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Review

Biological activities of fibroblast growth factor-2 in the adult myocardium

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Abstract

Fibroblast growth factor-2 (FGF-2) is a potent regulator of many cellular functions and phenomena, including cell proliferation, differentiation, survival, adhesion, migration, motility and apoptosis, and processes such as limb formation, wound healing, tumorigenesis, angiogenesis, vasculogenesis and blood vessel remodeling. In the adult myocardium, FGF-2 is expressed by various cell types, including cardiomyocytes, fibroblasts and smooth muscle cells. The biological effects of FGF-2 in the myocardium are mediated by the high-affinity tyrosine kinase receptor FGFR-1, the major FGF receptor in the heart. Here, we give an overview of current insights into the multiple roles of FGF-2 in the myocardium, as they pertain to two basic phenomena: ischemia–reperfusion injury and cardiac hypertrophy. The first category includes roles for FGF-2 in cardioprotection, the inflammatory response, angiogenesis and vascular remodeling, while the second includes myocyte hypertrophy, fibrosis, and gap junction functioning (conduction). Given the strong evidence for FGF-2 as both a cardioprotective and angiogenic agent, the therapeutic potential of FGF-2 in the ischemic myocardium is discussed.
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Keywords: Angiogenesis; Growth factors; Hypertrophy; Ischemia; Signal transduction

1. Introduction: fibroblast growth factor-2

''Only a handful of researchers paid much attention to the discovery of FGF at the time but it would turn out to be a big step in a landmark development for medicine'' [1]. This statement, referring more specifically to the purification of fibroblast growth factor-2 (FGF-2) [2], appears in a recent biography of Dr Judah Folkman, pioneer in the field of angiogenesis. Certainly research focused on FGF-2 has revealed an important place for this protein in cardiac cells in health and disease. FGF-2, also known as basic FGF because of its *p*I (>9.0), is one of 23 structurally related polypeptide growth factors (FGF-1 to FGF-23) [3], but because of its high affinity for heparin is also considered a member of the larger heparin binding growth factor family, which includes vascular endothelial growth factor (VEGF) and heparin-binding epidermal growth factor-like growth factor [4–6]. FGF-2 is highly conserved amongst species [7,8]. FGF-2 exists in high (HMW) and low molecular weight (LMW) forms due to alternative translation of the same messenger RNA from upstream leucine (CUG) sites or a conventional downstream methionine (AUG) site [9]. This was shown to be controlled by internal ribosomal entry sequences in the 5′-untranslated region in a cap-independent manner [10]. More recently, a larger 34-kDa HMW form of FGF-2 was identified in human cervical carcinoma HeLa cells arising from translation initiation at a more distal CUG site, involving a cap-dependent process [11].

Despite its name, FGF-2 is now known to modulate numerous cellular functions in multiple cell types, including cell proliferation, differentiation, survival, adhesion,

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migration, motility and apoptosis, and processes such as limb formation, vasculogenesis, wound healing, tumorigenesis, angiogenesis and blood vessel remodeling [12]. FGF-2 is expressed by various cell types of the myocardium, including cardiac myocytes and vascular cells at all developmental stages [13,14]. In cardiac myocytes, FGF-2 was shown to be associated with the basement and cell membranes, intercalated discs, Z-lines, cytoplasm, as well as the nucleus [14]. Both HMW and LMW FGF-2 species are expressed by cardiac myocytes [14,15]. HMW FGF-2 is largely targeted to the nucleus whereas LMW FGF-2 is predominantly cytoplasmic, although it can also be found in the nucleus [16–18]. The distribution of FGF-2 implies participation in diverse functions.

The biological functions of FGFs are mediated primarily by specific cell surface receptors of the tyrosine kinase family [12]. The predominant FGF-2 receptor in the heart is FGF receptor-1 (FGFR-1) [19–21]. The presence of functional FGFR-1 appears to be essential for normal cardiac development [22]. Although at reduced levels, FGFR-1 expression persists in the adult heart [20]. The presence of cell surface receptors implies the presence of FGF-2, and thus its release to the extracellular milieu. FGF-2 release occurs in spite of the absence of any 'classic' hydrophobic export signal peptide [12]. This is supported further by the detection of FGF-2 protein in the extracellular spaces/matrix surrounding various cells including cardiac myocytes, as well as in serum at low levels [8,23–25]. FGF-2 release may occur through exocytosis involving the Na^+/K^+ ATPase pump [26–28] and/or via passive processes [23]. The latter would include FGF-2 released as a result of cell lysis during tissue injury and cell death [29,30], complement-mediated injury [31], matrix-associated release via heparin, heparan sulfate and heparinase [32,33], as well as plasminogen activator-mediated proteolysis [34]. In the postnatal heart, there is also evidence to support a passive mechanism of FGF-2 release from adult cardiac myocytes on a beat-to-beat basis through contraction-induced transient remodeling or 'wounding' of the plasma membrane under normal physiological conditions [30,35]. FGF-2 is also released from endothelial and vascular smooth muscle cells through a similar mechanism involving non-lethal plasma membrane disruptions [36–38].

