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Fibroblast growth factor-2 and cardioprotection

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Abstract Boosting myocardial resistance to acute as well as chronic ischemic damage would ameliorate the detrimental effects of numerous cardiac pathologies and reduce the probability of transition to heart failure. Experimental cardiology has pointed to ischemic and pharmacological pre- as well as post-conditioning as potent acute cardioprotective manipulations. Additional exciting experimental strategies include the induction of true regenerative and/or angiogenic responses to the damaged heart, resulting in sustained structural and functional beneficial effects. Fibroblast growth factor-2 (FGF-2), an endogenous multifunctional protein with strong affinity for the extracellular matrix and basal lamina and well-documented paracrine, autocrine and intacellular modes of action, has been shown over the years to exert acute and direct pro-survival effects, irrespectively of whether it is administered before, during or after an ischemic insult to the heart. FGF-2 is also a potent angiogenic protein and a crucial agent for the proliferation, expansion, and survival of several cell types including those with stem cell properties. Human clinical trials have pointed to a good safety record for this protein. In this review, we will present a case for the low molecular weight isoform of fibroblast growth factor-2 (lo-FGF-2) as a very promising therapeutic agent to achieve powerful acute as well as sustained benefits for the heart, due to its cytoprotective and regenerative properties.

Keywords Fibroblast growth factor-2 · Cytoprotection · Cardioprotection · Regeneration

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General Introduction

Fibroblast growth factor-2 (FGF-2), previously termed basic FGF, is a prototypic and extensively studied member of a large family (FGF-1 to FGF-23) of highly conserved, structurally related heparin binding growth factors [1]. FGFs show developmental, tissue, and cell-specific regulation, and there seems to exist a need for certain members of this family during distinct early developmental stages [1, 2]. FGFs act by binding to plasma membrane tyrosine kinase receptors (FGFR1–4), albeit with different affinities, thus substantial redundancy between the various FGF ligands is to be expected. This redundancy can account for the fact that transgenic mouse models lacking FGF-1 and/or FGF-2 are viable, presenting nevertheless specific abnormalities and defects [3]. Preferential interactions between specific FGF ligands and specific FGFRs have also been noted, and are believed to contribute to cell, stage and tissue specificity as well as fine tuning of the action of FGFs [1, 2].

FGF-2 is ubiquitously expressed, and exists as 18 kDa low molecular weight (lo-FGF-2) and 20–34 kDa, high molecular weight (hi-FGF-2) isoforms. The vast majority

of studies to-date, especially in the heart, and all of the FGF-2 clinical trials, have tested the effects of lo-FGF-2, acting 'from the outside in'. Relatively little information exists about the activities of hi-FGF-2 isoforms, especially in the context of paracrine or autocrine signaling. Nevertheless, hi-FGF-2 isoforms have been found to possess certain distinct activities, as we reviewed recently [4]. Briefly, only hi-FGF-2 can stimulate cardiac and cardiomyocyte hypertrophy when acting on plasma membrane receptors; and only hi-FGF-2 can cause apoptotic cell death when acting in an intracrine fashion. Properties of hi- and lo-FGF-2, and also of specific mutant versions of lo-FGF-2, are summarized in Table 1. Figure 1 provides an overview of how subcellular location of hi- and lo-FGF-2 interrelates with their biological effects.

The low molecular weight (Lo) isoform

Function

Lo-FGF-2 is a potent mitogen for cells of mesodermal and neuroectodermal origin and a powerful angiogenic agent. It can also promote survival and prevent apoptosis in many cell types in addition to regulating differentiation and gene expression [1]. In the myocardium, FGF-2 is upregulated in response to injury and/or chronic stress at both the transcriptional and translational level [10], and this factor is implicated in most aspects and cell types associated with the injury-repair-regeneration response [11]. Cardiomyocytes express functional FGF-2 receptors at all developmental stages including the adult [12]; they respond to FGF-2 addition by activating numerous down-stream signals, leading to a number of end-points such as cytoprotection, activation of DNA synthesis, and also a negative inotropic effect [5, 13]. Lo-FGF-2 is a potent mitogen and survival agent for responsive, committed cells of various lineages, as well as bone marrow-derived, and tissue-derived (resident) human and rodent cells with stem-cell properties [14–20]. Lo-FGF-2 has been traditionally considered as an inducer of pathological myocardial hypertrophy [4]; our recent research however demonstrated that this is not true, and that only the hi-FGF-2 isoform is pro-hypertrophic [21]. Some of the effects of administered FGF-2 are summarized in Table 1.

