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# Preclinical and Clinical Aspects of Gene Therapy in Myocardial Infarction

Saurabh Bharti, Ashok Kumar Sharma,  
Bhaskar Krishnamurthy and Dharamvir Singh Arya  
*Department of Pharmacology,  
All India Institute of Medical Sciences,  
New Delhi,  
India*

## 1. Introduction

Clinical occurrence of acute myocardial infarction (AMI) has substantially increased over the last decade. More than 50% of the cases of coronary heart disease alone are covered under AMI. Thus, the prevalence of AMI poses a serious health care burden, demanding for more strategic approaches. Exploration in molecular mechanisms underlying myocardial infarction (MI) has radically improved over the last half century. Novel pathways have not only provided in-depth knowledge of mechanistic approach to understand the pathophysiology of MI but also offer new strategies for its treatment. Currently, polypharmacy comprising  $\beta$ -blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and aspirin are generally adopted for treating patients of MI. Surgical interventions include stenting procedures and percutaneous coronary interventions. Despite availability of these pharmacological and surgical therapies, they are associated with limitations encompassing side effects, recurrence, non-compliance and interactions with other drugs. The early mortality rate from AMI is about 30%, with more than half of these deaths occurring before the stricken individual reaches the hospital (AHA report, 2003). Therefore looking towards the 21st century, now the question arises, can we bring an ideal therapy for treatment of human MI where all the side effects, interaction and compliance issues can be eliminated? The answer might lie in an emerging area known as gene therapy. Cardiovascular gene therapy is one of those areas where administration of a particular gene would result in long term gene expression and preclude the heart from various insults. In general an ideal gene therapy should have multifaceted approach such as 1) It should be specific for the myocardial tissue 2) it should not cause potential adverse effects 3) it should not disrupt the vital genes 4) it should not increase the risk of other diseases such as predisposition to cancer and finally 5) it should be long enough to sustain and to prevent any recurrence of the disease. Most recent exciting development in gene therapy includes formation of new blood vessels and improved blood flow to ischemic tissues induced by growth factors such as VEGF, HGF, FGF and PDGF. Furthermore, improved calcium handling through SERCA2a, phospholamban, parvalbumin and S100A1 proteins offers a viable and attractive approach in the treatment of heart failure. In addition, modulation of  $\beta$ -adrenergic receptor signaling through  $\beta_2$  adrenergic receptor overexpression, GRK2/ $\beta$ ARKct and Adenyl cyclase 6 has yielded encouraging results in

experimental models of MI. Thereafter, various cardiovascular diseases where gene therapy has made its mark in pre-clinical studies are angiogenesis for myocardial ischemia, re-stenosis and bypass graft failure. Gene therapy with its specificity on myogenesis, cell cycle activation anti-oxidant and anti-apoptotic pathways upregulates the cellular defense system and provides augmented cardioprotective response to injury and cardiac remodeling. Success in animal models and in early phases of clinical trials of gene therapy in myocardial ischemic reperfusion setting further validates imminent use of gene therapy. Mechanistically, gene therapy for MI must be aimed at activating key molecular pathways in myocardium that significantly alleviate the cardiac injury by inducing neo-angiogenesis, strengthening anti-oxidant status of the myocardium, inhibiting necrosis/apoptotic pathways, inhibiting mitochondrial permeability transition pore and most importantly preserving the integrity of myocardium (remodeling).

This chapter will basically focus on the significant results of gene therapy obtained in myocardial ischemia reperfusion injury in pre-clinical studies, phase I/II clinical trials and their future implications to make gene therapy a completely bench to bed-side approach.

## **2. Preclinical aspect**

### **2.1 Role of angiogenesis in gene therapy**

Perhaps, the most highly investigated and promising application of gene therapy which has shown indispensable results in animal models of myocardial infarction is angiogenesis. Angiogenesis occurs as a result of multiple growth factors participating in the formation of new blood vessels. Growth factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and platelet derived growth factor (PDGF), either alone or in combination, result in induction of angiogenesis. Therefore gene therapy can act in very specific and targeted manner, i.e. either to activate the pathways which up-regulate endogenous growth factors like hypoxia inducible factor-1 alpha (HIF-1 $\alpha$ ), to inhibit endogenous anti-angiogenic processes or to induce growth factors exogenously. The various angiogenic growth factors which have shown significant results in animal models of myocardial infarction are as follows:

#### **2.1.1 VEGF**

Family comprises of five isoforms viz. VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor. The major function of VEGF is to regulate coronary vasculature development, blood vessel physiology, vasodilation, proliferation and migration of endothelial and smooth muscle cells.

#### **2.1.2 HGF**

HGF shows its diversified action through coupling with c-Met receptor following tyrosine kinase activation. HGF is a pleiotropic factor which promotes neo-vascularization, migration and proliferation of endothelial cells and inhibits apoptosis. In addition; morphogenesis, mitogenesis, motogenesis and neurite extension are the other effects shown by HGF.