The diverse functions of FGF-2 in the adult myocardium can be divided into two basic categories, related to the phenomena of: (1) ischemia–reperfusion injury and (2) cardiac hypertrophy. The first category includes roles for FGF-2 in cardioprotection, the inflammatory response, angiogenesis and vascular remodeling, while within the second is contained myocyte hypertrophy, fibrosis and cell conduction (gap junction functioning). In this review, we will explore the current knowledge with respect to these two categories of function, and discuss the therapeutic potential of FGF-2 in this context.

2. FGF-2 and ischemia–reperfusion injury

When coronary perfusion is blocked partially or completely, the myocardium is subjected to injury related to the deprivation of oxygen and other components carried by the blood. There is an influx of calcium and hydrogen ions in ischemic myocytes, and when flow is restored, the extent of the injury is exacerbated because the myocytes are forced to resume contractile function under conditions of elevated calcium and acidic tissue pH, in addition to osmotic strain [39]. Much research effort has been devoted to the discovery of factors and/or conditions which might protect the myocardium against such injury. Clearly, factors which preserve the viability of the ischemic myocardium (i.e. so-called 'cardioprotective agents') represent potential therapeutic agents for the treatment of patients at high risk of myocardial infarction, or for the prevention of lethal reperfusion injury caused by restoration of coronary flow [40].

Early evidence that FGF-2 is cardioprotective came from experiments by Kardami et al. [41] with neonatal rat cardiac myocyte cultures treated with hydrogen peroxide or starved for serum. Addition of LMW FGF-2 improved cell survival and decreased cardiac myocyte injury as evidenced by preservation of nuclear morphology and myofibrillar structure. Subsequently, administration of exogenous LMW FGF-2 before or during ischemic injury in various heart ischemia/reperfusion models was shown to increase myocyte viability and/or functional recovery in the rat or mouse heart [42–46]. In vivo, exogenous addition of FGF-2 stimulated myocardial function and/or reduced infarct size in ischemic porcine and canine hearts through increased angiogenesis and systolic function [47–50]. Increased production of endogenous FGF-2 through transgenic overexpression in the heart was also associated with a significant increase in myocyte viability following a period of global ischemia [45]. In this model, increased FGF-2 release in transgenic hearts overexpressing FGF-2 relative to wild-type hearts was observed [45]. This is consistent with the idea that endogenous FGF-2 is released during contractions under normal physiological conditions [30,35] and can interact with functional cell surface receptors [20,46]. Under these conditions, FGF-2 may play a role in the normal maintenance of a healthy myocardium, as well as possibly limiting the extent of injury. Further to this, a recent study reported that transcoronary arterial gene transfer of a secreted form of FGF-2 was beneficial for recovery of left ventricular systolic function as well as for the development of collaterals in the microembolized rabbit heart [51].

The therapeutic applications of a cardioprotective agent given prior to an ischemic insult are limited to the relatively rare cases of patients which present with clear risk of myocardial infarction, without having had an ischemic episode. Thus, it is desirable to seek out agents

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which may protect the myocardium against the exacerbating injury caused by reperfusing an ischemic area, since the restoration of blood flow is considered to be essential for recovery in cases of coronary occlusion [40]. In a Langendorff-perfused rat heart model, FGF-2 administered in the first 12 min of reperfusion following 30 min of global ischemia significantly improved functional recovery over a 60-min reperfusion period [46], an effect which was fully reversed with the PKC inhibitor chelerythrine. On the other hand, administration of FGF-2 at the time of reperfusion in isolated mouse hearts after stunning (causing severe ventricular dysfunction) did not improve myocardial recovery when compared to administration prior to stunning [52]. The amount of FGF-2 administered to isolated hearts is different between the two studies (1 μg [52] versus 10 μg [45,46]). Although this may reflect a dose correction per kilogram body weight, experiments from our laboratory with isolated mouse hearts demonstrated significant functional recovery after administration of 10 μg FGF-2 [45], the same amount used in isolated rat hearts [46]. Nevertheless, the difference more likely reflects the type and severity of injury to which the isolated hearts were subjected. Stunning, a transient condition, is not equivalent to the irreversible injury (necrosis and apoptosis) incurred during extended ischemia followed by reperfusion. Given that overexpression of FGF-2 in transgenic mouse myocardium resulted in decreased lactate dehydrogenase release but not improved function after ischemia and reperfusion [45], it is possible that FGF-2 cardioprotection relates more to myocyte viability than to contractile recovery, at least in the mouse. Thus, a model of myocardial stunning [52] would show less effect of FGF-2 than a model of more severe ischemic injury [46].

