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Re-modelling 'hostile' milieu of diseased myocardium *via* paracrine function of transplanted cells or relaxin

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Abstract

While the approaches of regenerating cardiac muscle remain undetermined, recent evidence indicates that paracrine function of transplanted cells contributes significantly to the beneficial effects of cell therapy. Combination of such paracrine function of grafted cells with extracellular matrix remodelling by relaxin represents a promising complement to cell-based therapy for cardiac repair and muscle regeneration.

Key words: cell therapy • myocardial infarction • fibrosis • extracellular matrix • angiogenesis • relaxin • myoblasts

The earlier hypothesis of cell-based therapy for heart disease has now become the mainstream of experimental and clinic concept albeit underlying mechanisms have been hinted but never completely elucidated. Numerous experimental studies have shown significant improvement of cardiac function together with inhibited cardiac remodelling. However, the efficacy achieved by cell therapy in many phase-1 clinical trials is modest and whether sustaining clinical benefits are present in treated patients is unclear. Many challenges remain in attempts to heal a diseased heart by muscle regeneration. Major problems that have been generally admitted include a very low viability of implanted stem cells regardless of cell type, insufficient nourishment, host inflammatory and fibrotic responses, poor functional coupling with viable host cardiomyocytes, and incomplete differentiation. All these might be underlying reasons for the limited efficacy of cell therapy achieved so far.

Thus, to improve the fate of stem cells and hence the outcome of cell therapy, it is essential to promote migration, survival, proliferation, differentiation and functional coupling of grafted stem cells or endogenous cardiac progenitor cells, which is critically dependent on interactions of local environment and stem cells. In fact, recent studies emphases paracrine action, rather than muscle regeneration, as the primary mechanism underlying the therapeutic effect of mensenchymal stem cells [1]. Nevertheless, the mechanisms by which the local milieu influences fate of stem cell are as yet undetermined.

Following myocardial infarction (MI), expression of inflammatory genes increased markedly whilst expression of factors critical for angiogenesis suppressed [2], changes unfavour cell therapy. The myocardial milieu in settings of chronic infarction and various types of cardiomyopathy is even more 'hostile' to implanted and endogenous stem cells. Thus, it would be helpful to re-model and improve the milieu prior to and after cell implantation. Along this direction, increasing studies have focused on approaches aimed at improving local environment at the time of cell therapy with encouraging findings. These include promoting expression of pro-angiogenic factors [3], increasing expression of cytokines or anti-apoptotic factors critical to stem cell homing and survival [1, 4, 5], and re-modelling extracellular matrix (ECM) [5]. In the infarcted mouse heart, therapeutic effects

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of treatment with granulocyte colony-stimulating factor (G-CSF) is largely attributable to increased density of blood vessels and non-bone marrow-derived cardiomyocytes [6]. Regional matrix metalloproteinase (MMP) activity also plays a key role in cell homing and migration [7]. Statins protect mesenchymal stem cells against apoptosis *in vitro* [8] and this action is worth to be tested *in vivo*. However, there has been lack of interventions that can remodel fibrotic tissues.

In this issue of *J Cell Mol Med,* Fromigli *et al.* [9] presented an interesting study on cell therapy of chronic MI using mouse skeletal C2C12 myoblasts. Their findings convincingly show that grafted myoblasts exhibit important paracrine function through which the chronically infarcted scar is remodelled. A further important finding is that after implantation of cells expressing relaxin gene *via* a lentiviral vector, survival of myoblasts was markedly improved together with better therapeutic effects in regard to microvessel density, MMP levels, fibrotic scar re-modelling and ventricular function. Several merits of this study are worth comments. *First*, a model of chronic MI was studied allowing the authors to explore the capability of remodelling the mature scar tissue. While majority of studies have been focused on cell therapy of acutely infarcted heart, chronic MI and cardiomyopathy represent conditions that are suitable for cell therapy. Indeed, recent studies indicate that delayed rather than immediate cell therapy following MI achieve better efficacy [10]. *Second*, C2C12 myoblasts with viral transfection of a control (GFP) or relaxin gene were examined. Since relaxin is a peptide hormone with well documented actions on ECM re-modelling [11], comparison of treatment with cells without and with relaxin gene transfection would indicate importance of ECM remodelling in the overall efficacy observed. *Third*, the authors used porcine MI model that better simulates clinical situation and allows for the use of a catheter-based cell delivery *via* coronary veins into the infarcted region, which is routinely doable in the clinic. *Finally*, the primary end-points include comprehensive morphological measures of collagen structure, MMPs and angiogenesis, as well as global functional assessment by echocardiography that were performed one month after cell therapy. Expression of relaxin by transfected cells was confirmed *in vitro* and *in vivo*.