#### **2.1.3 FGF**

Family comprises of 22 distinct polypeptide growth factors and seven single-chain tyrosine receptor kinases. FGF is also known as heparin binding growth factor because of its higher affinity towards heparin and heparan sulfate. FGF displays its activities through auto-phosphorylation via dimerization of its receptor. FGF actively participates in both the

phases of angiogenesis/arteriogenesis i.e. early invasive phase and late maturative phase. Functionally, it is involved in migration and proliferation of endothelial and smooth muscle cells, production of proteases and vessel maturation.

2.1.4 PDGF

Family comprises of five isoforms viz. PDGF-A, PDGF-B, PDGF-C, PDGF-D and PDGF-AB heterodimer; and acts through two distinct receptors namely  $\alpha$  and  $\beta$ . Its function is to regulate cell growth and division. PDGF is a potent mitogen for smooth muscle cells of mesenchymal origin.

Growth Factors	Species	Outcome	References
VEGF-165	Rats, rabbits	Increased neovascularization and improved fractional shortening after MI	Bull et al., 2003; Hao et al., 2007; Ruixing et al., 2006.
VEGF-165	Porcine	Increased myocardial blood flow, increased vasodilation with adenosine, improved wall thickening and strain, improved wall motion, increased ejection fraction and increased myocardial viability	Choi et al., 2006; Ferrarini et al., 2006; Jacquier et al., 2007; Tio et al., 1999; Zhang et al., 2002.
VEGF-121	Rats, porcine	Increased collateral circulation following MI	Lee et al., 2000; Mack et al., 1998.
VEGF-B186	Pigs, rabbits	improved myocardial perfusion and ejection fraction	Lahteenvuio et al., 2009
VEGF-C	Piglets	augmented collateral formation and decreased wall thickening after MI	Patila et al., 2006
VEGF-D	Porcine	improved perfusion when administered through a catheter mediated intra myocardial gene transfer method	Rutanen et al., 2004
VEGF gene constructs	Rat, mouse	Reduced infarct size and induction of angiogenesis	Lee et al., 2003; Su et al., 2002; Yockman et al., 2009
HGF	Rats, dogs	Induction of angiogenesis	Aoki et al., 2000; Funatsu et al., 2002; Wang et al., 2006a
HGF	Mice, Swine, canine, porcine	Improved remodeling, decreased apoptosis, improved mobilization of stem cells for cardiac repair, decreased fibrotic scar formation and improved contractility of the heart	Ahmet et al., 2002; Ahmet et al., 2003; Cho et al., 2008; Jayasankar et al., 2003; Jin et al., 2004; Li et al., 2003; Taniyama et al., 2002; Yang et al., 2007; Yang et al., 2010

Growth Factors	Species	Outcome	References
HGF + ultrasound mediated micro bubble destruction	Rat	Increased angiogenesis, limitation of infarct size, and prevention of LV remodeling	Kondo et al., 2004
FGF	Pig	Improved blood flow and MI by enhancing collateral formation	Post et al., 2006
FGF-2	Pigs	Improved LV functions and increased arteriogenesis	Horvath et al., 2002
FGF-4	Pigs	increased perfusion and decreased LV dysfunction	Gao et al., 2004
FGF-5	Pigs	Improved blood flow, Reduced pacing-induced regional myocardial dysfunction	Giordano et al., 1996; Suzuki et al., 2005
PDGF-AB	Rat	Promoted angiogenesis and minimized the extent of myocardial infarction	Edelberg et al., 2002
PDGF + basic FGF	Rat	Promoted angiogenesis with more stable capillaries	Hao et al., 2004a
PDGF-BB + VEGF-A165	Rat	Stimulated angiogenesis both at the capillary and arteriolar levels and transiently counteracted cardiac remodelling after MI	Hao et al., 2004b
PDGF	Rat	Improved cardiac function	Zheng et al., 2004
PDGF	Rats	Improved post-infarction ventricular function without pulmonary toxicity	Hsieh et al., 2006
PDGF-BB + FGF-2	Pigs	Enhanced myocardial collateral growth and significantly restored myocardial perfusion and function	Lu et al., 2007
PDGF-BB	Rats	Significantly decreased MI	Krausgrill et al., 2009

Table 1. The examples of various growth factors in myocardial infarction.

2.2 Role of oxidative stress in gene therapy

The human cells are replete with a number of molecules that function as antioxidants, i.e. which protect the cells from a group of molecules known as pro-oxidants. Anti-oxidants are molecules that are capable of inhibiting the oxidation of other molecules. In this process, they themselves get oxidized. On the other hand, pro-oxidants either generate free radicals or inhibit the anti-oxidant systems, thus predisposing the cell to oxidative damage. Oxidative stress represents a condition characterized by an imbalance between the pro-oxidant and anti-oxidant systems within the cell, leading to oxidative damage to the cell; ultimately leading to lipid peroxidation, protein degradation and DNA damage and has been implicated in a vast variety of disorders; atherosclerosis, diabetes, myocardial infarction, myocardial ischemia-reperfusion injury, hypertension, heart failure,

cardiomyopathy and rheumatoid arthritis being just a few of them. Examples of ROS include radicals like superoxide, hydroxyl, alkoxyl and peroxy; and non-radicals like hydrogen peroxide, hypochlorous acid and organic hydroperoxides.