The mechanism of cardioprotection by FGF-2 is the subject of ongoing investigation (Fig. 1). FGF-2 can act on most cardiac cells, including cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts. There is strong evidence for a direct FGF-2 receptor-mediated shielding effect on cardiac myocytes, independent of its effects on the vasculature [44,46]. Chelerythrine, a non-specific protein kinase C (PKC) inhibitor, blocked the action of FGF-2 when administered both prior to ischemia [44] as well as to ischemic myocytes with or without reperfusion [46]. The involvement of PKC in cardioprotection is well established, especially with respect to ischemic preconditioning [54–57]. In fact, several pieces of evidence suggest links between the pathways which mediate ischemic preconditioning and FGF-2-induced cardioprotection. The effects of ischemic preconditioning include activation of G protein coupled receptors as well as tyrosine kinases [55,58]. Cross-talk between FGF-2 tyrosine kinase receptors and G protein coupled receptors has been reported, since FGF-2 was able to induce PLCβ isoforms in adult hearts and cardiac myocytes [59], as well as in the regulation of myogenic differentiation [60]. The effects of FGF-2 administered to ischemic myocytes were dependent on the binding of FGF-2 to its major receptor in cardiac myocytes, FGFR-1 [46]. Downstream, PKCε was demonstrated to play an integral role in ischemic preconditioning [56,61] and studies in the rat heart have also implicated PKCε as the cytoplasmic intermediate involved in FGF-2-mediated cardioprotection, both prior to ischemia [44] and during reperfusion [46]. Activation of PKCε results in the opening of mitochondrial K^+/ATP channels [62], a proposed end-effector of ischemic preconditioning [58]. Glibenclamide, a specific K^+/ATP channel blocker, could block the cardioprotective effects of FGF-1 [63]. It remains to be determined whether mitochondrial K^+/ATP channels play a role in the cardioprotective effects of FGF-2, although this is a likely scenario. since both factors signal through the same receptor (FGFR1) in the heart [19–21,64]. Nitric oxide (NO) has also been proposed to be a prime mediator of delayed ischemic preconditioning [65]. FGF-2 was also shown to induce NO release into the coronary milieu as part of the vasodilatory response [66]. Collectively, these links suggest that FGF-2-induced cardioprotection is mediated by the ε subtype of PKC. It is possible that FGF-2 itself may play a role in ischemic preconditioning, though direct evidence to this end is not yet available.

An interesting link exists between FGF-2-mediated cardioprotection and myocardial gap junctions: FGF-2 decreased cardiomyocyte metabolic coupling by stimulating the phosphorylation of Cx43 on serine [67]. These effects were mediated by direct interaction of PKCε with Cx43 at intercalated disks, while the erk1/2 pathway was not involved [66]. FGF-2 is likely to affect adult myocyte gap junction coupling in a manner similar to the neonatal counterparts: in both systems FGF-2 can stimulate PKCε and cause Cx43 phosphorylation [44,68] (also see our unpublished data). It is, therefore, likely that FGF-2, released after myocardial injury, decreases channel permeability between myocytes. A similar effect is elicited by anesthetics and is considered to be protective overall, since it decreases the spreading of contracture (injurious stimuli) between myocytes [69,70]. Thus, FGF-2-induced, PKCε-mediated cardioprotection is likely to include effects on gap junction channels.

Aside from this direct signaling mechanism, FGF-2 may trigger other cellular events leading more indirectly to a cardioprotective response. Studies in the rat heart have demonstrated that FGF-2 administered to the non-ischemic heart can induce a negative ionotropic effect, which may contribute to cardioprotection through the preservation of energy stores or the suppression of the energy requirement for contraction [44]. A negative ionotropic effect of FGF-2 was also observed in isolated adult cardiac myocytes [71]. FGF-2 is reported to possess vasodilatory effects [49,66] and vasodilators have been shown to be cardioprotective in ischemia–reperfusion injury [72,73]. There is also some