Distribution, expression, release

Lo-FGF is present in most cells and tissues and is relatively elevated in highly vascularized organs such as the adult heart or slow skeletal muscles [22–24]. It is generally believed that lo-FGF-2 is localized in the extracellular space and the cytosol, translocating to the nucleus only during the G1-S transition of the cell cycle [25–27]. Nevertheless ectopic expression studies using tagged or untagged, human, rodent or avian lo-FGF-2 in a variety of

Table 1 Extracellular FGF-2

Properties	Lo-FGF-2	Lo-FGF-2 *(E104A)*	Lo-FGF-2 *(S117A)*	Hi-FGF-2
Binds to HSPG	xxxx	xxxx	xxxx	xxxx
Activates FGFR	xxxx	–	xxxx	xxxx
Activates PLC-PKC	xxxx	–	xxxx	xxxx
Activates all MAPKs	xxxx	–	xxxx	xxxx
Activates Akt	xxxx	–	xxxx	xxxx
Activates CK2	xxxx	–	–	xxxx
DNA synthesis	xxxx	–	–	xxxx
Chemoattraction	xxxx	?	?	-
Cytoprotection	**xxxx**	**–**	**xxxx**	**xxxx**
Pre-conditioning-like action	**xxxx**	**–**	**xxxx**	**xxxx**
Post-conditioning-like action	**xxxx**	**–**	**xxxx**	**xxxx**
Angiogenesis	**xxxx**	**–**	**–**	**–**
Hypertrophy	**–**	**–**	**–**	**xxxx**
Sustained benefits	**xxxx**	**–**	**–**	**–**

This table summarizes acute and long-term effects and end-points induced by extracellular (administered) lo- and hi-FGF-2 isoforms, as well as those of specific lo-FGF-2 mutants, (E104A; S117A). It is based on our published [5–9] and unpublished work. We have used specific lo-FGF-2 mutations to interfere with specific properties of the molecule: the E104A lo-FGF-2 mutant has diminished affinity for the FGFR, while the S117A lo-FGF-2 mutant is incapable of activating one branch of the FGF-2-triggered pathway (requiring nuclear CK2), but can still activate other pathways. These mutant lo-FGF-2 versions have shown that the ability to activate FGFR is essential for all effects of FGF-2; in addition, the ability to bind/activate nuclear CK2 is required for the induction of a mitogenic response, but it is not required for acute cardioprotection. Chemo-attractant ability is required for induction of angiogenesis. Only the hi-FGF-2 isoform induces cardiac and cardiomyocyte hypertrophy, and only the lo-FGF-2 isoform possesses the ability to exert both acute as well as sustained beneficial effects

