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Gene Therapy of the Heart through Targeting Non-Cardiac Cells

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1. Introduction

To combat ischemic heart disease in the clinical scenarios of open heart surgery, unstable coronary syndromes, percutaneous coronary interventions, or thrombolysis, different research approaches are used to improve clinical treatments. The most dreaded long term consequence of ischemic heart disease – heart failure – is another clinical diagnosis where the treatment we have to offer is less than optimal. Some researchers are attempting to omit the reason for cardiovascular disease through targeting the process of atherosclerosis. Others adress the pathophysiology of restenosis, which may occurr after balloon dilatation of atherosclerotic lesions. Yet others adress improved treatment of the myocardium which has undergone an infarction, where the building of new blood vessels, strengthening of the contractile apparatus, and recruitment of new cells to areas of necrosis may be therapeutical end-points. Arrthythmias may occur due to reperfusion injury, after long-term morphological changes in the heart, or due to endogenous causes related to changes of the conduction system; new therapies are required for improved treatment. Novel treatments for dysfunctional, calsified heart valves are subject to other lines of investigations. Gene therapy and cell therapy using genetic engineering of stem cells will be the focus of this chapter, in particular the current status of treatments directed towards the myocardium itself in ischemic heart disease will be discussed. Gene therapy and to a lesser extend cell therapy have been used both clinically and experimentally to combat acute ischemia, remodeling and heart failure. However, the protected location of the heart of the heart inside the thoracic cavity, the nature of cardiac cells with minimal ability of entering cell cycle, and the electrophysiological properties of the heart render this organ with some particular challenges for gene therapy.

2. Gene therapy for myocardial protection

Delivery of DNA to hearts as well as other organs has been performed in animal experiments, and clinical studies in "no-option" patients have been conducted. Many clinical trials with gene therapy in cardiovascular patients have recently been reviewed (Lavu at al., 2010, Lyon et al., 2011). A general challenge with gene delivery to the heart is low transfection efficacy (the cardiomyocyte does not enter cell cycle), cell injury/inflammation, and unwanted sideeffects. There are several options on routes of DNA

delivery to the heart. One alternative is intravascular delivery, which can be directed through coronary arteries or retrogradely into the coronary sinus. An arterial approach which requires open coronary arteries may not be suitable for patients with coronary artery disease if the target is treating cardiomyocytes rather than vascular cells. Pericardial gene delivery has been attempted, but there are rather few publications with that particular route of delivery. Another option is direct intracardiac delivery, which has been tried clinically and experimentally (Isner, 2002, Semenza, 2004, Vinge et al., 2008). In general it is difficult to achieve a lasting transfection through this invasive approach, which may be delivery of naked DNA or DNA ligated to a vector. Viral vectors used for cardiovascular therapy are most commonly adenovirus, adenoasscociated virus, and to a lesser extent lentivirus. A third possibility is systemic delivery with "something" that directs the DNA/RNA to a specific cell. The "something" in question may be adenovirus or adenoassociated virus, which have been most extensively used for genetic correction of cardiovascular disorders. Adenovirus have the advantage of being easy to manipulate, can be produced in high titers, and have a large transgene cloning capacity (Vinge et al., 2008). However, adenovirus elicit an inflammatory response. Development of so-called "gutted or gutless" adenovirus, where the immunogenic viral epitopes are removed, may become an option in the future (Vinge et al., 2008). Adenoassociated virus are not associated with any human disease, produce a stable and long-lasting gene expression, and easily transfect cardiac muscle cells. The latter is especially the case with some of the newer serotypes, of which serotype 9 is most cardiotropic (Bush et al., 2008, Zancarelli et al., 2008). A disadvantage is that only small constructs (less than 5 kb) can be packed into adenoassociated virus (AAV). Non-viral vectors are also in use and will be briefly discussed.

Further considerations in cardiac gene therapy are which cells are to be treated and what do we want to overexpress or silence (Vinge et al., 2008). The possibilities range from targeting the vasculature to stabilize atherosclerotic plaques, prevent neointima formation, reduce atherosclerosis, induce angiogenesis, to improve survival of cardiomyocytes, improve function of cardiomyocytes, to reduce pathologic remodelling, and to prevent arrhythmia generation. Choice of gene construct and delivery route will depend on this. Genes encoding for factors which have intracellular effects should be delivered to a large population of cells to correct the underlying pathology, while genes encoding for secretory factors require fewer successfully transfected cells provided gene expression lasts (Isner, 2002). RNA interference or silencing, a possibility for gene knockdown, is predominantly at an animal experimental level. Experimentally, RNA interference though short hairpin RNA silencing the RNA polymerase of Coxsackie B3 virus packed into AAV2 successfully treated cardiac dysfunction in mice with coxsackieB cardiomyopathy (Fechner et al., 2008). In that study, the AAV2-construct was given intravenously. The same group have also used phospholamban silencing in short hairpin RNA delivered systemically through a AAV9 vector to normalize left ventricular remodelling after phenylephrine-induced hypertrophy (Suckau et al., 2009). RNA silencing will not be discussed further in this chapter.