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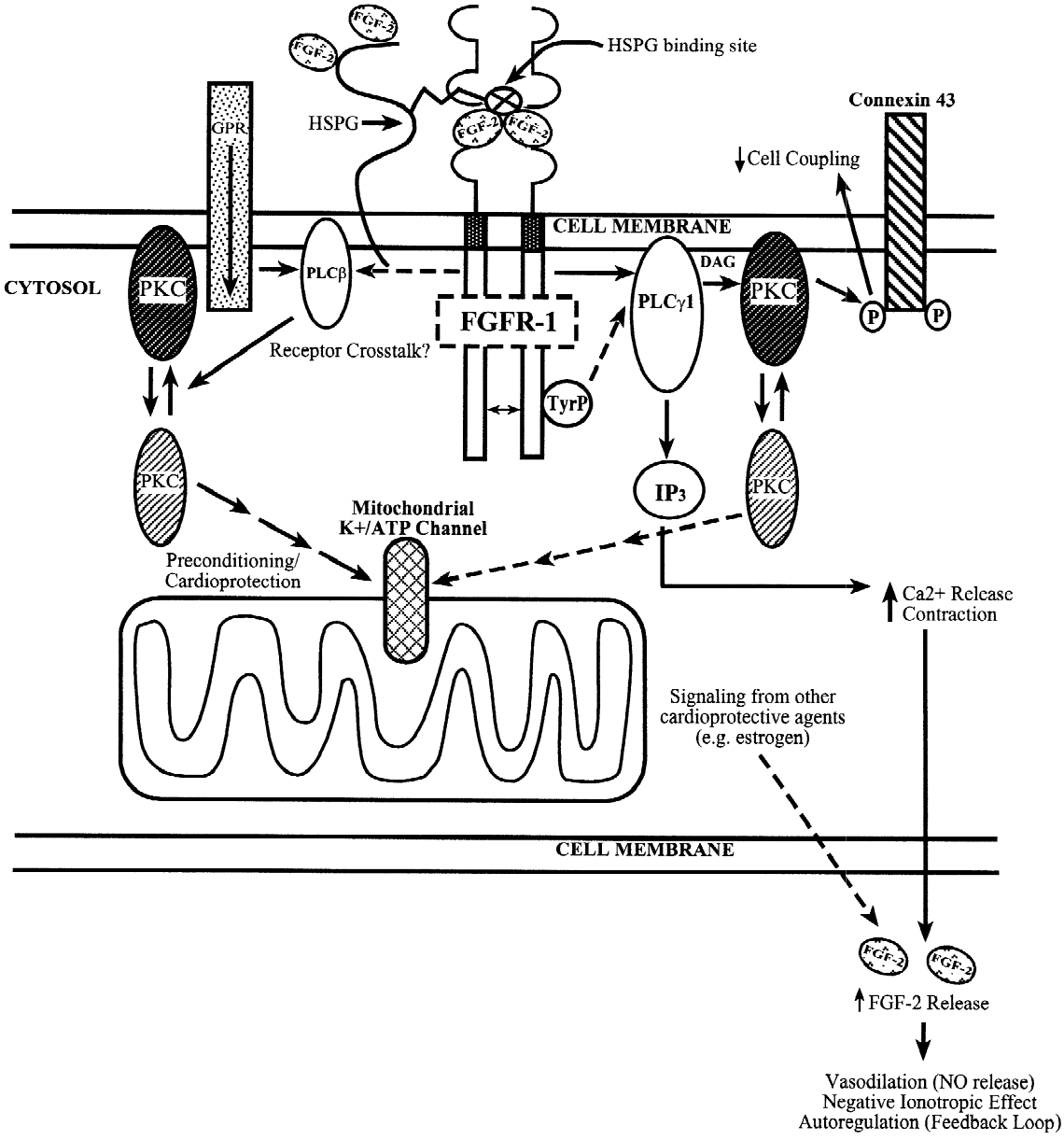

Fig. 1. Overview of the major signaling pathways involved in FGF-2 mediated cardioprotection in myocytes. FGF-2 binds to its high affinity receptor, FGFR1, and to cell-surface heparan sulfate proteoglycans (HSPG). Activation of FGFR-1 results in membrane translocation of protein kinase C (PKC) isoforms, possibly resulting in the opening of mitochondrial K^+/ATP channels, an end-effector of ischemic preconditioning. There has been a suggestion of crosstalk between FGFR and G-protein coupled receptors (GPR), which may contribute to the cardioprotective effect. PKC activation (specifically the ϵ isoform) also results in the phosphorylation of connexin43, which would decrease cell coupling and possibly slow the propagation of hypercontracture and other harmful effects of ischemia. Alternatively, the accumulation of IP_3 in the cytoplasm results in the release of Ca^{2+} ions from the sarcoplasmic reticulum; this in turn would lead to increased contraction and therefore increased FGF-2 release through transient disruption of the sarcolemma. FGF-2 release may also be invoked by other cardioprotective agents. The released FGF-2 could then act indirectly to elicit a cardioprotective effect through negative inotropy, vasodilation or even autoregulation of its own signaling or production. Solid arrows indicate established relationships; dashed arrows indicate proposed pathways. Adapted from Ref. [53]. Abbreviations: DAG, diacylglycerol; HSPG, heparan-sulfate proteoglycan; GPR, G-protein coupled receptor; IP_3, inositol-1,4,5-triphosphate; PKC, protein kinase C; PLC, phospholipase C; TyrP, tyrosine-phosphate.