The finding of a better efficacy by myoblasts pre-infected with relaxin gene is extremely interesting. As a peptide hormone known to induce ECM re-modelling in the reproductive tissues for many decades, recent findings strongly indicate relaxin as a promising agent for heart disease therapy [11]. Well-documented cardiac protective actions of relaxin include anti-fibrosis [12, 13], anti-oxidative stress [14], anti-apoptosis [14, 15] and vasodilation *via* nitric oxide synthase (NOS)/nitric oxide signalling [16]. In cultured cardiomyocytes, relaxin protects cells against oxidative stress-induced apoptosis by activating pro-survival molecules like phosphoinositide 3-kinase (PI3K)/Akt, ERK and Bcl-2 [15]. These actions might underlie relaxin's potent protection against myocardial injury *in vivo*, seen in a porcine model of acute MI [14]. Pro-angiogenic action of relaxin has only been reported in skin wound, cancer or reproductive tissues [11, 17, 18]. The anti-fibrotic effect of relaxin is attributable to its multiple actions by which it inhibits activation and proliferation of fibroblasts and promotes collagen degradation by up-regulating expression of MMPs together with down-regulation of certain subtypes of tissue inhibitors of metalloproteinases (TIMPs) [11]. In settings of heart disease and cell therapy, combination of these actions would certainly be desirable (Fig. 1). Interestingly, all these known actions of relaxin have been observed in the porcine infarcted cardiac tissue [9]. It is known that regional formation of a number of cytokines, chemokines, growth factors and MMPs are critical for stem cell homing, migration, myocyte-lineage formation and proliferation [19]. In addition, studies in non-cardiac tissues have revealed that relaxin is able to synergistically activate the insulin-like growth factor (IGF1) signalling [21]. While the importance of IGF1/phosphatidylinositol 3-kinase (PI3K) pathway in the outcome of myocardial regeneration has been demonstrated [6], the possibility that relaxin interacts with IGF1/PI3K signalling in cardiac cell therapy warrants further investigation.

The mature scar tissue that is absence of blood vessels and cellular components disadvantages stem cell homing, survival and proliferation. In this regard, re-modelling the milieu, specifically removal of scar tissue and promoting angiogenesis are of utmost importance. It is interesting to see that in the present study [9], implantation of myoblasts, especially those transfected with relaxin gene, significantly altered mature scar tissue in such a way that the structure of fibril collage was loosened and microvessel density increased. Survival rate of

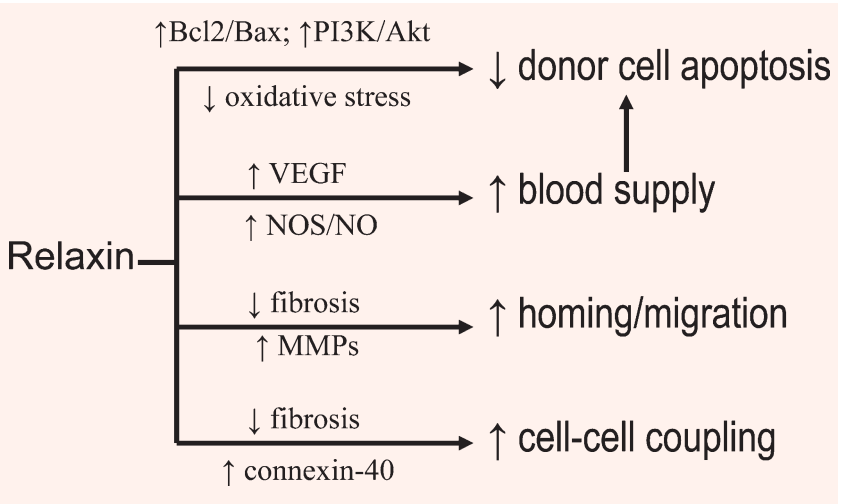

Fig. 1 Recent studies have revealed multiple cardiac actions of the peptide hormone relaxin, including anti-apoptosis, pro-angiogenesis and anti-fibrosis. These actions are expected to be invaluable in overcoming major problems in the setting of cell-based therapy of heart disease. PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor; NOS, nitric oxide synthase; MMPs, matrix metalloproteinases.

grafted myoblasts was better in relaxin-expressing than control cells.

Inhibited ventricular remodelling together with improved global function was detected by Formigli *et al.* [9]. However, the underlying mechanism for such functional benefit remains to be illustrated. Myoblasts are poor in plasticity making phenotypic conversion into cardiomyocytes unlikely. Thus, the beneficial actions seen in this study with grafted myoblasts are not attributable to muscle regeneration. A possibility that is worth testing is that improved local environment by the myoblasts/relaxin therapy favourites homing, migration and differentiation of either bone-marrow-derived or cardiac progenitor stem cells that are 'myogenic'. Indeed, presence of endogenous cardiac stem cells in the infarcted porcine heart has recently been documented [22]. Furthermore, cell coupling might also be improved in the presence of relaxin indicated by an earlier report from the same group that relaxin improves coupling of C2C12 myoblasts with neonate cardiomyocytes in co-culture by upregulating connexin-40 [23] (Fig. 1). Thus, the findings from this study points to the option of giving complementary treatment with relaxin or relaxin gene either to "pre-conditioning" the diseased myocardium for subsequent cell implantation or in conjunction with cell delivery. In this regard, the optimal timing of pre-treatment followed by cell implantation will need to be carefully determined to maximize the efficacy.

Collectively, these intriguing findings by Formigli *et al.* [9] suggest that the paracrine function of transplanted cells contributes significantly to the therapeutic effects. By showing potent actions of relaxin-expressing myoblasts on ECM remodelling, pro-survival and pro-angiogenesis in the chronically infarcted heart [11], we now have good reason to include relaxin as a promising therapeutic agent complementary to cell-based therapy of heart disease. Appreciation of significant paracrine function of engrafted cells, as shown by Formigli *et al.* [9], might be critical in the interpretation of therapeutic efficacy, considering the fact that almost every cell type tested so far appears equipotent, indicative of some common mechanisms irrespective of cell types since majority of them are not 'myogenic'.

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