The observation that the level of antioxidants is increased during periods of oxidative stress has led to the finding that the synthesis of antioxidants is increased at the times of oxidative stress. This has led to the concept that administration of genes for antioxidants can combat the role of oxidative stress and thus protect cells and tissues from damage. Various studies and clinical trials have been carried out in this regard and the role of gene therapy for antioxidants in myocardial ischemia prevention has been established in animal models. The intramyocardial delivery of heme oxygenase-1 (HO-1) into the left ventricle of rats using AAVs (Adeno-Associated Virus) several weeks before myocardial infarction resulted in approximately 80% decrease in infarct size in association with decreases in oxidative stress, inflammation, and interstitial fibrosis and was accompanied by postinfarction recovery and normalization of ventricular dimensions (Melo et al., 2002). Similar results were obtained after transfer of gene for extracellular superoxide dismutase (EC-SOD); wherein recombinant adeno virus transfer into the myocardium of conscious rabbits of the gene encoding EC-SOD, three days before the induction of myocardial ischaemia by a 30-minute coronary artery occlusion using a balloon occluder, afforded cardioprotection with a decrease in the region at risk and infarct size as compared to controls (Li et al., 2001). Its intracellular counterparts (Mn-SOD and Cu/Zn-SOD) have also been shown to decrease infarct size following ischaemia-reperfusion injury, as well as decrease apoptosis and delay induction of NF- $\kappa$ B (Michiels et al., 1994; Chen et al., 1998). Thioredoxins are potent antioxidants that decrease p38MAPK signaling and superoxide anion generation and hence, combat oxidative stress. Trx-1 gene therapy administered post-MI has been shown to promote angiogenesis, decrease apoptosis, reduce ventricular remodeling, and improve ejection fraction in diabetic rats (Samuel et al., 2010). Below are mentioned some important anti-oxidants which have shown remarkable results in experimental models of myocardial infarction.

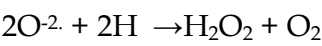
### 2.2.1 HEME oxygenase-1 (HO-1)

Heme oxygenase cleaves the heme ring at the alpha-methene bridge to form biliverdin, iron and carbon monoxide. Its activity is highest in the spleen, where old RBCs are sequestered and destroyed, leading to liberation of hemoglobin and its subsequent separation into heme and globin. It has three isoforms: HO-1 (which is also referred to as heat-shock protein 32K or HSP-32K) is inducible in response to oxidative stress, hypoxia, heavy metals, cytokines and a variety of other compounds. HO-1 has also been suggested to induce VEGF formation. HO-2 is constitutive and is expressed under normal or homeostatic conditions. HO-3, unlike the other two isoforms, is not catalytically active, but has been postulated to work in oxygen sensing. AAV delivery of HO-1 performed in MI-rat models demonstrated an increase in cardiac remodeling and an improvement in cardiac function (Melo et al., 2002). Studies performed in animals with human heme oxygenase-1 (hHO-1) administered 6 – 8 weeks prior to induction of ischaemia resulted in decreased mortality at 1 year, along with a decrease in infarct size, reduction in ventricular thinning, decreased inflammation stress and interstitial fibrosis, decreased lipid peroxidation, and was accompanied by post-infarction recovery and normalization of ventricular functions (Melo et al., 2002).



2.2.2 Superoxide dismutase (SOD)

The enzyme superoxide dismutase (EC: 1.15.1.1) was first discovered by Fridowich and McCord (McCord & Fridovich, 1988). It scavenges the superoxide (O<sup>-2</sup>) radical and causes its dismutation into water and hydrogen peroxide by the following reaction –



The enzyme has three isoforms – 1. Cytosolic (Cu/ Zn-SOD) or SOD-1, dependent on copper and zinc (Cao, et al., 2008). 2. Mitochondrial (Mn-SOD) or SOD-2, dependent on manganese (Borgstahl, et al., 1996) and 3. Extracellular (EC-SOD) or SOD-3. The genes for these are located on chromosomes 21q22.1, 6q25.3 and 4p15.3 respectively. Cu/ Zn-SOD is dimeric and the other two are tetrameric in structure. All of them have improved left ventricular function and increased survival in rat ischaemia-reperfusion models (Michiels et al., 1994, Chen et al., 1998; Li, et al., 2001). Studies on EC-SOD in rat ischaemia-reperfusion models have demonstrated decreased myocardial stunning and infarct size (Li et al., 2001). Mn-SOD and eNOS administered together have also led to decreased infarct size in animal ischaemia-reperfusion models (Abunasra et al., 2001).

2.2.3 Thioredoxins

Thioredoxins are another group of antioxidant proteins that are ubiquitously found in many organisms and are encoded by the TNX gene in humans (Wollman et al., 1988). They have a 12 kDa tertiary structure, that contains a dithiol-disulfide active site and facilitate the reduction of other proteins by a cysteine thiol-disulfide exchange. The amino acid sequence of thioredoxins is characterized by two vicinal cysteines in a CXXC motif which provide the enzyme the ability to reduce various substrates, such as ribonucleases, chorionic gonadotrophins, coagulation factors, glucocorticoid receptors and insulin. The thioredoxins also act as electron donors to peroxidases and ribonucleotide reductase (Arner & Holmgren, 2000).