evidence to suggest that other cardioprotective agents may act by releasing FGF-2. For example, estrogen increases FGF-2 release from endothelial cells [74]. It was suggested that estrogen enhances endothelial cell-basement membrane interactions that lead to the release of 'trapped' FGF-2 from the extracellular matrix [74] and/or by mechanisms involving the chaperoning of intracellular FGF-2 via heat-shock protein 27 [75]. It is not known whether estrogen increases the release of endogenous FGF-2 from cardiac myocytes. Regardless, FGF-2 released from any cardiovascular cell type would theoretically be made available to act on cardiac myocytes in an intracrine

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manner. In fact, an increase in the early growth response protein (Egr)-1, a transcription factor associated with stress response, was reported in neonatal cardiac myocytes following stimulation with estradiol [76]. This may be significant as overexpression of Egr-1 was shown to increase rat FGF-2 promoter activity in neonatal rat cardiac myocytes [77], and Egr-1 was shown to be important for the autocrine regulation of FGF-2 expression in human glioma cells [78].

Finally, it is possible that FGF-2 may function along with other members of the FGF family to exert a cardioprotective effect. Specifically, FGF-1 (or acidic FGF) is also a well-established cardioprotective agent [43,63,79,80]. Since FGF-1 also functions through FGFR-1 in the myocardium [64], it is conceivable that the two factors work along similar pathways. Recent work with transgenic mice overexpressing FGF-1 linked the cardioprotective effect observed after coronary artery occlusion to ERK1/2 [80]. The role of PKC was not assessed in this study. However, the 'cardioprotective effect' as it was assessed in this study was simply delayed infarct development, with no improvement in overall infarct size, and post-ligation cardiac function was not assessed. Work with FGF-1 exogenously added to an in vivo rat model of ischemia–reperfusion has focused largely on more downstream targets, such as the mitochondrial K^+/ATP channel or nitric oxide synthase [63,81]. Thus, further work is necessary to establish whether FGF-1 and FGF-2 act through overlapping (either cooperative or competitive) mechanisms to induce their cardioprotective effects.

A key event following myocardial reperfusion injury is the rapid initiation of a cascade of events very similar to the inflammatory response [82]. The heart's inflammatory reaction to injury may also include a 'misguided' attack mounted against host tissue, without the regular checks and balances to distinguish 'self' from 'non-self' [83]. The cellular component of this response is mediated immediately and largely by neutrophils [84], but also includes the participation of monocytes and T lymphocytes [84,85]. Specifically, neutrophils contribute to tissue injury through the release of oxidants and proteases that cause cell death and, perhaps, by perpetuating the recruitment process [83].

Although a role for FGF-2 in the cardiac inflammatory response following injury remains to be defined, several pieces of evidence suggest a possible link between FGF-2 signaling and infiltrating T cells. Studies from our laboratory using transgenic mice, have demonstrated that chronic overexpression of FGF-2 in cardiac myocytes can exacerbate the cardiac inflammatory response following isoproterenol-induced cardiac injury in vivo [86]. A component of isoproterenol-induced injury is T-cell dependent, since suppression of T cells with cyclosporin A or anti-CD3ϵ antibodies greatly reduced the level of myocardial injury observed [86]. Also in this study, cells positive for both CD4 and FGFR-1 were detected in FGF-2-overexpressing as well as wild-type mouse hearts, suggesting a link between FGF-2 signaling and T cells. This raises the possibility that FGF-2 release at the time of injury contributes to the 'normal' inflammatory process caused by T cell infiltration, and that in the presence of chronic excess FGF-2, T-cell infiltration and cell injury is exacerbated.

FGF-2 might affect the recruitment and/or the expansion (i.e. proliferation) of T lymphocytes. FGF-2 is a known chemoattractant affecting cell attachment and migration [23,87], and accumulates at sites of myocardial injury in vivo [88]. Many infiltrating cells, including $CD4^+$ T lymphocytes, express FGFR-1 [86,89]. However, there is no direct evidence to suggest that FGF-2 can stimulate the proliferation of these cells, especially since FGF-1 alone failed to act [89]. Further study will establish what role FGF-2 may play in T-cell infiltration, and the relative importance of recruitment versus amplification in the myocardial inflammatory response.

In addition to its action directly on myocytes, FGF-2 plays a key role in the vascular response to myocardial ischemia and reperfusion. During development, the formation of the vasculature involves two separate but overlapping processes: (1) vasculogenesis, or the formation of major vessels through the proliferation and migration of smooth muscle cells, fibroblasts and endothelial cells, and (2) angiogenesis, a process specific to endothelial cells and resulting in the formation of small vessels and capillaries [90]. Through the use of antisense strategies, FGF-2 was demonstrated to be essential for embryonic mouse vascular development [91]. FGF-2 can stimulate proliferation of all three principal vascular cell types (endothelial cells, vascular smooth muscle cells and fibroblasts), and its role in both developmental vasculogenesis and angiogenesis is well established [90,92,93].