2.1 Viral vectors

The first experimental studies on cardiac gene therapy used intramyocardial delivery with plasmid DNA, demonstrating the feasibilty of envisioning cardiac gene transfer (Acsadi et al., 1991, Lin et al., 1990, Burrick et al., 1992). Although those studies were successful in

the terms of being able to cause transgene expression up to six months later in cardiomyocytes, the number of transfected myocytes was estimated to be as low as 60-100 cells (Ascadi et al., 1991). This lead to the search for vectors to enhance nuclear uptake, where viral vectors have been most extensively studied. Adenovirus was first attempted. Guzman and coworkers injected an adenoviral vector containing β-galactosidase (1993) into the myocardium, and was able to see a stronger signal than that evoked by plasmid containing the same molecular marker. However, the expression lasted only one week, and was accompanied by an inflammatory response (Guzman et al. 1993). Subsequently viral titers and protein production have been extensively studied and optimized, as have anatomic location and duration of adenoviral based gene expression in the heart (French et al., 1994, Magovern et al., 1996, Barr et al., 1994). Delivery of therapeutic genes with adenoviral vectors has been performed with success. For instance, adenoviral based delivery of DNA encoding for β2-adrenoceptors enhanced cardiac function in hamsters with cardiomyopathy (Tomiyasu et al., 2000). However, although adenovirus was the first vector to be used for cardiac gene therapy and has been useful for "proof of concept" as well as some initial clinical trials (Lavu et al., 2010), it may not be of large scale therapeutic use for the future. Adenovirus are double-stranded DNA viruses, with a high effeciency of delivery and expression of their genome in nuclei of dividing and non-dividing cells (Voplers & Kochanek, 2004). They are relatively large viral structures, with the capacity to carry constructs of up to 30 kB (Lyon et al., 2011). However, despite the fact that they are relatively cheap to produce in high titers and with a reasonably high purity, a major issue is that they evoke an immune response. As naturally occurring pathogens, patients are likely to have encountered them previously. Thus immune responses leading to destruction of cells containing adenovirus in the heart is a likely outcome. The latter factor also limits the time frame of therapeutic gene expression (Lyon et al., 2011). However, since work on gene therapy of the heart started with adenoviral vectors, the experience in use of this vector is high, and it is an excellent tool for basic science studies to evaluate the therapuetic potential of novel genes.

Attempts are being made to reduce the immunogenicity of adenoviruses, removing the viral genome and viral proteins. The third generation of "gutless" adenovirus have low immunogenicity, and longer transgene expression (Chen et al., 1997). Direct myocardial delivery of gutless adenovirus resulted in less inflammation than the first generation virus, but the gene expression was not high and it was short-lasting (Fleury et al., 2004). Another still remaining problem with adenovirus in the heart is the affinity for other organs such as gastrointestinal tract, liver, respiratory tract, and muscle, causing side effects in clinical trials (Lavu et al., 20120, Lyon et al., 2011).

Adenoassociated viruses (AAV) are currently without comparison the most suitable vectors for cardiovascular gene transfer. AAVs are not associated with any human pathology although 20-40% of all humans may have antibodies to them, making them attractive and safe for clinical treatment. AAVs exist in different seroforms, which have different affinity for the heart. The most recent serotyope, AAV9, is more cardiotropic than any other known virus and will transfect nearly 100% of all heart cells (Vandendriessche et al., 2007). AAV9 causes a sustained cardiac expression of the delivered gene, with little leakage to other organs (Bish et al., 2008, Inagaki et al., 2006, Zincarelli et al., 2008, Pacak et al., 2006). AAV1, 6, and 8 also have relatively high tropism to the heart, and since they have been around for a longer time, they have come further into clinical studies. AAV have been used for

intracardiac, intravascular, and systemic gene delivery. Hitherto more than 20 clinical trials using AAV vectors have delivered the vectors to hundreds of patients without observing any adverse effects (Lyon et al. 2011, Leon et al. 2010). A major advantage of AAV9 is that a systemic approach to gene delivery can be used, thus avoiding some of the challenges of the other viral vectors.

Retroviruses are RNA viruses which integrate into the host cell chromosome after enzymatic conversion to DNA. Retroviral vectors are modified to retain the part of the genome which is neccessary to initiate reverse transcription into the target cell, while the rest of the viral genome is removed (Lyon et al., 2011). Integration of virus into the cell requires cell division, which is why this vector can be suitable for therapies against endothelial or smooth muscle cells such as in avoiding atherosclerosis or restenosis, but less suitable for cardiomyocytes which have a low division rate. However, the insertion of retrovirus into the host genome may cause mutations, potentially leading to malignancies which can be passed on into the germline to offspring.

Lentiviruses belong to the retroviridae family, and include vectors derived from the human immunodeficiency virus type I (HIV-1). Wild-type HIV-1 have an affinity for T-cell subpopulations, limiting their usability for cardiovascular purposes. Hybrid "pseudotyped" lentivirus have been produced to expand their tropism for other cell types. In the context of transfecting cardiomyocytes, lentiviral-based vectors are as effecient as adenoviruses, with transgene expression lasting longer (Yoshimitsu 2006). They can incorporate constructs up to 8 kB in size (Yoshimitsu). Lentivirurses are especially favoured in studies targeting transfection of endothelial cells or smooth muscle cells (Sakoda et al., 2007). The major obstacle towards a large-scale employment of lentivirus is currently uncertainties regarding safety. Modifications of the virus to avoid any risk of human disease are being performed, and may in the future lead to a larger therapeutic potential (Lyon et al. 2011).