The summary of the researches carried out on antioxidant gene therapy in MI is summarized in the table 2.

Gene	Model	Outcome	References
HO-1	Rat I/R	Decrease in infarct size. Decrease in oxidative stress, inflammation and interstitial fibrosis. Post-infarction recovery with normalization of ventricular dimensions	Melo et al., 2002
SOD	Rat I/R	Decreased stunning. Decreased infarct size following I/R injury. Improved left ventricular function. Increased survival	Michiels et al., 1994 & Chen et al., 1998, Li et al., 2001
TRX-1	Post-MI diabetic rats	Angiogenesis. Decreased apoptosis. Decreased ventricular remodeling. Improved ejection fraction.	Samuel et al., 2010
SPHK1	Rats	Improved systolic and diastolic functions of the heart and improved peak contraction velocity	Jin et al., 2007

Table 2. Examples of various anti-oxidants studied for gene therapy.

Gene	Species	Outcome	Reference
Bcl-2	Rabbits	Reduced apoptosis, reduced ventricular dilatation and decreased wall thinning	Chatterjee et al., 2002
Apoptosis repressor gene	Rabbits	Inhibition of apoptosis, decreased LV dilatation with preserved ejection fraction	Chatterjee et al., 2003
Cardio-trophin-1 (CT-1)	Mouse	Decreased apoptosis, decreased infarct size, decreased caspase-3 activation and improved ventricular pressure indices	Ruixing et al., 2007
Akt	Rats	Limitation of infarct size, improved myocardial contractility and reduced infarct size	Cittadini et al., 2006; Miao et al., 2000
sTNFR1 (TNF- $\alpha$ antagonist)	Mice	Reduced infract size and improved cardiac function	Sugano et al., 2004
HSP 20	Rats	Improved LV end systolic and end diastolic pressures, reduced apoptosis and decreased infarct size	Zhu et al., 2005
HSP 70	Rabbits	Decreased infarct size	Okubo et al., 2001
HSP 72	Rats	Decreased apoptosis and prevented MI	Suzuki et al., 2002
Kallikrein gene	Rats	Decreased apoptosis, endothelial dysfunction and preservation of cardiac output	Agata et al., 2002
Sonic hedgehog homolog (Shh)	Mice, Rats	Preserved ventricular function by enhancing neovascularization, reduced apoptosis and fibrosis	Kusano et al., 2005
Troponin I type 3 interacting kinase	Mice	Decreased MI and inhibition of remodeling	Lai et al., 2008
Leukemia inhibitory factor (LIF)	Rats	Preservation of rat myocardium post-MI	Berry et al., 2004
Cluster of Differentiation 151 (CD151)	Pigs and rats	Promoted neovascularization and improved cardiac function	Wang et al., 2006b, Zuo et al., 2009
p38 kinase + active MAP kinase kinase 3b	Rats	Reduced infarct size, decreased apoptosis, increased capillary density, decreased fibrosis and improved ejection fraction	Tenhunen et al., 2006

Table 3. The examples of various proteins involved in apoptosis studied for gene therapy.



### 2.3 Role of apoptosis in gene therapy

Apoptosis means programmed cell death. It is a physiological phenomenon that normally occurs during the embryonic development in humans and also serves as a protective response in case of irreparable DNA damage to avoid the multiplication of defective cells. Two various pathways are involved in induction of apoptosis. The extracellular pathway involves stimulation of death receptors that transduce signals to the nucleus to activate caspases. The intracellular pathway involves increase in the permeability of the inner mitochondrial membrane that causes leakage of pro-apoptotic molecules like cytochrome c into the cytoplasm that directly activate the caspases. The end-result is the activation of caspases that digest the protein cytoskeleton of the cell. The distinct family of Bcl proteins is involved in apoptosis, with Bcl-2 and Bcl-x acting as anti-apoptotic factors; while Bax and Bak act as pro-apoptotic factors.

The pathways of apoptosis provide an excellent target for gene therapy as inhibition of apoptosis allows the cells to survive. Gene therapies with pro-apoptotic molecules and genes that transcribe proteins that suppress the anti-apoptotic proteins have been tried in animal models with sufficiently encouraging results, which are yet to be supplemented by results from clinical trials (Table 3).