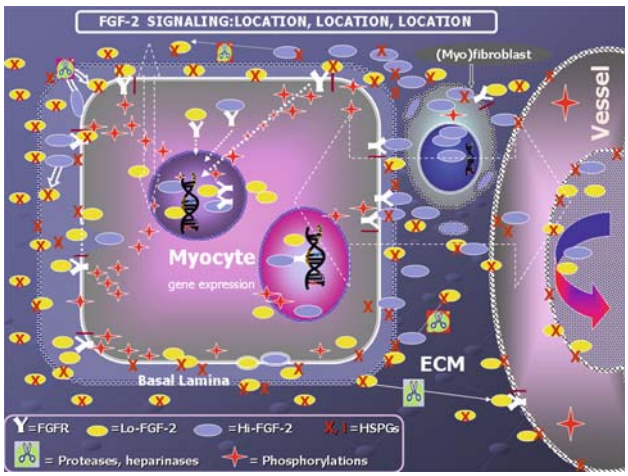

Fig. 1 The various sites of endogenous FGF-2 location and action in the myocardium. Cardiac myocytes express both hi- and lo-FGF-2, localizing to nuclear, cytosolic and extracellular sites. Cardiac fibroblasts accumulate and release predominantly the hi-FGF-2 isoforms [46]. Released FGF-2 is bound by HSPGs at the plasma membrane, basal lamina and extracellular matrix; matrix and basal lamina-bound FGF-2 can be 'liberated' by heparinases and can then act on cell surface FGFR. Extracellular, HSPG-bound hi-FGF-2 can be converted to lo-FGF-2 through limited proteolysis by proteases that are also associated with HSPGs [41]. Released FGF-2 can find its way into the circulation. *Extracellular FGF-2* (either type of isoform) affects cells by binding to plasma membrane FGFR and thus activating a multitude of downstream cascades inducing phosphorylation of many targets; *at the same time* it also becomes internalized, translocates into the nucleus and influences gene expression. The pattern of FGF-2-induced gene expression is isoform-specific [47]. Endogenous FGF-2 may also find its way into the nucleus without exiting the cell, and thus exert isoform-specific *intracrine* effects: intracrine action of hi-FGF-2 in myocytes includes induction of apoptosis [48]

cell types from a variety of species have consistently detected lo-FGF-2 in cell nuclei, in a manner not necessarily relating to cell cycle stimulation [22, 28–31].

FGF-2 gene expression is upregulated by many stress stimuli, including hypoxia and ischemia [32], and also in response to angiotensin-II [33] and adrenergic stimulation [34]. In addition, there is some evidence to suggest that FGF-2 stimulates its own gene expression in a positive feed-back loop [35, 36]. Further to transcriptional regulation, FGF-2 accumulation is regulated at the translational level: cells need to be directed to employ either a cap-dependent mechanism of translation, or the less conventional internal ribosome entry site (IRES) mode; and they also need to decide whether to initiate translation from AUG or CUG start sites [27]. These decisions will determine which FGF-2 isoforms will accumulate within the cell. It is theorized that factors/complexes binding to the 5′ untranslated, as well as the usually extremely long 3′ untranslated regions of the FGF-2 mRNA are ultimately responsible for determining relative levels of translation of the various FGF-2 isoforms. In human cells, standard cap-dependent translation produces the 34 kDa (hi; CUG initiated) FGF-2, while IRES-dependent translation produces lo-FGF-2 (AUG-initiated) as well as 21–25 kDa (hi; CUG initiated) isoforms [37–39]. A potential third level of regulation, converting hi-FGF-2 to lo-FGF-2, by limited degradation of the N-terminal extension, has also been considered but has not been addressed in any detail to-date [40, 41].

The FGF-2 protein lacks a classic export signal sequence but is nevertheless released from viable cells in an ongoing manner; it is also released by dying cells during tissue injury. Thus, FGF-2 is found in association with heparin sulphate proteoglycans (HSPGs) at the basal lamina and the extracellular matrix; it can also be detected in the serum and other body fluids [42]. In fact a number of acute or chronic pathologies are associated with increased FGF-2 levels in the blood or urine [43–45]. It is important to note, however, that because these studies rely almost exclusively on ELISA-based FGF-2 detection with antibodies that are not isoform-specific, it is not known if they actually report on lo-FGF-2, hi-FGF-2, or both types of isoforms [4]. Figure 1 illustrates the various cellular, extracellular and subcellular sites of localization and action of FGF-2.

The mechanism of FGF-2 release from viable cells is not well understood. It is ATP-dependent and does not involve the endoplasmic reticulum/Golgi transportation pathway; pharmacological inhibitors of the Na^+/K^+ ATPase pump can prevent FGF-2 export from a number of cell lines, implicating the Na^+/K^+ ATPase in this process [49, 50]. FGF-2 can also be released through plasma membrane vesicle shedding [51], and non-lethal transient membrane disruptions [52]. Contractile cells such as cardiomyocytes release FGF-2 on a beat-to-beat basis, under normal conditions. Cardiac fibroblasts, a rich source of released FGF-2 and a major cardiac population existing in close association with myocytes, would be stretched passively by myocyte contraction, and thus also release FGF-2 to the extracellular matrix. This is quite significant since (myo)fibroblasts accumulate and release predominantly the pro-hypertrophic hi-FGF-2 isoforms [46]. Increased contractility, and increased endogenous FGF-2 expression result in increased levels of heart-released FGF-2 [53, 54], increasing the opportunity for exerting effects in an autocrine–paracrine manner. As summarized in Fig. 1, extracellular FGF-2 becomes free to interact with cell surface receptors subsequent to the action of heparinase(s) and/or plasminogen activator-mediated proteolysis, events that are upregulated during injury, repair, and pathological situations associated with cell invasion [55, 56].