2.2 Intrapericardial gene delivery

In theory, injection of DNA into the intrapericardial space may offer an environment which is relatively constant (no blood flow), and would be a relatively non-invasive approach for getting DNA to the heart. However, an intrapericardial injection can not lead to directed gene delivery, in the sense that there is no control over uptake in a specific type of cell or a specific area of the heart such as into the border zone of myocardial infarction. It is noteworthy that few publications exist using this option. Zhang and coworkers delivered adenoviral based LacZ into the pericardium of neonatal mice through a percutaneous puncture, and three days later found LacZ activity in the endocardium, epicardium, and myocardium (Zhang et al., 1999). However, the same regimen did not lead to wide-spread expression in adult hearts, in which hepatic transduction was found in high levels (Zhang et al., 1999). Using a transdiagfragmatic approach, Fromes et al. (1999) delivered adenoviral based β-galactodase intrapericardially in rats. Positive staining was found exclusively in pericardial cells. Mixing the virus with proteolytic enzymes increased transgene expression intramyocardially within a short time later, but the expression did not last, and there was leakage to other organs (Fromes et al., 1999). In the canine myocardium, March and coworkers (1999) delivered adenovirus based LacZ through a penetrating catheter. This lead to a pericardial-located activity of LacZ. The abscence of publications using this delivery approach for the last decade suggests that this is not a delivery route for the future.
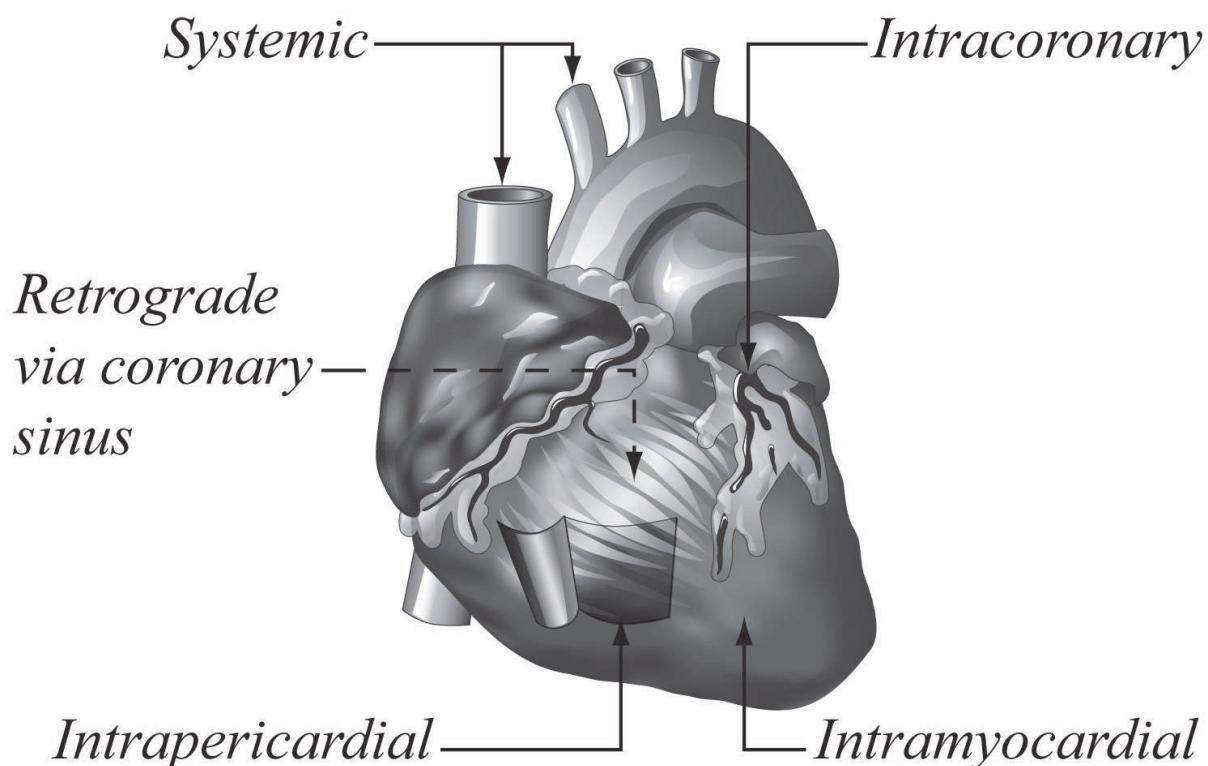
Fig. 1. The cartoon depicts possible routes of delivery of either stem cells or DNA with or without a vector to the heart. Systemic delivery is suitable only when DNA is ligated to a cardiotropic vector.

2.3 Intramyocardial gene delivery

Gene delivery to the heart of either plasmid DNA or DNA ligated to a vector has been performed for decades both in experimental and in clinical trials (Lavu et al., 2010, Katz et al., 2010). Regardless of whether the injection is of plasmid DNA or DNA ligated to a vector, intramyocardial injections are invasive and do not have a clinical appeal. One can envision injection of DNA during open heart surgery when the heart is exposed anyway, or catheter-based delivery when a patient is undergoing invasive arterial procedures. However, except for open heart surgery with direct visualization the accuracy of such an approach is not high - if the intention is delivery of genes i.e. into an ischemic border zone to induce angiogenesis, it will be very difficult to control where exactly the injection site is in relation to where it would be wished to be. The approach has, however, given us invaluable research information on the therapeutic potential and limitations of genes thought to correct underlying pathologies. Many studies have used intramyocardial injections of DNA to induce angiogenesis. Delivery of the transcription factor GATA-4 ligated to an adenoviral vector before coronary artery ligation resulted in improved left ventricular function and reduced infarct size (Rysä et al., 2010). This was due to increased angiogenesis, decreased apoptosis, and mobilization of cardiac stem cells in GATA-4 treated hearts. AAV-based transfection with angiogenin in an *in vivo* infarction model reduced remodelling, induced angiogenesis, and attenuated cardiac dysfunction four weeks later (Zhao et al., 2006). Therapeutic use of AAV9-vascular endothelial growth factor-B is cardioprotective in canine