### 2.4 Role of calcium signaling in gene therapy

The handling of  $\text{Ca}^{2+}$  during excitation-contraction (EC) coupling is an important feature of the cardiomyocyte contraction. In cardiomyocytes, EC coupling begins with the initiation of action potential, whereby,  $\text{Ca}^{2+}$  enters the cell through voltage-gated L-type  $\text{Ca}^{2+}$  channels and triggers the ryanodine receptor (RyR) to extrude  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR) into the cytosol. This  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release trigger is a crucial step in cardiomyocyte contraction through  $\text{Ca}^{2+}$  binding to troponin C within the myofilaments of the sarcomere. Furthermore, it is again imperative to remove the  $\text{Ca}^{2+}$  from the cytosol to initiate the relaxation of sarcomere which is mainly dependent on sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase 2a (SERCA2a) and sarcolemmal  $\text{Na}^{+}$ - $\text{Ca}^{2+}$  exchanger (NCX). Moreover, distortion of  $\text{Ca}^{2+}$  handling in cardiomyocytes occurs due to decreased SR  $\text{Ca}^{2+}$  store and a prolonged  $\text{Ca}^{2+}$  transient, which is generally a consequence of increased NCX, reduction in SERCA2a, decreased phospholamban (PLN)/SERCA2a ratio and increased open probability of the RyR. In addition, these abnormalities in  $\text{Ca}^{2+}$  handling cause dysfunctional contractile performance and may increase the risk of cardiac arrhythmias and cardiac remodeling. Because myocardial contractility is dependent on ventricular  $\text{Ca}^{2+}$  handling, therefore, genetic modification of molecules involved in dysfunctional cardiomyocyte  $\text{Ca}^{2+}$  handling could be a viable and attractive target in the treatment of heart failure. The major proteins which participate in handling of calcium during cardiomyocyte contraction are discussed briefly:

#### 2.4.1 Sarco/endoplasmic reticulum $\text{Ca}^{2+}$ -ATPase (SR $\text{Ca}^{2+}$ -ATPase, SERCA)

SERCA, a calcium ATPase (type P-ATPase) exists in the SR within muscle cells and transfers  $\text{Ca}^{2+}$  from the cytosol of the cell to the SR at the cost of ATP hydrolysis during muscle relaxation. There are three isoforms of SERCA viz. SERCA1-3, which have been shown to play distinct roles in various cells. Of the three isoforms available SERCA2 plays a significant role in calcium handling during myocyte contraction. Of note, SERCA2a activity is reduced in heart failure, resulting in decreased calcium uptake and impaired relaxation. Furthermore, SERCA2a activity in myocytes is controlled by phospholamban (PLN), a small

inhibitory peptide that inhibits SERCA2a in its dephosphorylated form; whereas phosphorylated PLN reduces this inhibition. Therefore, SERCA2a gene therapy would aim to increase SERCA2a activity, which in turn would increase calcium uptake and thus improve diastolic relaxation. In addition it would also increase contractile reserve because of higher SR calcium concentration.

#### 2.4.2 Phospholamban (PLN)

PLN is a protein which is encoded by the PLN gene in humans. The important function of PLN is to regulate  $\text{Ca}^{2+}$  pump in skeletal and cardiac muscle cells. It has been observed that dephosphorylated phospholamban (PLB) inhibits SERCA2a activity, while proteins such as calcium-calmodulin-dependent protein kinase (CAMkinase) and protein kinase A (PKA) cause phosphorylation of PLB to relieve this inhibition. This cross-talk between PLB and SERCA2a controls the calcium content of the SR and normal myocardial contractility. The role of PLB in cardiac  $\text{Ca}^{2+}$  handling has been elucidated through PLB-knockout mice; that displayed enhanced  $\text{Ca}^{2+}$  kinetics and showed significantly increased cardiac contractility. Paradoxically, PLB overexpressing mice showed impaired  $\text{Ca}^{2+}$  cycling associated with depressed contractile parameters. Thus, PLB plays a nodal role in the regulation of SR  $\text{Ca}^{2+}$  homeostasis through its potent action on SERCA2a activity which in turn leads to slower cytosolic  $\text{Ca}^{2+}$  decay and prolonged diastolic relaxation.

#### 2.4.3 Parvalbumin

Parvalbumin is a calcium-binding albumin protein that resides in fast-contracting muscles (highest levels), in the brain and some endocrine tissues. Gene therapy with parvalbumin, a  $\text{Ca}^{2+}$  sequestering protein, potentially provides an energy-independent removal of cytosolic calcium and thus improves the functioning of the heart. Adenovirus (AdV) parvalbumin delivery in hypothyroid rat hearts led to an increased rate of calcium removal and an improved rate of diastolic relaxation. Thus, parvalbumin may constitute a potentially attractive mode of correcting the prolonged diastolic  $\text{Ca}^{2+}$  decay generally seen in heart failure without further energy deprivation. Likewise, AdV-parvalbumin delivery to isolated cardiomyocytes from dog hearts after thoracic aortic coarctation resulted in improved relaxation kinetics but depressed sarcomere shortening at higher parvalbumin concentrations. This was probably due to inadvertent calcium removal during systole. Although potentially promising target for gene therapy in heart failure, further studies addressing the impact of long-term parvalbumin expression in relevant models of HF is warranted to clarify its role.

#### 2.4.4 S100A1

S100A1 belongs to the S100 protein family (the largest  $\text{Ca}^{2+}$  binding protein subfamily) and appears to play multiple and inimitable roles in cardiomyocyte  $\text{Ca}^{2+}$  handling. It is highly expressed in the heart and localized to SR, sarcomere and the mitochondria. It is demonstrated by some researchers that myocardial levels of S100A1 are decreased in heart failure. So, S100A1 gene delivery to cardiomyocytes may result in an increased isometric contraction followed by an increase in the amount of  $\text{Ca}^{2+}$  pumped into the SR. Adrenergic receptor stimulation in the presence of S100A1 overexpression enhanced maximal contractile performance. Furthermore, S100A1 decreases the  $\text{Ca}^{2+}$  concentration during diastole and augments  $\text{Ca}^{2+}$  release during systole by regulating both RyR and SERCA2a. In addition, S100A1 also augments SERCA2a activity during the relaxation phase and improves diastolic relaxation.