Signal transduction

The biological effects of extracellular FGF-2 are mediated by binding to high-affinity FGF receptors (FGFR1-4) of the

tyrosine kinase family [57]. FGFR1 is the best-studied FGFR in embryonic, neonatal, and adult cardiomyocytes [12, 58]. Heparan sulphate proteoglycans (HSPGs), which function partly to sequester FGF-2 in the extracellular matrix until signaling is triggered, are also present at the plasma membrane and act as lower affinity 'receptors' to facilitate interaction of the ligand with FGFR [57, 59]; see also Fig. 1. Plasma membrane HSPGs may in fact carry their own signaling capacity in response to FGF-2 binding [60]. Activated FGFRs recruit and phosphorylate other signaling molecules culminating in the activation of major signal transduction pathways such as all three branches of the mitogen activated protein kinase (MAPK pathway), the phospholipase C-protein kinase C (PKC)- and Src-associated pathways [10].

An additional, *concurrent* pathway for extracellular FGF-2-triggered signalling is initiated by binding and activating FGFR1 at the plasma membrane, followed by FGF-2-FGFR1 internalization and nuclear translocation. Activated nuclear FGFR1 may then activate a number of genes directly [61, 62]. Thus the end-effects of extracellular FGF-2 are brought about by signals activated downstream of the plasma membrane FGFR *as well as* by those triggered by internalized FGF-2 and FGF-2/FGFR complexes that translocate to the nucleus and affect gene expression directly [4].

Internalization followed by nuclear translocation is reported to be essential for the mitogenic response to lo-FGF-2. It occurs during the G1 stage of the cell cycle and is mediated by lo-FGF-2 binding to translokin, an ubiquitous cytosolic protein [25]. Activation of casein kinase 2 (CK2) through the interaction of its beta-subunit with lo-FGF-2 in the nucleus is also essential for the induction of the mitogenic response [25, 63, 64].

Pre-conditioning-like cardioprotection

Exposure to short periods of ischemia protects the heart from a subsequent severe insult, a phenomenon termed *ischemic preconditioning* [65]. Protection manifests as preservation of tissue viability and overall function, and can be observed in two stages: an early phase developing within minutes of the brief ischemic episode(s) and lasting several hours, and a late phase, the ''second window'', emerging 12–24 h later and lasting several days [66]. Early preconditioning does not require novel gene expression but is dependent on the activation of particular signal transduction cascades, eliciting post-translational modifications (phosphorylation) and changing the behavior of a multitude of target proteins [66]. These cascades eventually promote changes in gene expression resulting in upregulation and/or downregulation of molecules mediating the second window of protection. Considerable research effort has been applied to the identification of triggers and mediators of cardioprotection with the intent to realize therapeutic potential through activation of the pre-conditioning response (pharmacological pre-conditioning) [67–70].

Several models have been used by us and other laboratories to examine the ability of exogenously administered lo-FGF-2 to confer protection from cardiac injury (reviewed in more detail in [10, 11]). Addition of FGF-2 protected neonatal cultured cardiomyocytes from H_2O_2-induced cell injury and death [71]. In the isolated adult rat or mouse heart model, lo-FGF-2 given by retrograde perfusion, entered the myocardium and distributed in a basement-membrane fashion, increasing the overall heart-associated FGF-2 levels nearly 4-fold [6]; it also protected the heart from subsequent ischemia-reperfusion induced contractile dysfunction and myocardial tissue loss, and preserved cardiac energy metabolites [6]. Perfusion-administered lo-FGF-2 prevented the ischemia-induced dephosphorylation of the gap junction protein connexin-43 suggesting an ability to preserve pre-ischemic patterns of intercellular communication and rhythm [72], in agreement with reports addressing this issue directly [73]. We have also shown that chronic overexpression of lo-FGF-2 in transgenic mouse myocardium resulted in increased resistance to myocardial ischemic injury, and increased levels of peri-cellular FGF-2 [53]. Our data have since been confirmed by other investigators [74].