pacing-induced dilated cardiomyopathy, but not due to formation of new vessels (Pepe et al., 2010). Delivery of adenoviral vector-ligated vascular endothelial growth factor B to rats with angiotensin II-induced hypertrophy leads to reduction of diastolic dysfunction, increasing capillary area but not density (Serpi et al., 2011). In a chronic ischemia model in rats, AAV2-based delivery of both vascular endothelial growth factor A and – B were protective (Zentilin et al., 2010). Vascular endothelial growth factor B was more protective than A, reducing apoptosis and remodelling and preserving heart function in the abscence of angiogenesis. Hepatocyte growth factor delivered by adenovirus into the myocardium following myocardial infarction preserved cardiac function, reduced remodelling and apoptosis, and induced angiogenesis (Jayasankar et al., 2003). Other studies have used antiinflammatory agents injected into the myocardium to combat ischemic heart disease and its consequences. Adenoviral-based expression of inhibitory kappa B-alpha in a rat infarction model improved heart function six weeks later (Trescher et al., 2004). AAV9 based delivery of heme oxygenase-1 into the myocardium before myocardial infarction had infarct reducing, anti-inflammatory, and antiapoptotic effects (Melo et al., 2002). Intramyocardial injection with inducible nitric oxide synthase ligated to adenovirus had an infarct-reducing effect both short-term and long-term (Li et al., 2006). This effect was mediated by inducible cyclooxygenase and nuclear factor kappa B (Liet al., 2007). Other cardioprotective genes in various models of heart disease are the inhibitor of matrix metalloproteinase TIMP-1 (Jayasankar 2004), the cell cycle regulator cyclin A2 (Woo et al., 2006), the regulator of organ development sonic hedgehog (Kusano et al., 2005); Notch1, regulator of cell proliferation and differentiation (Kratsios et al., 2010), the beta adrenoceptor receptor betaARKct (Rengo et al., 2009), and sphingosine kinase 1, a protective protein kinase (Duan et al., 2007). Thus, a major insight into possible therapeutic genes has been provided by this gene delivery route. Intramyocardial gene delivery is likely to remain a powerful research tool for testing the therapeutic potiential of genes in experimental models in the future. However, the future clinical gene therapies are unlikely to involve intramyocardial delivery at a large scale.

2.4 Intravascular delivery

Cardiac intravascular gene delivery has been performed through antegrade coronary artery delivery, non-selective intracoronary delivery (i.e. left ventricular injection), and retrogradely through the coronary sinus (Katz et al., 2010). Common for these approaches is the need to occlude the coronary circulation temporarily to allow virus to migrate into cells. The attractive aspect of this approach is the possibility of a minimally invasive delivery procedure through a catheter well within established clinical procedures (at least the antegrade technique) and the possibility to deliver into all four heart chambers. The first studies using coronary artery delivery resulted in very few transfected cells (Longeart et al., 2001, Hayase et al., 2005, Kaplitt et al., 1996). Later studies have refined delivery methods to some degree. With a recombinant AAV2 vector ligated to deliver enhanced green fluorescent protein, Kaspar and collegues (2005) used rats for indirect intracoronary delivery. Rats had transgene expression lasting up to 12 months, with a gradient of expression across the left ventricular wall, the epicardium expressing much more than the endocardium. There was evidence of AAV2 vector genome in liver and lungs of injected animals (Kaspar et al., 2005). Lai and coworkers (2004) delivered DNA encoding for adenylyl cyclase 6 ligated to an adenoviral vector into all three major coronary arteries of pigs with heart failure, using a vasodilator at the time of delivery, and compared with

delivery of saline. Three weeks later left ventricular function was improved in the pigs recieving adenylyl cyclase 6. Gene expression in left ventricular biopsies evaluated with PCR was increased, although in which cells was not adressed (Lai et al., 2004). The success of intravascular gene delivery may depend on the target cell; if it is vascular, the chance of success may increase compared with a cardiac cell target. However, anything that enters the coronary circulation must enter the general circulation, reducing the clinical appeal of this approach. A special situation where this mode of delivery may be attractive is during open heart surgery with cardioplegic arrest.

2.5 Gene therapy using non-viral vectors

Although improvements are made in modifying viral vectors, reducing immunogenicity and increasing duration and amount of gene expression and narrowing the expression to target cells, researchers are travelling on alternative routes to deliver genes to the heart. Several non-viral techniques are used to improve the transfection efficacy of plasmids such as liposomes, polymers, electroporation, and nanotechnology (Holladay et al. 2010, Lukyanenko 2007). The status of these approaches are recently reviewed elsewhere (Holladay et al. 2010, Lukyanenko 2007).

3. Cardioprotection by cell therapy

Stem cell therapy for protecting hearts is a large topic. For readers particularly interested in the field, the recent reviews by Novotny et al. (2008), Beeres at al. (2008), and Atoui et al. (2008) are excellent. Stem cells are divided into committed and uncommitted cells, where the latter are the true stem cells in the sense that they are undifferenciated, capable of self-renewal, and multipotent (Novotny et al., 2008, Beeres et al., 2008, Atoui et al., 2008). These types of cells include multipotent bone marrow or adipose tissue derived mesenchymal stem cells and embryonic stem cells. Commited progenitor cells are more differentiated, and include endothelial progenitor cells, fetal cardiomyocytes, and autologous skeletal myoblasts. Experimental studies have successfully been able to induce neovascularization, increase cardiomyocyte survival, and improve postinfarct function through using cell transplantation. However, why it works is not completely clarified. Some investigators believe that stem cells dedifferentiate into cardiomyocytes, but not all studies confirm this finding (Silva et al., 2005, Cinnaird et al., 2004, Cocher et al., 2001, Murry et al., 2004). Possibly there is a fusion between the transplanted cells and the endogenous cardiomyocytes (Beeres et al., 2008). Possibly also the transplanted cells lead to recruitment of resident cardiac progenitor cells (Novotny et al., 2008, Beeres et al., 2008). Paracrine effects may be of importance. As transplanted cells have a short life span in their new environment, these effects will be transitory. Some suggested mechanisms of action are autocrine or paracrine release of cytokines and growth factors that will stimulate new vessel formation, inhibit apoptosis, rescue injured cardiomyocytes, and reduce pathologic remodelling. Recently, endogenous cardiac stem cells are reported to have even more promising potential for correcting cardiac pathologies. These cells are a large topic beyond the scope of this chapter (Bolli & Chaudrey, 2010).