Target Gene	Species	Outcome	References
SR CA <sup>2+</sup> ATPASE	Isolated cardio myocytes	In vitro restored the contractile function of cardiomyocytes isolated from failing human hearts.	del Monte et al., 1999
	Rats	Improved systolic and diastolic function along with improved survival (63% versus 9%).	del Monte et al., 2001a
		Improved left ventricular systolic pressure, enhanced ventricular pressure rise decline (dp/dt), normalized rate of isovolumic relaxation.	del Monte et al., 2001b
	SERCA2a	Adenoviral gene transfer of SERCA2a in a rat model of heart failure (aortic banding) improved left ventricular function.	Miyamoto et al., 2000
PHOSPHO LAMBAN	Mice	Impaired Ca <sup>2+</sup> cycling associated with depressed contractile parameters .	Kadambi et al., 1996
	Human myocardial cells	Improvement in contraction and relaxation velocities.	del Monte et al., 2002
	Silencing of PLB sheep	Improved SERCA activity, improved systolic and diastolic LV function.	Kaye et al., 2007
	Mice	Enhanced Ca <sup>2+</sup> kinetics.	Kiriazis et al., 2000
PARV ALBUMIN	Dog cardio myocytes	Improved relaxation kinetics but depressed sarcomere shortening at higher parvalbumin concentrations.	Hirsch et al., 2004
S100A1	Cardiomyocytes	Increase of isometric contraction followed by an increase in the amount of Ca <sup>2+</sup> pumped into the SR.	Remppis et al., 1996  Most et al., 2006  Pleger et al., 2007
	Mice	Substantially worsened LV function, transaortic constriction and MI with significantly lower survival.	
	Intracoronary delivery of AAV6-S100A1Post-MI rat heart	LV dysfunction and HF was evident initially and 2 months after gene delivery, S100A1-treated HF rats presented with significantly enhanced cardiac function and a reversal of LV remodeling compared to control HF rats.	
	AdV-mediated S100A1 gene transfer to failing rat cardiomyocytes	Restoration of disturbed Ca <sup>2+</sup> handling by increasing reuptake of SR Ca <sup>2+</sup> during the relaxation phase and a lowering of the RyR-mediated Ca <sup>2+</sup> leak.	

Table 4. The examples of various proteins involved in calcium signaling studied for gene therapy.

2.5 Role of cell cycle activation in gene therapy

The normal cell passes through the stages of G1-S-G2-M in that orderly sequence as a part of the cell cycle; with a quiescent G0 phase before entering the S phase from G1. This cell cycle is highly subject to strict regulation by a number of molecules that either enhance or retard the progression of the cycle. The two important check-points G1-S and G2-M demand the requirement of a group of proteins called cyclins to overcome them; that do so by combining with a group of cyclin-dependent kinases (CDKs). The expression of cyclins is enhanced by dephosphorylation of Rb protein; mutations in which are found to be responsible for the ocular tumour retinoblastoma. Similarly, the CDKs are inhibited by the proteins of the Cip/Kip family as well as those of the p16/INK4a locus. A major role is also played by p53 protein, that halts the cell cycle, allowing time for the DNA damage to be rectified; if successful, terminates its own action by inducing autocatalysis through induction of Mdm2 and if the DNA damage cannot be repaired, initiates apoptosis. Cell cycle activation is another promising application of gene therapy which results in the induction of endogenous myocardial regeneration. In various pre-clinical set ups it has resulted in activation of the cardiomyocyte cell cycle, thereby limiting the infarct size and improving LV dysfunction. The cyclins and the CDKs are the various candidate genes, the induction of which permits the cell to overcome the endogenous checkpoints and continue with the replication, thus allowing the cardiomyocyte growth. Studies in animals have yielded encouraging results, as listed in the following table; while clinical trials in humans are still awaited (Table 5).

Target Gene	Species	Outcome	Reference
Cyclin -A2	Rats	Increased border-zone myofilament density and improved myocardial function	Woo et al., 2006
CDK4	Rats	Improved left ventricular function	Tamamori-Adachi et al., 2008

Table 5. The examples of proteins involved in cell cycle studied for gene therapy.

2.6 Role of  $\beta$ -adrenergic system in gene therapy

$\beta$ -adrenergic receptor blockers have been shown to exert favorable effects in heart-failure patients. Numerous clinical and experimental studies have shown that molecular targeting of various proteins within the cardiac beta-adrenergic receptor ( $\beta$ -ARs) pathway may be beneficial in heart failure. Chronic heart failure due to MI is associated with increased sympathetic discharge. However, this increased sympathetic activity is compensated mechanistically, but is more injurious in the long term. The  $\beta$ -adrenergic system is affected by multiple modifications including  $\beta$ -AR down-regulation, up-regulation of  $\beta$ -AR kinases and increased Gi function leading to  $\beta$ -AR desensitization and decreased  $\beta$ -AR signaling activity in heart failure. Several gene-based experiments tested and established that cardiac functions are improved or enhanced by genetic manipulation of the myocardial  $\beta$ -AR system.

