Does growth differentiation factor 11 protect against myocardial ischaemia/reperfusion injury? A hypothesis

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Abstract
The pathogenesis of myocardial ischaemia/reperfusion injury is multifactorial. Understanding the mechanisms of myocardial ischaemia/reperfusion will benefit patients with ischaemic heart disease. Growth differentiation factor 11 (GDF11), a member of the secreted transforming growth factor-β superfamily, has been found to reverse age-related hypertrophy, revealing the important role of GDF11 in cardiovascular disease. However, the functions of GDF11 in myocardial ischaemia/reperfusion have not been elucidated yet. A number of signalling molecules are known to occur downstream of GDF11, including mothers against decapentaplegic homolog 3 (SMAD3) and forkhead box O3a (FOXO3a). A hypothesis is presented that GDF11 has protective effects in acute myocardial ischaemia/reperfusion injury through suppression of oxidative stress, prevention of calcium ion overload and promotion of the elimination of abnormal mitochondria via both canonical (SMAD3) and non-canonical (FOXO3a) pathways. Since circulating GDF11 may mainly derive from the spleen, the lack of a spleen may make the myocardium susceptible to damaging insults. Administration of GDF11 may be an efficacious therapy to protect against cardiovascular diseases in splenectomized patients.

Keywords
Myocardial ischaemia/reperfusion, growth differentiation factor 11, mitochondria

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Introduction
Ischaemic heart disease (IHD) is a leading cause of death worldwide and a number of risk factors have been identified, including ageing, diabetes and hypertension.¹ It is well

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known that early coronary artery reperfusion via either fibrinolysis or angioplasty can attenuate ischaemic injury. However, there is strong evidence that reperfusion leads to additional myocardial injury beyond that induced by the ischaemia alone. The pathogenesis of ischaemia/reperfusion-induced myocardial injury is multifactorial and includes the effects of oxidative stress and calcium ion (Ca$^{2+}$) overload. In recent years, strenuous efforts have been made to identify the mechanisms responsible for this type of cardiovascular injury. However, to date it is still not possible to prevent myocardial ischaemia/reperfusion injury in patients with IHD who receive vascular recanalization. Further elucidation of the mechanisms of myocardial ischaemia/reperfusion and the development of efficacious therapy to increase the net cardiac benefits of reperfusion are areas of great ongoing interest.

Growth differentiation factor 11 (GDF11) is a member of the transforming growth factor-β (TGF-β) superfamily of secreted growth factors. The GDF11 gene has a broad expression pattern in mice, being found, for example, in skeletal muscle, intestine, pancreas, kidney and the developing nervous system. GDF11 has been detected in a range of tissues, including serum and the myocardium. The spleen has been found to have the highest concentration of GDF11, and a secretory defect in the spleen was shown to lead to a reduction in circulating GDF11 in mice. The amino acid sequence of GDF11 is 90% homologous to myostatin, which is another secreted member of the TGF-β superfamily. Both GDF11 and myostatin stimulate the TGF-β signalling pathway through activin receptors type 2A (ACVR2A) and 2B (ACVR2B), and are antagonized by the activin-binding protein follistatin. Myostatin-null mice do not develop changes in cardiac mass associated with ageing, but treatment with a soluble ACVR2B antagonist leads to increased cardiac muscle mass, suggesting that the cardiac effects of this antagonist may arise from inhibition of GDF11 signalling independent of the effects on myostatin. Thus, despite similar activity in stimulating activin receptors, GDF11 and myostatin may exhibit many non-overlapping functions. Their differential effects might be due to differences in the activity of endogenous inhibitors and subtle differences in receptor affinity. Growth differentiation factor-associated serum proteins 1 and 2 have been reported to delicately regulate the activities of GDF11 and myostatin. In addition, deletion of the GDF11 gene in mice caused abnormal development of many organs, including the stomach and the pancreas. More recently, Loffredo et al. reported that restoring GDF11 to youthful levels in old mice reversed age-related cardiac hypertrophy, suggesting therapeutic potential for GDF11 in cardiac ageing. However, whether GDF11 exhibits protective effects in acute myocardial ischaemia/reperfusion injury remains unclear.

The hypothesis
As illustrated in Figure 1, the presented hypothesis is that circulating GDF11 exerts cardioprotection in acute myocardial ischaemia/reperfusion through the canonical SMAD3 and the non-canonical FOXO3a pathways to suppress oxidative stress, prevent Ca$^{2+}$ overload and promote elimination of abnormal mitochondria. In addition, as a major source of circulating GDF11, the spleen may have an important role in the state of the heart under both normal and abnormal conditions. Consequently, GDF11 has interesting therapeutic potential in cardiovascular disease.

Evaluation of the hypothesis
Like other members of the TGF-β superfamily, GDF11 is produced from precursor
proteins by proteolytic processing. The binding of activin to ACVR2A or ACVR2B induces the recruitment and phosphorylation of an activin type 1 receptor, which then phosphorylates the intracellular signalling proteins SMAD2 and SMAD3. Bujak et al. reported that SMAD3 signalling was critically involved in myocardial infarct healing and played an important role in the pathogenesis of cardiac remodelling. They also demonstrated that the profibrotic actions of TGF-β on cardiac fibroblasts were mediated by SMAD3. Previous studies have suggested TGF-β signalling is an important protective pathway against ischaemia/reperfusion injury. However, the role of secreted GDF11 in myocardial ischaemia/reperfusion injury remains unclear. In vitro research has demonstrated that GDF11 administration increased the phosphorylation of SMAD3 and decreased the phosphorylation of FOXO3a, both of which may modify the effects of myocardial ischaemia/reperfusion.