Therapeutic use of stem cells is now a clinical reality, but there is need for more laboratory work before this field can become useful in patients at a large scale. At the moment we do not know the optimal cell for delivery, the optimal amount of cells, or which route of delivery (as for gene therapy, intramyocardial, intravascular through artery or vein, pericardial and other approaches have all been performed) that will give the best outcome.

Genetically modifed cells may act as transgene carriers and be used to deliver therapeutic targets to cardiac tissue. Transfected cells of different origins have been used in animal experiments to induce angiogenesis, increase contractility, decrease fibrosis, improve remodelling, and improve graft cell survival.
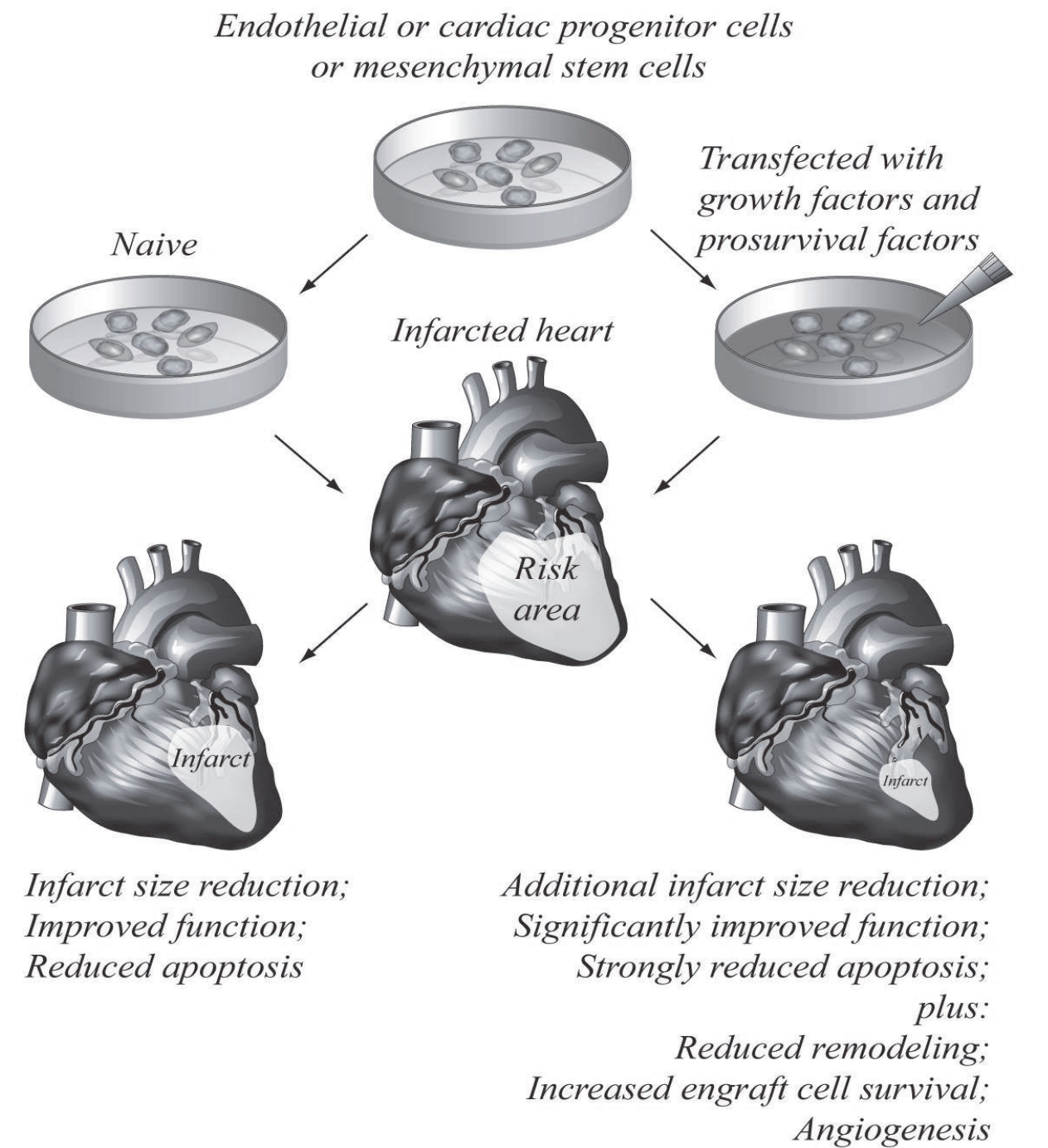

Fig. 2. The advantages of using genetically engineered stem cells versus naive stem cells are illustrated. Naive stem cells do rescue myocardium, but the effects are much more pronounced when stem cells are genetically engineered.