2.6.1  $\beta$ 2-adrenergic receptor overexpression

Although  $\beta$ 2-AR overexpression in mouse hearts results in improved systolic and diastolic function; but at significantly high levels, mice developed fibrotic cardiomyopathy and heart

failure. Moreover,  $\beta$ 2 signaling stimulates cell-survival and protects myocyte damage from apoptosis. This fact led to the use of  $\beta$ 2-AR gene delivery in various experimental models of heart failure (Table 6).

2.6.2 Inhibition of G protein-coupled receptor kinases (GRKs)

Homologous desensitization (agonist dependent) is mediated by G protein-coupled receptor kinases (GRKs). GRK-2 upregulation is responsible for  $\beta$ -AR desensitization in heart failure and these kinases dampen the interaction between  $\beta$ -receptors and their G proteins. In addition,  $\beta$ -ARKct is a peptide within the carboxy terminus of GRK2 that inhibits GRK-2 mediated  $\beta$ -AR desensitization.  $\beta$ ARKct gene transfer to isolated failing human cardiomyocytes improved their contractile function.

2.6.3 Adenylatecyclase type 6

$\beta$ -AR stimulation activates adenylate cyclase (AC) through G-protein activation and AC then activates protein kinases to exert its downstream effects. AC6 is the predominant cardiac isoform and its overexpression in cardiac tissue leads to increased left ventricle contractility and function.

Target Gene	Species	Outcome	References
$\beta$ -ARs	AdV $\beta$ -AR intracoronary delivery mice	Enhanced cardiac function.	Shah et al., 2000
Inhibition of GRK-2by $\beta$ ARKct gene transfer	Isolated failing human cardiomyocytes  Intracoronary delivery of AdV- $\beta$ ARKct in mice	Improved their contractile function.  Improved cardiac function post-MI.	Jameel & Zhang, 2009  Iaccarino et al., 1998
Adenylate cyclase type 6	AdV-AC6 gene delivery in pigs	Improved LV contractility and function.	Lai et al., 2004

Table 6. Proteins involved in  $\beta$ -adrenergic system studied for gene therapy.

3. Clinical aspects

The human quest and efforts to expand the boundaries of his knowledge into the arena of gene therapy is endless. Genes responsible for cardiovascular events or diseases, myocardial



Clinical Trial	Sample size = n	Outcome	References
Intramyocardial injection of adenovirus (Ad.VEGF-121) by thoracotomy & CABG / by minimally invasive thoracotomy	21	Both groups showed reduction in angina but no improvement in exercise duration. Proper conclusions could not be drawn due to absence of control group.	Rosengart et al., 1999
Injection of ph.VEGF-165 into LV myocardium by minimally invasive thoracotomy	5  20	Reduction in angina and nitroglycerine use in 60 days in all 5 patients. Results limited because of a small sample size and absence of controls. Reduction in angina and nitroglycerine use in 90 days in 16 out of 20 patients. Results limited due to absence of controls.	Losordo et al., 1998
Administration of VEGF-165	20	Significant symptomatic improvement in patients with inoperable CAD	Symes et al., 1999
Catheter-mediated VEGF plasmid/liposome (P/L) gene transfer	15	Catheter-mediated intracoronary gene transfer performed after angioplasty significantly prevents restenosis and myocardial ischemia.	Laitinen et al., 2000
Percutaneous catheter-based gene transfer of naked plasmid DNA for VEGF-2 to LV myocardium	19	Decrease in angina & improvement in symptoms. Drawback: sample size is low.	Losordo et al., 2002

Table 7. Clinical trials for gene therapy in myocardial infarction. Ad.VEGF-121: Adenovirus encoding Vascular Endothelial Growth Factor-121; ph.VEGF-165: Plasmid vector encoding Vascular Endothelial Growth Factor-165; Ad.FGF-4: Adenovirus encoding Fibroblast Growth Factor-4; LV: Left ventricle; LacZ: Z gene of lac operon; HIF- $\alpha$ : Hypoxia-Inducible Factor- $\alpha$ .

infarction in particular, have been identified and targeted as a means of curative approach. An example can be illustrated of the study conducted by Doney and his colleagues (Doney et al., 2009) who identified an allele of rs9939609 gene, also referred to as FTO (Fat mass and obesity associated) gene. This gene increased the risk of Type II diabetes mellitus, an atherogenic lipid profile (including decreased high density lipoprotein and increased triglycerides) and myocardial infarction. This allele presents a target which if suitably replaced by the wild type can decrease the risks for all the above conditions. One of the main drawbacks for designing gene therapy for myocardial infarction is the ethical concerns pertaining to the selection of participants. The participants are among those who have suffered an attack of myocardial infarction and conducting studies on such a



cohort of patients with a risk of failure of therapy raises a substantial debate. Apart from this, the set-up for these studies also demands technical and financial support on a tremendously large scale. It is for these very precise reasons that a large number of these studies have not yet been conducted so far and hence the relative paucity of these studies in literature as compared to other studies. However the recent advances in the study of human genome and identification of genes associated with the risk factors, pathogenesis as well as outcome of myocardial infarction, has given a great impetus for designing clinical trials for gene therapy. As a result, a good number of trials are currently underway, for instance, the endocardial gene therapy with VEGF-D for severe coronary heart disease.