In terms of mechanism we found that acute cardioprotection by extracellular lo-FGF-2 required interaction with and activation of FGFR1, and activation of the PLC-PKC signal cascade [5, 7]. PKC activation, in particular the ε isoform, is a central mediator of the injury-resistant phenotype, modifying targets at the plasma membrane, mitochondria, and other subcellular sites [75–80]. Chronic overexpression of lo-FGF-2 in transgenic mouse hearts also resulted in increased levels of membrane associated α-PKC and total ε-PKC [53]; upregulation of these signals would be expected to elicit protection.

Activation of extracellular signal regulated kinase (ERK) has been reported to mediate a pro-survival phenotype [81] and thus could be expected to contribute to lo-FGF-2-induced protection. In agreement, cardioprotection induced by overexpressed FGF-2 in a transgenic mouse model was reported to be dependent on the ERK activating pathway [82]. We have found that, by inhibiting PKC, it was possible to completely block FGF-2-induced cardioprotection without affecting activation of ERK. Our data suggested that ERK activation may occur upstream of the PKC pathway and/or that ERK activation is not sufficient, in and of itself, to substitute for lo-FGF-2-induced resistance to injury. In addition to activation [5] lo-FGF-2 increased the interaction of ε-PKC with the gap junction protein connexin-43 (Cx43) at the intercalated discs [83],

and caused hyper-phosphorylation of Cx43 at PKC target amino-acid sites including serine 262 in vitro [84] and in the adult perfused heart [85]. In view of recent reports that Cx43 is required for the development of a pre-conditioning response our data imply that the lo-FGF-2-induced Cx43 phosphorylation is an important component of an injury-resistant phenotype. Additional signals that upregulate and are upregulated by lo-FGF-2 and that are involved in pre-conditioning type cardioprotection include nitric oxide and the nitric oxide synthases as reviewed in more detail previously [4, 10].

The potent protective actions of administered or over-expressed lo-FGF-2 have indicated that its endogenous counterpart may play a similar role. FGF-2, released on a beat-to-beat basis is likely contributing to the maintenance of a healthy myocardium, and would be expected to confer increased capacity to resist injury and function under stress. A component of its protective action likely includes effects on contractility and thus energy consumption and requirement: positive inotropism induced by a variety of agents, catecholamine stimulation for example, results in increased release of FGF-2 [34] which, by exerting a negative inotropic effect [5, 13], would be expected to normalize force of contraction, and thus bring FGF-2 release back to baseline levels. Studies using FGF-2-deficient mice, lacking both hi- and lo-FGF-2 isoforms, have offered strong support to the notion that endogenous FGF-2 is mediating resistance to injury: House and colleagues showed that, compared to wild type, FGF-2-deficient hearts are more vulnerable to ischemic injury, while overexpression of human FGF-2 in the FGF-2-defficient mouse conferred significant protection [74].

Secondary injury prevention

Pre-conditioning-type protection, be it ischemic or pharmacological, is impractical as a general approach since it requires to predict when the need for protection will arise [86]. This issue is no longer a concern in situations where ischemic injury is developing or on-going, and also in the context of ischemia-reperfusion induced pathologies [87]. Acute myocardial infarction (MI), resulting from an interruption of blood flow to the heart, is a major cause of mortality and morbidity worldwide; its management requires re-establishment of blood flow by surgical and/or pharmacologically means [88]. Reperfusion of the ischemic myocardium carries added risks since it is associated with exacerbation of cell injury and death in the acute stage [87]. Interventions and factors that can reduce damage during development of MI and during reperfusion have thus attracted much attention over several years [67, 89–91].

Using a rat model we demonstrated that lo-FGF-2, administered by intra-cardiac injection to the ischemic left ventricle during the development of MI caused by permanent coronary ligation remained near the injection sites for several hours in association with both viable and necrotic tissue, and exerted significant protection from acute (4–24 h from occlusion) muscle loss and contractile dysfunction [7]. Using a variety of animal models other laboratories have reported similar findings, as reviewed in detail previously [92]. It is evident that acute FGF-2 protection reflects a direct effect on myocardial tissue and is not dependent on angiogenesis [93, 94]. In fact, a mutant, non-angiogenic and non-mitogenic lo-FGF-2 (S117A-FGF-2) remained capable of eliciting acute cardioprotection [8]; see also Table 1.