There is evidence that collateral development does occur naturally to some extent in response to coronary artery occlusion [94,95]. Within the last 5 years, therapeutic angiogenesis to enhance this process has been advocated as a promising treatment strategy for patients with advanced ischemic heart disease who are not candidates for standard revascularization, since it results in the generation of a new blood supply in the diseased heart [95,96]. Unfortunately, progress in this area has been limited by, among other things, a poor understanding of the mechanisms involved in adult collateral development and angiogenesis, as opposed to developmental processes [97]. However, the ability of FGF-2 to induce angiogenesis in mature ischemic myocardium has been demonstrated in various injury models, including porcine, canine and rabbit [47–51]. Although the mechanisms involved in FGF-2 mediated angiogenesis have not been fully elucidated, the p38 MAP kinase signaling pathway has been implicated [98]. FGF-2 induces VEGF expression in vascular endothelial cells via both paracrine and autocrine pathways to mediate angiogenesis [99]. In fact, the roles of FGF-2 and VEGF in angiogenesis are inextricably linked and dependent one upon the other [100,101], a relationship which is believed

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to relate to the observed synergistic response when both growth factors are used in combination [102,103].

A number of clinical trials, focused on exploiting the angiogenic effects of FGF-2, have been recently published or are currently underway to assess the potential therapeutic effects of FGF-2 in the ischemic myocardium (Table 1). Several strategies have been used to deliver FGF-2 into the human heart. While gene therapy approaches using adenoviral FGF-2 delivery vectors have been explored in animal models with some success [104–106], human gene therapy trials have focused mainly on VEGF [107,108]. Clinical trials involving FGF-2 have instead utilized a number of methods to introduce the protein directly into the myocardium. Initial studies used a heparin alginate FGF-2 delivery system in patients with coronary disease undergoing coronary artery bypass surgery [109,110]. Both studies demonstrated the safety and feasibility of this mode of therapy and patients in the FGF-2 group were found to be symptom free (i.e. no angina) after 3 months of surgery [109,110], although these effects were shown to be dose-dependent in a placebo-controlled group [110]. Intracoronary and intravenous FGF-2 deliveries were also found to be feasible and tolerable in patients with severe coronary disease [111–113], although a dose-dependent hypotensive effect was reported in two studies [111,113], and this was dose-limiting in one instance [111]. Consistent with a favorable effect of angiogenesis, delivery of FGF-2 resulted in attenuation of stress-induced ischemia and an improvement in resting myocardial perfusion up to 180 days after treatment [112]. In addition, intracoronary delivery of FGF-2 was also shown to significantly improve symptom assessment, as assessed by angina frequency and exertional capacity (i.e. exercise tolerance) [111]. Intracoronary FGF-2 injection was also demonstrated to be well tolerated in patients with stable angina [113]. Clearly, these results suggest that FGF-2 delivery is well tolerated in the human myocardium producing functionally significant benefits in the ischemic myocardium [96]; however, adverse effects and toxicity are also issues which must be addressed when considering long term therapies with FGF-2. A recently published phase II clinical trial, FIRST [114], failed to show improved exercise tolerance or myocardial perfusion with a single intracoronary infusion of recombinant FGF-2. However, at the end of this study it was concluded that further trials are warranted, given the favorable safety profile for FGF-2 from previous trials, and the apparent improvement in groups of highly symptomatic patients compared to patients with less severe angina at baseline [114].

It has been suggested that the usefulness of FGF-2 in human therapy is dependent upon our ability to selectively enhance its endothelial effects and minimize smooth muscle effects [115]. Indeed, vascular cell growth is a double-edged sword, as another phenomenon associated with this effect of FGF-2 is vascular remodeling. This is a response of blood vessels to physiological or pathophysiological stimuli, resulting in either vessel enlargement (positive or outward remodeling) or reduction (negative or inward remodeling). Inward remodeling is associated with predisposition to cardiovascular diseases such as atherosclerosis and restenosis. Flow-induced changes in arterial wall structure and vessel size are linked with increased FGF-2 expression [116], and the remodeling associated with the formation of atherosclerotic plaques is postulated to be flow-related [117,118]. Interestingly, clinical trials using FGF-2 and other angiogenic factors, specifically VEGF, indicate no outstanding problems related to vascular remodeling [108]. Nevertheless, it