3.1 Genetically engineered stem cells as cardioprotective agents

Based on the assumption that the major effect of stem cells is through their paracrine effects, quite a few works have focused on genetically engineered stem cells to produce angiogenic factors, with the perspective to both increase survival of the transplanted cell and to enhance the formation of new blood vessels in the infarcted heart. For instance, bone-marrow derived endothelial progenitor cells were expanded and transduced with AAV to overexpress insulin-like growth factor 1. Then the autologous cells were transplanted into the infarct area of rats (Sen et al., 2010). Three months later rats receiving insulin-like growth factor 1 transduced cells as opposed to LacZ-transduced cells had improved myocardial function, reduced apoptosis, increased number of capillaries, and increased cardiomyocyte proliferation in the infarct area. There was no dissemination of transduced cells into other organs (Sen et al., 2010). In a model of neointima formation in hypercholesterolemic rats, endothelial progenitor cells transduced to overexpress hepatocyte growth factor were delivered. The transduced cells homed to the vascular site of injury more than untreated cells, and this caused a decreased neointima formation and increased endothelialization (Song et al., 2009). Colony stimulating factor-1 was used to transfect primary autologous rat myoblasts, which were transplanted into the myocardium of rats with postinfarction heart failure (Aharinejad et al., 2008). Left ventricular function evaluated by echocardiography was improved in hearts of rats treated with with autologous colony stimulating factor myoblasts. This protection was not found after delivery of untransduced myoblasts or plasmid DNA encoding for colony stimulating factor. In a similar model myoblasts transduced with human growth factor were able to improve heart function, increase capillary density, and reduced apoptosis (Rong et al., 2008). Mesenchymal stem cells engineered to overexpress adrenomedullin transplanted after myocardial infarction improved cardiac function more than naive mesenchymal stem cells (Jo et al., 2007). The growth factor angiopoietin-1 in modified mesenchymal stem cells has reduced ischemic damage when injected shortly after ischemia in rat hearts (Sun et al., 2007). In pigs, mononuclear cells were extracted from peripheral blood and induced to overexpress vascular endothelial growth factor retrogradely delivered through the coronary sinus. The transduced cells induced angiogenesis and reduced postischemic ventricular dysfunction four weeks later (Hagikura et al., 2010). Vascular endothelial growth factor ligated to mesenchymal stem cells under the control of a hypoxia response element induced ischemia-responsive production of vascular endothelial growth factor when transplanted into the ischemic myocardium (Kim et al., 2010). This caused an increased retainment of genetically altered mesenchymal stem cells in the infarcted heart compared with naive cells, reduction of apoptosis, and reduced remodelling. Also hypoxia-regulated heme oxygenase-1 overexpressing mesenchymal stem cells transplanted into the infarcted ventricular wall improved survival of transplanted cells, improved heart function, and reduced cell death (Tang et al., 2005).

Genetic modification of stem cells also improves cell survival and outcome of ischemia models when the gene in question is not considered to be a secretory molecule. Treatment of mesenchymal stem cells to overexpress connexin 43 followed by injection into infarcted myocardium improves left ventricular function and reduces cell death (Wang et al., 2010). Mesenchymal stem cells overexpressing heat shock protein of the 20 kDa family has similar beneficial effects (Wang et al., 2009). In the latter study, the authors provide evidence that the protective effect could be through increased secretion of proteins, where vascular

endothelial growth factor, insulin-like growth factor, and fibroblast growth factor were released from transfected cells. The authors speculate that the relased growth factors were due to a detected activation of the protein kinase Akt (Wang et al., 2009). Indeed, mesenchymal stem cells overexpressing Akt itself transplanted into the ischemic myocardium improved left ventricular function, reduced infarct size, reduced apoptosis, increased mobilization of cardiac progenitor cells (c-kit+), and reduced collagen deposition (Mangi et al., 2003). The beneficial effects were dependent on the amount of transplanted cells. In a follow up study, the authors found that the mechanism for cardioprotection was not through stem cell fusion with cardiomyocytes, which occurred infrequently, and not due to differentiation of stem cells into cardiomyocytes (Noiseux et al. 2006). Another protein kinase associated with myocardial protection, Pim-1 kinase, was transfected into cardiac progenitor cells before injection into ischemic myocardium (Fischer et al., 2009). When animals were observed up to 32 weeks later, improved function and reduced infarct size was accompanied by increased survival of engrafted cells, increased vascularization, and increased number of c-kit+ cells (Fischer et al., 2009). Consequently, secondary secretory effects of genetic manipulation with a factor acting intracellularly is indicated. The antiapoptotic molecule Bcl2 has been used to transfect cardiomyoblasts (Kutcha et al., 2006) and mesenchymal stem cells (Li et al, 2007) before transplantation into infarcted myocardium, leading to improved function and survival of both engrafted cells and infarcted myocardium. Mesenchymal stem cells transfected with Bcl2 had an increased secretion of vascular endothelial growth factor in vitro, and an increased capillary density in vivo (Li et al., 2007). Finally, a few studies have used genes coding for antiinflammatory factors as enrichment of stem cells to improve survival of engrafted stem cells and the heart. Mesenchymal stem cells overexpressing the interleukin-18 binding protein, the naturally occurring inhibitor of the proinflammatory cytokine interleukin 18, improved cardioprotection more than that observed with unmodified stem cells (Wang et al., 2009). The beneficial effects observed on heart function, remodelling, and infarct size could have been due to increased secretion of vascular endothelial growth factor and decreased interleukin 6 levels in hearts of animals treated with genetically modified cells. Mesenchymal stem cells have also been used to overexpress the chemokine receptors CCR1 and CXCR2 before intramyocardial injection into infarcted heart (Huang et al., 2010). Stem cells with overexpression of CCR1 had increased survival intramyocardially, which was accompanied by less cardiac remodelling, increased capillarization, and improved cardiac function in both acute and chronic (4 weeks) observation times. The effect was not found when cells were overexpressing CXCR2, which lead to similar findings as with naive mesenchymal stem cells (Huang et al., 20010).