It is noteworthy that exogenous lo-FGF-2, whether injected directly into the tissue, or administered by perfusion, shows similar distribution to the basal lamina and extracellular matrix as the endogenously expressed molecule [6, 7, 53, 95]. This is likely due to its very high affinity for HSPGs present at the cell surface, basal lamina and matrix; illustrated in Fig. 1. Locations enriched in HSPGs would thus be expected to absorb FGF-2 from both intracellular and extracellular origins. Muscle-linked repair-regeneration studies conducted over many years have shown that basement membranes may remain relatively intact after cellular demise, thus serving as a scaffold and a blueprint informing tissue rebuilding [96–98]. Basal laminae can provide local ‘cues’ and spatial guidance for cells homing in from the circulation and/or neighboring tissue. Strong retention of the FGF-2 protein by basement membranes and extracellular matrix would be expected to serve as a natural reservoir for prolonged stimulation of reparative processes [96]. Binding to HSPGs not only sequesters FGF-2 to sites of actual and ‘pending’ action, it can also protect it from degradation. This is not quite the case for hi-FGF-2: binding to heparin protects its core 18 kDa sequence from degradation by proteases that are also heparin-bound, while allowing limited proteolysis of its N-terminal extension. This process effectively converts hi-FGF-2 to lo-FGF-2, can be regulated by second messengers such as calcium [99], and may reduce hi-FGF-2 detectable at the extracellular matrix.

To examine the potential of FGF-2 for specifically protecting during the reperfusion phase, we subjected ex vivo perfused hearts to 30 min of global ischemia, followed by 10 min of reperfusion in the presence or absence of lo-FGF-2 and 50–110 min of reperfusion with standard perfusion medium. Administration of lo-FGF-2 during reperfusion resulted in significantly improved contractile recovery and reduced apoptotic cell death [7, 8]. It also resulted in re-establishment of pre-ischemic levels of phosphorylated Cx43, even though this protein becomes dephosphorylated after 30 min of global ischemia [100, 101]; without added FGF-2, reperfusion achieved low level

of recovery in phosphorylated Cx43 [72]. FGF-2 given during reperfusion re-established localization of Cx43 mostly to intercalated disks [72], supporting the notion that it can prevent reperfusion-linked conduction abnormalities and arrhythmias. Exogenously administered hi-FGF-2, or non-mitogenic lo-FGF-2, were equally effective as lo-FGF-2 as survival and protection agents during reperfusion, again demonstrating a dissociation between the mitotic and cytoprotective pathways induced by FGF-2 [8].

Lo-FGF-2 administered during reperfusion stimulated a number of intracellular signals (PKC; ERK; Akt) which are expected to mediate its beneficial effects ([74, 94] and unpublished observations). Apoptotic cell death is a feature of reperfusion-induced injury since stimulation of signaling pathways capable of preventing apoptosis elicits cardioprotection during reperfusion [90, 102]. Such protective signaling pathways include PKC-dependent cascades [7, 103]; and the ERK as well as the phosphatidylinositol-3-OH kinase (PI3K)—Akt axis [89].

We propose that the ability of FGF-2 to exert cytoprotection when administered during reperfusion, in combination with its particular 'spatial-retention' properties (Table 1, Fig. 1), represents a very attractive features for any reperfusion regime in the clinic. A relatively low dose of the FGF-2 protein (we would hypothesize this to be at about 10–100 μg/human heart), could be introduced *once* during, for example, balloon angioplasty, and would be expected to prevent ischemic and reperfusion-induced myocardial damage. Selecting the non-mitogenic (S117A) FGF-2 mutant (see Table 1) would be equally effective as a cytoprotective agent with the added bonus of being devoid of any possible side effects linked to mitogenic stimulation, such a stimulating vascular proliferative events (restenosis), or when treating cancer patients. FGF-2, a ubiquitous protein acknowledged by our immune system as 'one of our own', would not be expected to elicit adverse immune responses, especially when used as a brief treatment. Finally, the non-mitogenic lo-FGF-2 mutant could also be considered for systemic and/or prolonged usage, when a generalized cytoprotection is required, for example as a supplement to cardioplegic solutions in surgery and organ transplantation; in muscle degenerative diseases; and in various types of heart failure.