As a member of the forkhead transcription factors, FOXO3a can be phosphorylated by Akt, leading to inactivation of the FOXO3a pathway. Phosphorylation of FOXO3a results in its translocation from the nucleus to the cytoplasm, deactivating its ability to regulate transcriptional targets in the nucleus. By decreasing FOXO3a phosphorylation, GDF11 may activate the FOXO3a pathway, protecting the heart against insults. Moreover, selective dephosphorylation/activation of FOXO3a is able to upregulate the expression of manganese superoxide dismutase, a reactive oxygen species scavenging protein.

Mitochondrial Ca²⁺ homeostasis is known to play a critical role in maintaining cardiac cell survival. FOXO3a can activate caspase recruitment domain expression by directly binding to its promoter,
consequently attenuating the release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum and inhibiting mitochondrial Ca\textsuperscript{2+} overload in cardiomyocytes.\textsuperscript{28} Furthermore, phosphatase and tensin homologue-induced putative kinase 1 (PINK1) has been shown to have a central role in eliminating dysfunctional mitochondria by promoting the recruitment of the ligase parkin to depolarized mitochondria.\textsuperscript{29} Chen and Dorn\textsuperscript{30} have demonstrated that PINK1 can phosphorylate mitofusin 2 and promote its parkin-mediated ubiquitination in cardiac tissues, suggesting an important role for the PINK1/mitofusin 2/parkin pathway in cardiovascular pathologies. Most importantly, PINK1 serves as an important downstream mediator of FOXO3a, and FOXO3a activation can lead to PINK1 upregulation.\textsuperscript{31} Therefore, it is conceivable to suppose that GDF11 can enhance the elimination of abnormal mitochondria through a FOXO3a/PINK1/mitofusin 2/parkin pathway in a heart subjected to ischaemia/reperfusion. Consistent with the hypothesis presented here, Siddall et al.\textsuperscript{32} reported that PINK1 increased the heart’s resistance to ischaemia/reperfusion injury.

Activation of SMAD3, a downstream mediator of GDF11, has been shown to participate in the cardioprotective effects of TGF-\beta1, whereas inhibition of SMAD3 blocks the preventive effects of TGF-\beta1 on cardiac fibroblast apoptosis in myocardial ischaemia/reperfusion.\textsuperscript{33} Moreover, SMAD3 has been reported to be associated with oxidative stress in the pathogenesis of kidney fibrosis\textsuperscript{34} and hyperglycaemia.\textsuperscript{35} Inhibition of SMAD2 can prevent SMAD2-mediated downregulation of Ca\textsuperscript{2+} ATPase \textit{in vivo} and in cardiomyocytes.\textsuperscript{36} SMAD3 is closely associated with FOXO signalling in various diseases.\textsuperscript{11} Nodal, another member of the TGF-\beta family, can stimulate FOXO3a mRNA and protein expression via the SMAD3 pathway, and SMAD3 overexpression enhances FOXO3a-induced cyclin G2 promoter activity in human epithelial ovarian cancer cells.\textsuperscript{37} Therefore, GDF11 may play an important role in myocardial ischaemia/reperfusion via the canonical SMAD3 pathway.

If the hypothesis that GDF11 exerts cardioprotection in acute myocardial ischaemia/reperfusion through the SMAD3 and FOXO3a pathways is correct, it offers the potential for new therapeutic strategies in the treatment of IHD.

The role of the spleen

As the spleen is a source of circulating GDF11,\textsuperscript{11} it may have protective effects in hearts subjected to ischaemia/reperfusion via its derived cytokines rather than its well-known immunological functions. Therefore, patients who have undergone splenectomy may have increased susceptibility to cardiac damage. Aydinok et al.\textsuperscript{38} reported that splenectomized patients have a higher incidence of myocardial siderosis than those with an intact spleen. It is possible that supplementation of exogenous recombinant spleen-derived cytokines such as GDF11 after splenectomy may increase cardiac resistance to damaging insults.

In addition, GDF11 levels exhibit an age-dependent decline in both the spleen and the general circulation.\textsuperscript{11} It is well known that ageing is one of the main risk factors for IHD and cardiac hypertrophy.\textsuperscript{39} It is therefore possible that administration of recombinant GDF11 in the elderly may help maintain cardiac health and decrease the incidence of cardiac diseases. Consistent with our theory, GDF11 can successfully activate the FOXO3a pathway,\textsuperscript{11} which has been shown to negatively regulate cardiac hypertrophy.\textsuperscript{40} A number of studies have shown that many organs and tissues play important functions in cardioprotection, such as the protection against myocardial ischaemia/reperfusion injury provided by insulin from the pancreas\textsuperscript{5} and adiponectin from adipose tissue\textsuperscript{41} and the protection against
diabetes-induced cardiac microvascular injury provided by intestine-derived glucagon-like peptide 1.42 Notably, the renin–angiotensin–aldosterone system closely links the kidney and heart, and significantly affects blood pressure and cardiac remodelling.43,44 It is suggested that the spleen may be another organ with an important role in the heart’s adaption to surroundings and its response to injury. Consistent with this hypothesis, Rezende et al.45 reported that splenectomy resulted in increased atherosclerotic lesions in apolipoprotein E-deficient mice. To date, there is no direct experimental evidence supporting the role of spleen in myocardial ischaemia/reperfusion, and further studies are needed to confirm this association.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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