Table 1
Clinical trials using FGF-2 for the treatment of coronary artery disease

Trial name, date, [Ref], type	Mode of delivery	Number of patients	Results
Sellke et al., 1998 [109], Phase I	Heparin–alginate slow release device	8	Safety and technical feasibility demonstrated
Laham et al., 1999 [110], Phase I	Heparin–alginate slow release microcapsules	24	Safety and technical feasibility demonstrated
Unger et al., 2000 [113], Phase I	Bolus injection in main left coronary artery; 3–100 μg/kg	25	Acute hypotension, some sustained hypotension (1–3 days); no long-term adverse effects
Udelson et al., 2000 [112], Phase I	Intracoronary or intravenous injection; 0.33–48 μg/kg	59	↓ stress-induced ischemia ↑ resting myocardial perfusion
Laham et al., 2000 [111], Phase I	Single 20-min intracoronary infusion; 0.33–48 μg/kg	52	Hypotension was dose-limiting (max. 36 μg/kg) Some evidence of improved quality of life and exercise tolerance. ↑ regional wall thickening ↓ extent of ischemic area
Simons et al. 2002 'FIRST', [114], Phase II	Single intracoronary infusion; 0–30 μg/kg	337	No improvement in exercise tolerance or myocardial perfusion; some symptomatic improvement at 90 (but not 180) days.

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was demonstrated that neutralizing antibodies to FGF-2 could inhibit lumen narrowing and negative remodeling in the event of intimal lesion formation in a coronary ligation model in mice [119]. A recent study demonstrated that normal NO production is essential for the enhanced vascular remodeling induced by FGF-2, along with another angiogenic factor, in a rat model of experimental peripheral arterial insufficiency [120]. In addition, FGF-2 has been suggested to be a survival factor for vascular smooth muscle cells and endothelial cells [121–125]. Adenoviral delivery of antisense FGF-2 in a rabbit model of arterial injury reduced neointimal thickening [126–128], suggesting an application of antisense strategies to counter restenosis following balloon angioplasty. Thus, both increasing and decreasing FGF-2 levels in the vasculature could have potential therapeutic effects in the myocardium, either by promoting angiogenesis to re-perfuse ischemic myocardium through FGF-2 addition, or by reducing restenosis to prevent blockage of a vessel by inhibiting FGF-2 activity. The mode of delivery chosen (i.e. local versus systemic) will likely be crucial in reconciling the undesirable (remodeling) versus desirable (angiogenic) vascular effects of FGF-2.

3. FGF-2 and the hypertrophic response

It is generally accepted that once damaged, the myocardium retains only a very limited ability to regenerate through the proliferation of myocytes [129,130]. Instead, highly proliferative fibroblasts will infiltrate the area and form a scar [131]. FGF-2 has been shown to increase both fibroblast and myofibroblast proliferation [24,132], which could conceivably have an adverse effect in the heart leading to increased scar formation or a ‘stiffer’ heart in the event of cardiac injury in vivo. On the other hand, studies have demonstrated that FGF-2 potently inhibits collagen fiber production by human smooth muscle cells [133]. It is thought that this represents a mechanism for thinning the local collagen environment during vascular remodeling, which could in turn be important in intimal accumulation of smooth muscle cells or destabilization of an atherosclerotic plaque [134]. The role of FGF-2 and its potential to act as a therapeutic agent during cardiac fibrosis remains to be determined.

To compensate for the increased workload, the remaining myocytes hypertrophy; that is, they increase in size rather than number. Evidence that FGF-2 may play an important role in cardiac hypertrophy was obtained over a decade ago in cultured cells. Addition of (LMW) FGF-2 to cultured neonatal cardiac myocytes alters the gene profile of contractile proteins from an ‘adult’ to ‘fetal’ program [135], which is characteristic of pressure overload-induced cardiac hypertrophy in vivo. Indeed, FGF-2 was also shown to decrease overall myosin accumulation in embryonic cardiac myocytes in vitro [14,16]; it may also cause re-expression of vimentin in cardiac myocytes surrounding the fibrotic region subsequent to cardiac injury in vivo [88]. It was also demonstrated that paced adult cardiac myocytes (i.e. increased FGF-2 release) but not non-paced control myocytes exhibited a ‘hypertrophic’ response (characterized by increased protein content and cell size) which could be mimicked by exogenously administered FGF-2 and blocked by neutralizing antibodies to FGF-2 [30]. Furthermore, both added FGF-2 and human pericardial fluid containing high levels of FGF-2 were able to induce adult cardiac myocyte hypertrophy in vitro [136]. In a mouse model of pressure-overload hypertrophy induced by aortic coarctation, mice deficient in FGF-2 exhibited a reduced hypertrophic response, resulting in compensatory hypertrophy as indicated by a slight preservation of function relative to wild-type coarcted mice [137]. Hypertrophy induced by coarctation occurs independently of the renin–angiotensin system. Subsequently, an angiotensin-dependent model of cardiac hypertrophy was produced, also in FGF-2-deficient mice, by chronic hypertension due to renal artery banding [138]. In this case, hypertensive mice deficient in FGF-2 showed no hypertrophy compared to wild-type controls. Therefore, FGF-2 appears to play a more central role in angiotensin II-dependent hypertrophy, which is not surprising given the direct activation of FGF-2 gene expression by angiotensin II demonstrated previously, at least in vascular smooth muscle cells [139].