Long-term benefits

In addition to its ability to elicit acute, direct cardioprotection, lo-FGF-2 is also capable of exerting sustained benefits to the heart. Once-only intramyocardial delivery of the lo-FGF-2 protein into the ischemic, akinetic left ventricle during the development of MI exerted substantial beneficial effects detectable 8–16 weeks post-MI: these included smaller scars and improved contractile function compared to a vehicle-treated, infarcted group ([21, 74] and unpublished data); see also Table 1. These long-term effects were dependent on the ability of lo-FGF-2 to increase infarct and peri-infarct vascularity by promoting angiogenesis [8, 21], an activity requiring that FGF-2 possesses *both* mitotic and chemo-attractant properties. Thus the non-mitotic and non-angiogenic S117A-lo-FGF-2 mutant, used in an identical fashion, was incapable of conferring sustained benefits beyond 1 week post-administration [8], while the hi-FGF-2 isoform, which cannot stimulate cell migration but is still capable of stimulating mitosis did not promote neovascularization and its beneficial effects were not sustainable in the long-term [21]. The magnitude of sustained beneficial effects exerted by a once-only administration of the lo-FGF-2 protein (at 2 μg/rat heart; in 50 μl of saline) is broadly similar to that reported in several experimental models dependent on: stem-cell transplantation and/or recruitment [104] and/or the strong paracrine component of stem-cell based therapy [105, 106]. Thus it is worth examining the notion that lo-FGF-2 can induce a truly regenerative response (see section below). If proven to be true, administration of the lo-FGF-2 protein could represent an effective, quick as well as inexpensive strategy to promote better heart repair. This approach would not require any cell isolations, purification, expansion or transfection, gene therapy, transplantation, or immunosuppression, although it could complement all of these procedures. We propose that the delivery of a few microgram of lo-FGF-2 protein into the infarcted myocardium would simply boost and guide what is already there.

Potential to induce regeneration

Lo-FGF-2 possesses properties considered desirable in the context of stimulating and sustaining the repair/regeneration process [15, 18, 20, 107–111]. Thus it can promote cell migration, homing, proliferation, and survival; it also allows differentiation into cardiogenic and other lineages; prevents senescence; its strong binding to HSPGs retains it within the area of administration (or injury, for the endogenous molecule) and protects it from degradation, while allowing slow diffusion to adjacent areas; it stimulates its own expression, effectively prolonging its influence. Importantly the safety of lo-FGF-2 for clinical use has been studied in several clinical trials (reviewed by us previously [10, 11, 92]) that pointed to lack of side effects such as hypotension, hypertrophy, tumorigenesis when this protein was administered by intracoronary bolus injection and used within an effective concentration range. We would like to also bring particular attention to reports showing that lo-FGF-2 can upregulate expression of stem cell factor (SCF), the ligand for the c-kit plasma membrane receptor in mesenchymal stem cells and a potent homing signal for these cells to areas of injury [16, 112, 113].

While the angiogenic properties of lo-FGF-2 have been documented extensively, and there is little doubt that its long-term beneficial effects include an angiogenic response (please see detailed review in [10, 11, 92]) it is somewhat surprising how relatively little attention has been paid to lo-FGF-2 by the scientific and clinical communities in regards to its potential to act as a safe, relatively easy to administer, and relatively inexpensive agent of a true regenerative response. We have already shown that a once only administration of lo-FGF-2 to a developing infarct elicited sustained benefits including smaller scars and improved function [7, 8, 21]. Using the same rat model and experimental design, our recent pilot studies (n = 4/group), indicated that infarcts injected with lo-FGF-2 also contained a statistically significant, 3-fold increase in the fraction of presumptive stem cells (positive for the c-kit antigen), as well as cells undergoing cell division (cells positive for phosphorylated histone H3), compared to saline-injected infarcts, at 3 days post-MI (unpublished observations). We also detected ample presence of the 'homing signal', SCF, in the lo-FGF-2-injected infarcts (Fig. 2). Thus we suggest that the pronounced and prolonged benefits elicited by lo-FGF-2 are very likely to include a regenerative component.