To conclude, we still have a long way to go to fully understand the mechanisms by which stem cells may protect hearts and which cell type and number that should be used for future therapies. However, it is well documented that genetic engineering of stem cells with both secretory factors and primarily intracellularly acting factors improve engrafted cell survival as well as survival of the myocardium. Many of the studies mentioned above using a primary intracellularly acting factor have documented secondary secretory effects.

4. Cardiac gene therapy using a peripheral approach

A downside with intracardiac delivery of either genes or genetically modified cells is the relative invasiveness of the method. It is possible to envision effects in the heart through a
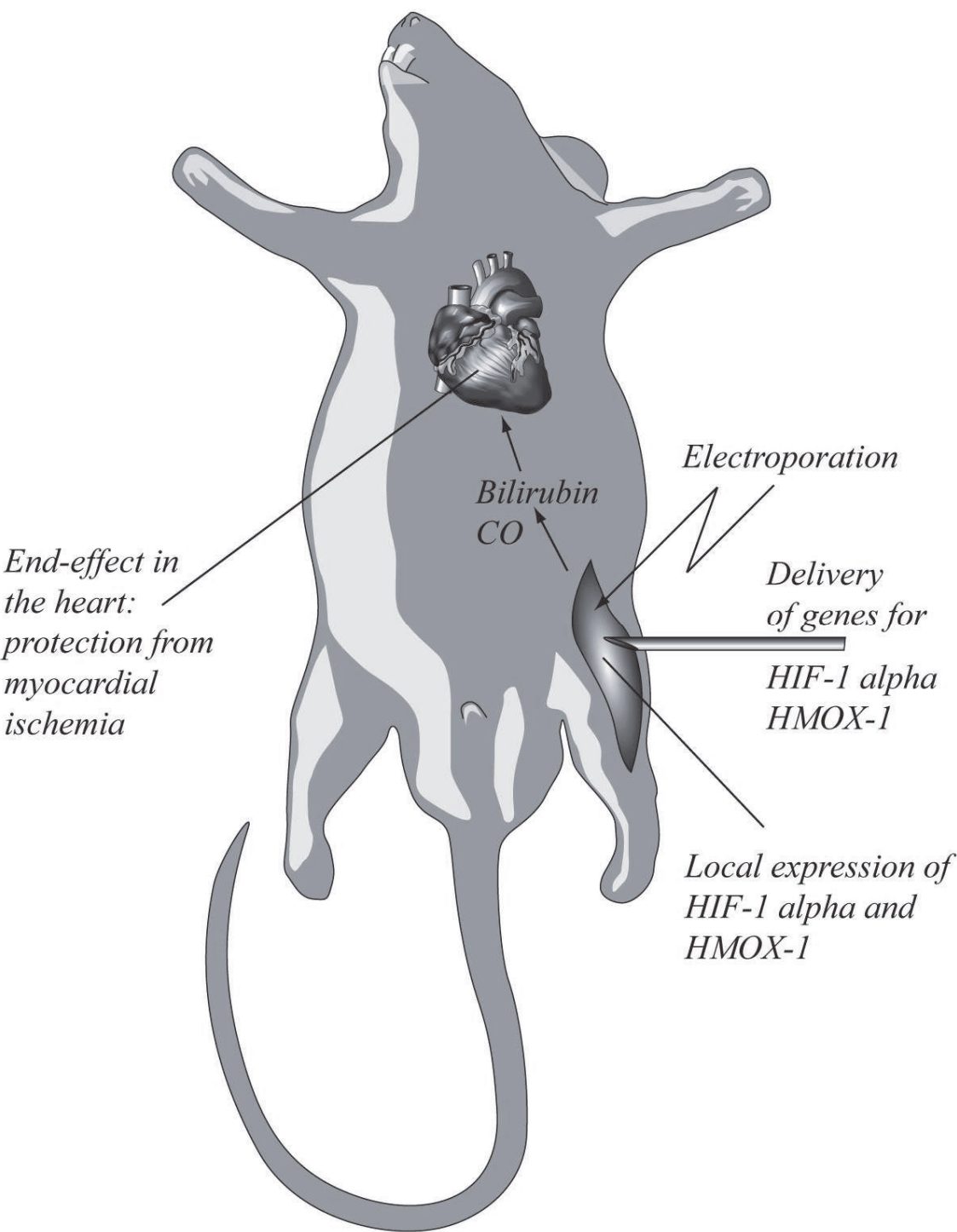
Fig. 3. The cartoon depicts the principle of remote gene therapy delivering plasmid DNA into the skeletal muscle, increasing nuclear uptake by electroporation, and achieving myocardial protection.

peripheral approach, building upon the principle of general organ protection evoked by pre- or postconditioning (Przyklenk et al., 1993). Preconditioning is the observation that brief episodes of ischemia and reperfusion to an organ will protect the organ against a later

