenesis, targeting TGF β may affect collateral and microvessel formation, remodelling and perfusion following MI and cause increased burden of ischemic tissue. In addition, TGF β 1 is a pleiotropic cytokine with vital homeostatic functions. Broad TGF β inhibition is likely to have adverse side effects, such as the development of autoimmune diseases, delayed wound healing, and tumour formation. Selectively targeting specific disease-driving aspects of TGF β signalling at the right dose and timing and for an appropriate period is therefore critical in producing desirable therapeutic effects.

Our recent study led to the identification of a novel angiogenic factor, LRG1. In addition to ECs, LRG1 is also expressed in cardiac fibroblasts (Ifkovits et al. 2014) and cardiomyocytes (Chen et al. 2004). It is likely that LRG1 mediates TGF β signalling through the ubiquitously expressed type I TGF β receptor, ALK5, in non-ECs. There is convincing evidence that a decreased expression of LRG1 is

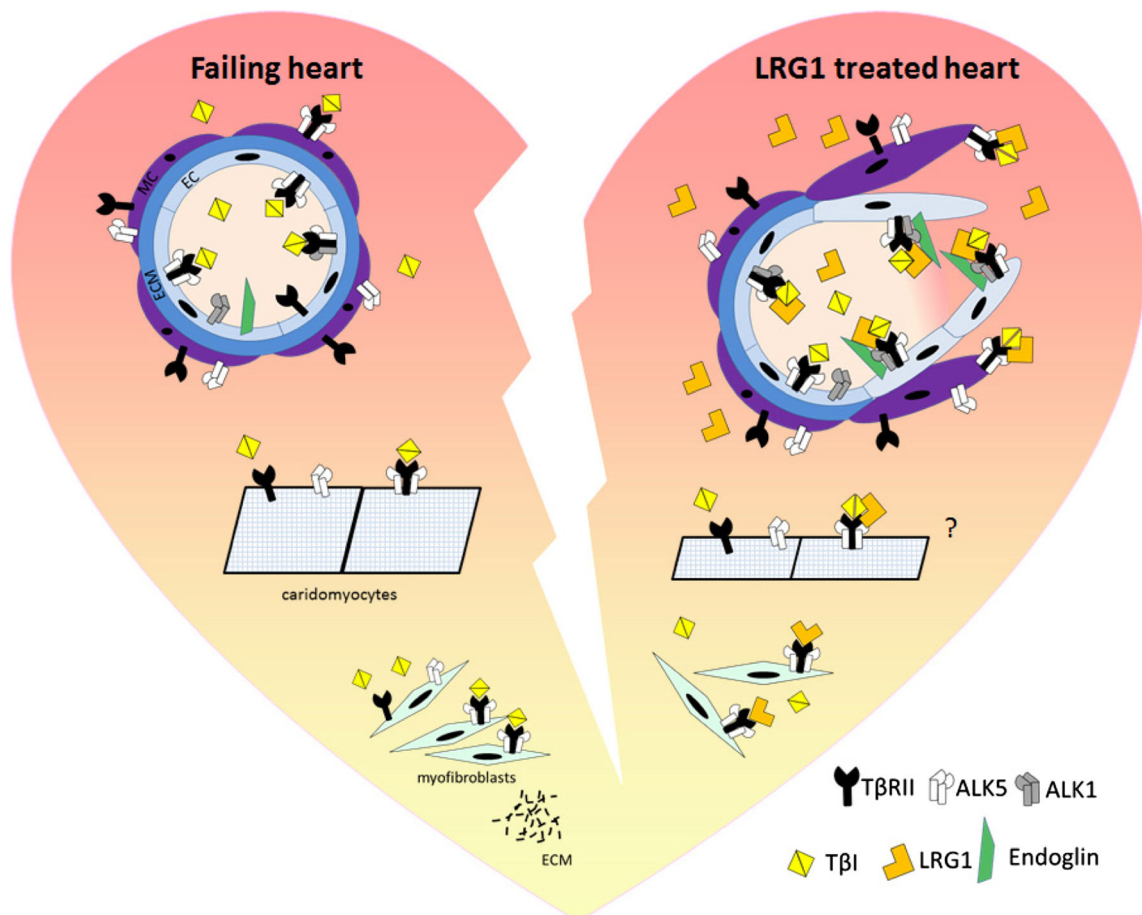


Fig. 2 Potential role of LRG1 in cardiac remodelling. In the failing heart, the ability of blood vessels to respond to angiogenic factors is compromised, fibroblasts acquire myfibroblast phenotype by expressing increased ECM protein, and cardiomyocytes are enlarged and undergo increased apoptosis. TGF β 1 mainly signals through ALK5 in ECs, cardiomyocyte and myofibroblasts leading to cardiac remodelling.