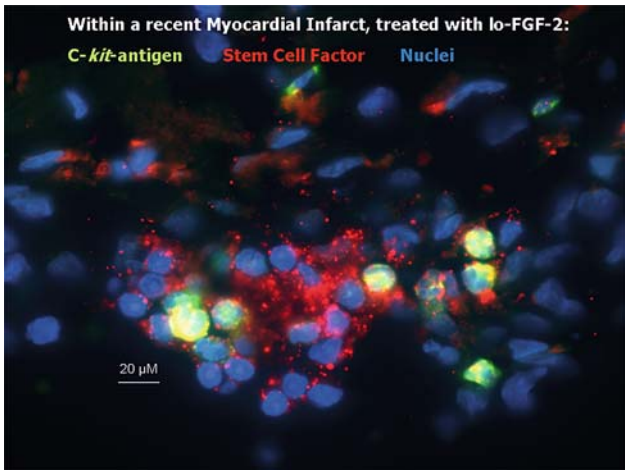

Fig. 2 Localization of presumptive mesenchymal stem cells and stem-cell factor (SCF) in FGF-2-treated myocardial infarcts. Myocardial infarction was induced by permanent coronary occlusion in a rat model; lo-FGF-2 was injected directly into the ischemic left ventricle within 15 min from occlusion, exactly as we described previously [7, 8]. Animals were euthanized at 3 days post MI, and hearts were processed for cryosectioning and immunofluorescence. Sections were stained for c-kit (green; fluorescein-labeled anti c-kit antibodies), SCF (red; Texas-red-labeled anti-SCF primary antibodies) and nuclei (blue; Hoechst stain). Staining with the secondary fluorescent antibodies, without pre-incubation with primary antibodies, displayed only very low background staining, indicating that the patterns observed with the primary antibodies were specific. Clusters of round c-kit-positive cells can be observed within the infarct, in association with pools of SCF at the pericellular space

Concluding remarks

In the preceding sections, we have built a case for lo-FGF-2 as a very promising therapeutic agent for both acute (pre- and post-conditioning) as well as long-term interventions (tissue repair-regeneration). What makes lo-FGF-2 stand out, in our opinion, amongst several mitogenic and angiogenic factors and cytokines that have been reported as having a variety of beneficial effects, is: (A): its very strong affinity for heparin and HSPGs that allows it to be retained where it is needed, even if given during reperfusion and subjected to continuous 'wash-out' [6] and (B): its proven ability not to cause detrimental effects in the long run (see also Table 1). This point was brought home by our recent studies on the *long-term* effect of a once-only administration of hi-FGF-2: our initial studies had suggested that this isoform was as beneficial as lo-FGF-2, because it exerted acutely cytoprotective effects, reduced infarct size, and elicited overall functional improvement for up to 6 weeks post-MI. More long-term studies told another story: unlike lo-FGF-2, hi-FGF-2-treated groups developed significant cardiac and cardiomyocyte hypertrophy, and any functional improvement was lost after the 6th week post-MI. The effects of hi-FGF-2 (but not lo-FGF-2) were accompanied by significant upregulation of cardiotrophin-1, a cytokine that, while cytoprotective as an acute agent, is also linked to the development of pathological hypertrophy and heart failure [114, 115]. Other members of the FGF family (FGF-1, FGF-5) have been investigated in the context of inducing beneficial effects to the heart [116–118], highlighting the potential for these factors to be used therapeutically.

In considering lo-FGF-2 for therapeutic usage, a number of specific issues should be resolved. Most importantly, the optimal strategy for administering this protein should be established in preclinical models for *specific* diseases. Dosage, timing, and the particular lo-FGF-2 mutant to be used in relation to a particular pathology should also be investigated. Study of potential side effects, acute as well as chronic and even when the protein is administered for only a brief period, should not be neglected: a good reminder for remaining vigilant is provided by the hi-FGF-2 example mentioned in the preceding paragraph. Finally, the potential of lo-FGF-2 and/or its mutant versions to be of benefit in early and even late stages of heart failure, caused by a variety of conditions, demands attention.

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