ischemic event (Murry et al., 1986), while postconditioning is the observation that brief episodes of ischemia and reperfusion at the start of reperfusion will reduce organ damage (Zhao et al., 2003). It is shown that the protection afforded by these brief episodes of ischemia and reperfusion provide an universal organ protection termed remote preconditioning (Przyklenk et al., 1993). In a series of experiments we have delivered plasmid DNA encoding for hypoxia-inducible factor 1alpha (HIF-1α) into an easily accessible peripheral organ, the quadriceps skeletal muscle. Others have shown that the skeletal muscle may serve as an endocrine organ, stably secreting endocrine factors into the blood stream after delivery of plasmid DNA and enhancing nuclear uptake by electroporation (Mathisen et al., 1999). This gives a very local increase of gene expression, transfecting a few skeletal muscle fibers in the treated muscle and with no leakage to other organs (Czibik et al 2009a, 2009b). The skeletal muscle expression of HIF-1α lasted for 8 weeks (not investigated longer) (Czibik et al., 2009a). When hearts were isolated and Langendorff-perfused with global ischemia and reperfusion, they had improved function and reduced infarct size compared with hearts of mice which were not pretreated with HIF-1α (Czibik et al., 2009a). To attempt to unravel mechanisms underlying the beneficial effects of HIF-1α, a Taqman low density array of some 47 HIF-regulated genes was performed on samples of the transfected skeletal muscle one week later. Several genes encoding for growth factors were increased in the transfected muscle, among them insulin-like growth factor 2, heme oxygenase-1, adrenomedullin, and platelet derived growth factor B (Czibik et al. 2009a). When these factors were used to protect the cardiomyocyte cell line HL-1 cells against injury evoked by hydrogen peroxide, heme oxygenase-1 (HMOX-1) was beyond comparison most protective, with effects similar to that of HIF-1α (Czibik et al. 2009b).

HMOX-1 is an inducible member of the heme oxygenase family of proteins, also consisting of the constitutive heme oxygenase-2 and the less well characterized heme oxygenase-3 (Durante et al., 2010, Wu et al., 2010). HMOX-1 expression is induced by its substrates: heme, oxidants, heavy metals, cytokines, growth factors, hemodynamic forces, gases, hypoxia, and hormones (Wu et al., 2011). Many transcription factors may be involved in its regulation. Some of them are HIF-1α, nuclear factor kappa B, activator protein 1, and nuclear factor E2-related factor (Wu et al., 2011). HMOX-1 catalyzes the degradation of heme into biliverdin, free iron, and carbon monoxide (Maines et al., 1986). Biliverdin is subsequently rapidly reduced to bilirubin by the enzyme biliverdin reductase. HMOX-1 is expressed in a plethora of cell types, including cardiac and vascular cells. Carbon monoxide, most known as a toxic gas, is recognized as an intracellular signalling molecule (Maines et al., 1986, Verma et al., 1993). Carbon monoxide has many cellular effects which have recently been reviewed elsewhere (Abraham & Kappas, 2008); in this context, it can be summarized that it may have antiinflammatory and antiapoptotic effects, lead to vasorelaxation, reduce lipid peroxidation and proliferation of vascular smooth muscle cells, and possibly induce angiogenesis. Bilirubin was shown to have antioxidant effects already in 1987 (Stocker et al.). Since then evidence supports that bilirubin regulates cellular redox states, reduces the formation of reactive oxygen species, and has antiinflammatory effects through decreasing the expression leukocyte adhesion molecules and neutrophil adhesion. Free iron may induce ferritin expression leading to iron sequestration. Thus, HMOX-1 through its downstream products is potentially very suitable for protection of cardiomyocytes.

When the HIF-1α gene was delivered in vivo into skeletal muscle of rats, the expression of HMOX-1 was increased, accompanied by increased serum bilirubin (Czibik et al., 2009b, Czibik et al., 2011). When a HMOX-1 blocker was given together with plasmid DNA encoding for HIF-1α and the hearts isolated and perfused with induced global ischemia, the beneficial effect of gene therapy was abolished (Czibik et al., 2009b). Delivery of plasmid DNA endocing for HMOX-1 before isolated heart perfusion mimicked the beneficial effects of HIF-1α (Czibik et al.,2009b). Gene delivery of HIF-1α into the skeletal muscle protected the heart ex vivo, and in vitro, and was also evaluated to be highly cardioprotective in an in vivo model of cardiac ischemia-reperfusion with remodelling six weeks later (Czibik et al., 2009a, 2009b, 2011). Unfortunately, systemic delivery of HIF-1α induced a general angiogenesis evident as increased CD31 positive staining in the electroporated muscle with gene delivery, the contralateral muscle, and in the heart (Czibik et al., 2009a, 2009b, 2011). Downstream factors to hypoxia inducible factor may turn out to be cardioprotective without the unwanted side-effects. Delivery of plasmid DNA encoding for HMOX-1 into the skeletal muscle before in vivo infarction protects against postinfarct remodelling without causing angiogenesis (manuscript in progress). Thus, these promising results from mice experimental studies should now be tested in larger animals as a bridge to human therapy.

5. Conclusion

For the treatment of cardiovascular disease, gene therapy may become an alternative in the near future. Gene delivery through intravascular approaches, intramyocardial injection, and pericardial route have been tried using plasmid DNA, adeno-, retro-, lenti-, and adenoassociated viral vectors. Of the viral vectors, adenoassociated virus serotype 9 is the most promising, as it is cardiotropic and can be delivered systemically. Stem cells are another approach to novel therapies against ischemic heart disease. Stem cells can be delivered through the same routes as genes. At the moment the mechanism of stem cell-induced protection of the heart is not well understood - the cells tend to stay shortly in the myocardium, and to a low degree fuse with cardiomyocytes or differentiate into cardiomyocytes. Possibly paracrine effects of stem cells are the reason for cardioprotection. Genetic engineering of stem cells improves the therapeutic effect of transplanted cells, both when the engineering is for a secretory factor and when it is overexpressing a factor primarily working intracellularly, and secondarily secretory. Gene therapy of the heart can also be evoked through using the skeletal muscle as a site of gene transfer, where delivery of plasmid DNA encoding for both hypoxia-inducible factor 1 alpha and its downstream target heme oxygenase-1 protects cardiomyocytes in vivo, ex vivo, and in vitro.

6. Acknowledgement

7. References

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Targets in Gene Therapy

Edited by Prof. Yongping You

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