An important and often detrimental result of scar formation and hypertrophy is arrythmias which can occur as the conduction system is compromised. Gap junction channels, composed mostly of connexin-43 (Cx43) in cardiomyocytes, are essential for the coordinated action of the heart pump. In fact, loss of cardiac Cx43 in conditional knockout models causes lethal arrhythmias, providing a direct link between gap junctions and cardiac function [134]. Factors affecting intercellular communication and connexins therefore have a direct impact on cardiac performance. Evidence from in vitro studies indicated that FGF-2 may play a role in regulating the function and/or levels of Cx43 in the heart: when added to cardiac fibroblasts, FGF-2 stimulated Cx43 expression and accumulation, and intercellular communication [140]. Cardiac fibroblasts can form gap junctions with cardiomyocytes [141]; increased FGF-2 during cardiac injury would be expected to increase the connectivity of fibroblasts, and these cells may compensate to some degree for the interruption of conduction (and resulting arrhythmias) that occur after infarction.

4. Summary and conclusions

Fig. 2 summarizes the key components of cardiovascular pathophysiology we have discussed and the contributions made by FGF-2 to each. When all the data are considered

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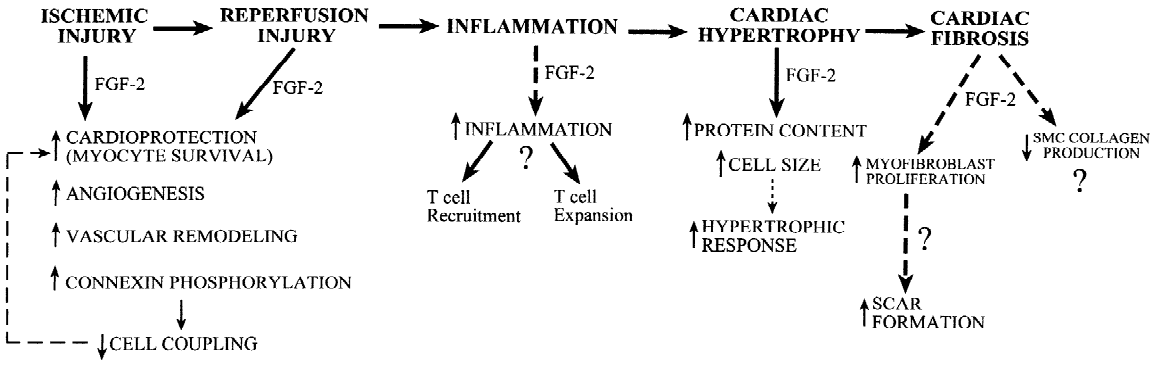

Fig. 2. Schematic of the major roles for FGF-2 in the myocardium as they relate to pathophysiological processes. In addition to its proposed role for the maintenance of a healthy myocardium as it is released into the extracellular space with normal contractile activity, the presence (or addition) of FGF-2 at various stages of injury may affect, positively or negatively, the disease process. The usefulness of FGF-2 as a therapeutic agent is dependent on precise targeting of FGF-2 activity in a dose- and time-dependent manner. Adapted from Ref. [53]. SMC, smooth muscle cell.

together, it becomes clear that the multitude of effects exerted by FGF-2 in the heart have to be weighed carefully in evaluating the therapeutic potential of such a multipotent factor. FGF-2 has many properties that make it a likely therapeutic target, positively or negatively, in addressing such problems as ischemia, restenosis or fibrosis. However, the importance of precise targeting, both spatially and temporally, cannot be overstated. FGF-2 is a highly potent cytokine which acts in many tissue types, and because of this the potential for unwanted side effects is significant. For example, FGF-2 has been implicated in many types of cancer [142–146], and recent attention has been given to the possibility that inhibition of FGF-2 signaling can quell tumor angiogenesis [147]. Thus, for example, if angiogenesis is a desirable effect for a patient with ischemic heart disease, clearly care must be taken to restrict this angiogenic effect to the myocardium. Future success of therapeutic strategies will depend on an increased understanding of the basic biological effects of FGF-2, the dissociation of its diverse activities as well as refined delivery systems and technologies that will allow spatial and temporal targeting of controlled doses.

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