In LRG1-treated heart, LRG1 switches TGF β 1 signalling towards the proangiogenic ALK1 signalling in ECs and promotes blood vessel formation. In myofibroblasts, LRG1 competes with TGF β to bind ALK5 and antagonise TGF β -induced ECM synthesis and to prevent fibrosis. With the presence of LRG1, there is reduced cardiomyocyte apoptosis and size

associated with increased fibrosis, aberrant vascular properties, and altered cardiomyocytes characteristics in ageing heart, and in failing heart induced by genetic modification, pressure overload, or hypertension (Fig. 2). Understanding the molecular mechanism underlying the role of LRG1 and its regulation in cardiac remodelling process will assist the development of novel treatments for heart failure.

LRG1, a potential target for therapeutic angiogenesis

LRG1 promotes EC proliferation, tube formation and vessel outgrowth through regulating the endothelial TGF β signalling (Wang et al. 2013). As it binds to the ubiquitously expressed type I TGF β receptor, ALK5, LRG1 might mediate the signalling and function of other types of vascular cells and regulate blood vessel remodelling. TGF β 1 interacts with VEGF signalling at the receptor level (Glinka et al. 2011) and regulates VEGF expression in macrophages (Jeon et al. 2007) and ECs (Ferrari et al. 2006). Understanding the role of LRG1 in TGF β 1 and VEGF crosstalk may provide valuable information regarding the molecular mechanism of angiogenesis, and facilitate the development of novel therapeutic angiogenesis strategies. Angiogenic factors are normally released in a tightly controlled and timely manner. Lessons learned from previous studies suggested that the hypoxia condition of the ischemic tissue during treatment dramatically affects the benefit of therapeutic angiogenesis. Virus-based transgene delivery systems offer an opportunity for site-specific administration at the right location, time and dose. The route of delivery may also affect the efficacy of treatment. Direct delivery to the myocardium may be an effective method for therapeutic angiogenesis in the heart. Comorbidities are known to have a great impact on the outcome of therapeutic angiogenesis. It is therefore important to evaluate the impact of LRG1 on angiogenesis under heart failure-associated disease conditions such as diabetes, hypertension and obesity. As oedema can impose further burdens on ischemic tissue, studies are required to investigate the impact of LRG1 on blood permeability. It is worth noting that excessive angiogenesis contributes to cancer growth and metastasis, atherosclerotic plaque expansion and instability, arthritis and blinding eye diseases. Understanding the involvement of LRG1 in other vascular complications will help to predict potential side effects of the treatment. Taken together, LRG1 is an attractive target for therapeutic angiogenesis. Further studies are required to evaluate the efficacy and toxicity of LRG1 treatment for heart failure.

LRG1, a potential modulator of cardiac remodelling process

Cardiac fibrosis is a key contributor to the morbidity and mortality in heart failure. Despite intense research efforts, no effective treatment is available to control this detrimental

process. Evidence showed that a reduced expression of LRG1 is associated with an increased TGF β 1 activity during the cardiac remodelling process in response to injury. However, it is not clear whether TGF β 1 exerts its function by inhibiting the expression of LRG1. As LRG1 binds ALK5 and TGF β R2 independently of TGF β 1, it may exert its function by competing with TGF β 1 for binding with the ALK5/TGF β R2 receptor complex. In addition to its role in mediating TGF β 1 signalling in endothelial cells, ENG plays an important role in cardiac fibrosis (Kapur et al. 2012). Unveiling the interaction between ENG and LRG1 in fibrosis will advance our understanding of the molecular mechanism underlying the cardiac remodelling process. It is widely accepted that context-dependent TGF β 1 signalling is defined by extensive interaction with other signalling pathways including IGF1 and AngII. Understanding the involvement of LRG1 in this highly complex network will shed light on the molecular mechanism of cardiac remodelling. Studies have shown that non-ECs including cardiac fibroblasts and cardiomyocytes also express LRG1. It will be interesting to see novel binding partners of LRG1 in these cells, and it will help to elucidate the TGF β 1-independent role of LRG1 in non-ECs in the heart. On the other hand, ECM is important for maintaining atherosclerotic plaque stability. Augment of collagen degradation has been correlated with ruptured plaques in patients (Cheng et al. 2009). Further studies are required to unveil the molecular and cellular mechanism of LRG1 in atherosclerotic plaque development and progression, which will provide valuable insights into the potential side effects of LRG1-targeted treatment.

Summary

At the moment, the focus of heart failure treatment is directed at risk factor management and alleviating symptoms; however, not all patients are suitable or will benefit from current therapeutics. As the magnitude of heart failure continues to accelerate globally, there is a pressing need for new treatments. Direct intervention on structural abnormalities of myocardium is a promising strategy. A reduced expression of LRG1 is associated with cardiac remodelling characterised by hypertrophy, fibrosis, and abnormal vasculature in various conditions leading to heart failure. However, it is not clear whether altered LRG1 expression is the cause or the consequence of these detrimental processes, or how LRG1 is correlated with specific pathologies and evolution of the disease. Although a great deal of work is still needed to fully understand the underlying mechanisms, targeting LRG1 and its regulators might offer a unique approach to treating heart failure by simultaneously targeting different pathologies of the disease.

Compliance with Ethical Standards

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Conflict of interest Weihua Song declares that he has no conflict of interest. Xiaomeng Wang declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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