Apart from the ethical and financial constraints, there are practical difficulties as well, for instance, the delivery of viral particles in to the human body may stimulate the immune system that stimulates the T lymphocytes and cytokines to clear the virus. The naked DNA administered may be lysed by DNases present in the serum. The permeability of the naked DNA across cell membranes is also restricted, necessitating the requirement for intracellular transfer. On the other hand, an intravenous injection may result in transfection of other tissues apart from the heart and lodgement of the vectors in other organs such as the lung or kidney. To circumvent these possibilities, the technology required for gene transfer needs to be highly advanced and sophisticated to ensure that the vector containing the therapeutic gene transfects only the desired myocardial cells. An example of such technology is the UTMD (ultrasound targeted microbubble destruction), in which ultrasound contrast agents used as gas microbubbles, are packed with the therapeutic gene containing DNA and are destroyed within the myocardium by subjecting them to high ultrasound frequency. A very high number of clinical trials have not been conducted for gene therapy in MI. Some of the clinical trials that have been undertaken in this regard are enlisted in table 7.

#### **4. Limitations of gene therapy**

As with most experimental therapies, safety of gene therapy for ischemic heart disease is of paramount importance. Though clinical trials have shown short-term safety, long-term surveillance over a period of decades is lacking. The question still remains as to which therapy benefits what sub-population of patients. Inclusion of a wide selection of patients in studies over time may lead to improvement in subgroups of patients if not the entire population. Confounding factors such as use of concurrent medications and concurrent medical conditions lead to difficulty in standardizing groups of patients. Objective end points of assessment need to be used uniformly as exercise testing may be subjective and is victim to high variability in the same patient on different days. Frequency of testing for objective improvements may need to be ramped up as the effects of therapeutic genes may have abated at the time of a single test. Another surprising factor that confounded results of clinical trials was a strong placebo effect. This might be minimized when objective and not subjective end points are used when assessing outcomes. Drug related issues such as the dose, gene transfection efficiency, pharmacokinetics and pharmacodynamics of individual therapies are valid as these may differ in different populations of patients. Also cost-effectiveness analysis has to be considered, as production of gene therapy vectors itself is cumbersome, requires specialized equipment and personnel and the administration of gene therapy is invasive in nature. Besides, specific gene therapy may not compare favourably to available pharmacological agents in use to treat ischemic heart disease in terms of cost-benefit ratio.

## 5. Conclusions and future directions

As of now, the applications of gene therapy for treatment of human diseases, including cardiovascular conditions, though appears to be extremely promising and fascinating, is, in the true sense of the term, still in its infancy. A vast magnitude of studies, not only on humans, but further studies on animal models, are still necessary to validate the practical efficacy of this widely-sought-after approach. Overcoming the practical difficulties discussed above is not an easy task and a tremendous amount of finance and infrastructure is an inevitable requirement to achieve this goal. In spite of that, there still remains a possibility that the gene therapeutically administered may not be expressed in the target individual, subject to an inability of the vectors to reach the target tissue, metabolism of the vectors by the immune system or the presence of other intracellular substances that may exert a negative regulation on the gene expression.

It is possible that extensive use of small animals for pre-clinical research may have led to excessive enthusiasm too early. Gene therapy testing on larger animals may provide a better insight into the true efficacy of specific therapies.

However, these shortcomings should not prove to be a permanent obstacle as the prospects offered by the advent of gene therapy have been extremely fascinating. The elucidation of the human genome has exposed a vast array of genes that are responsible for umpteen number of diseases; such as cystic fibrosis, familial hypercholesterolemia, Lesch-Nyhan syndrome, Alzheimer's disease, Parkinson's disease, Duchenne muscular dystrophy, multiple sclerosis and others. These genes offer potential candidates for regulation that can alter the course of the disease as against the current medications available that mainly provide symptomatic relief. Thus, the future for gene therapy appears to be very bright. A good political establishment to provide the necessary infrastructure and monetary support might go a long way to circumvent the existing shortcomings and empower gene therapy to grow as the most preferred and successful therapeutic approach to most of the diseases that were considered incurable till the past few decades.

## 6. References

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## **Gene Therapy - Developments and Future Perspectives**

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The aim of this book is to cover key aspects of existing problems in the field of development and future perspectives in gene therapy. Contributions consist of basic and translational research, as well as clinical experiences, and they outline functional mechanisms, predictive approaches, patient-related studies and upcoming challenges in this stimulating but also controversial field of gene therapy research. This source will make our doctors become comfortable with the common problems of gene therapy and inspire others to delve a bit more deeply into a topic of interest.

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Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
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Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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