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7

Gene Therapy in Cardiovascular Disease

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1. Introduction

Gene therapy is defined as the transference of nucleic acids to somatic cells of an individual resulting in a therapeutic effect (Yla-Herttuala & Martin, 2000). Among the advantages of gene therapy over the existent modalities of treatment are: the selective treatment of affected tissues, the possibility of using locally endogenous proteins in cases where its systemic application would incur in serious adverse secondary effects, and the possibility of therapeutic effects in the long term after a single application (Yla-Herttuala & Alitalo, 2003).
Despite medical advances in the last 30 years, cardiovascular diseases (CVD) constitute the main cause of death on the developed world. Ischemic Heart Disease (IHD) is one of the main morbidities in an ageing population. Over 5 million Americans are believed to have symptomatic Heart Failure (HF), and every year ~0.5 million patients are diagnosed, with a prevalence of 0.5-2% of the general population; the estimated direct and indirect costs of HF in the United States is ~$30 billion. HF is a disabling chronic disease and the most frequent discharge diagnosis for hospitalization among older adults. Despite the significant resources for its treatment, outcomes remain poor. The five-year survival for individuals diagnosed with HF is less than 50%, and in end-stage HF one-year survival may be as low as 25% regardless of medical therapy, which constitutes one of the dimmest prognosis compared with of any malignant disease, with the exception of lung and pancreatic carcinomas (Levy et al., 2002; Lloyd-Jones et al., 2002).
HF constitutes the end stage of most CVD, it has become one of the most researched fields in the last 10 years, with significant advances to understand its key mechanisms; and today presents a great challenge to modern medicine, because of its persistent high mortality and increasing incidence. This can be attributed partially, to the absence of therapies that are focused on the key molecular basis of the disease, although the therapeutic approach has improved in last the two decades, existing treatments are not ideal due to its broad scope and do not have a significant impact on the overall mortality, since they are not sufficient to support to the myocardium performance and to increase the global cardiac function. It is for this reason that new therapeutic approaches are required, focused to correct the molecular defects of HF.
Among the molecular defects observed during HF, that have been explored as therapeutic targets, are the alterations in Ca^{2+} handling during the excitation-contraction coupling (Hoshijima et al., 2002; Iwanaga et al., 2004; Kaprielian et al., 2002; Michele et al., 2004; Miyamoto et al., 2000; Most et al., 2004b; Most et al., 2007; Pleger et al., 2007; Pleger et al.,

2005; Szatkowski et al., 2001), alterations in the β-adrenergic receptors and their interaction with G proteins (Jones et al., 2004a; Koch, 2004; Maurice et al., 1999; Munch et al., 2005; Tevaearai et al., 2002), alterations of cellular signaling, including the members of the protein kinase C (PKC) family (Hambleton, 2006), and to the production of second messengers by the enzyme adenylyl cyclase (Lai et al., 2004). The apoptosis of cardiac myocytes also has been mentioned (Chatterjee et al., 2002; Tenhunen et al., 2006). Finally, the use of over-expressing angiogenic factors, also has been analyzed as alternatives in patients with HF secondary to ischemic cardiomyopathy.

As a therapeutic strategy for CVD, gene therapy is a field that during the last decade has experienced a substantial increase in research. Some diseases like hypertrophic cardiomyopathy, have a clear-cut genetic basis, arrhythmogenic right ventricular dysplasia, and as such constitute a theoretically obvious target. Nevertheless, gene therapy can also be used to normalize the expression of genes that undergo down regulation in pathological ventricular hypertrophy, where the cardiac myocyte responds to a physiological tension, such a pressure or volume overload, by means of the activation of a fetal genetic program, with a subsequent downgrade of performance, thus starting a deleterious cycle, with further deterioration of cardiac function, as observed in HF. The hypertrophic response is associated with a decrease of gene expression of several key proteins, such α– and β–myosin heavy chains, the Ca^{2+}-ATPase pump of the sarcoplasmic reticulum (SERCA2a) and its regulatory protein phospholamban (PLB), and the S100A1 regulatory protein.

There are diverse strategies used for the treatment of HF, mainly constituted by pharmacological therapy that it is mainly focused on a neurohormonal blockade; further treatment can be offered with more invasive therapies such as cardiac re-synchronization; mechanical unloading with different surgical strategies and the use of left ventricular assist devices, which can induce myocardial remodeling at the molecular level normalizing gene expression. Currently the only definitive strategy for treatment of end stage HF is cardiac transplantation. However, despite of the advances in the treatment of HF with the addition of new drugs and very effective devices, the number of patients with HF is increasing (Hamad et al., 2007). Without a great scale effective strategy, regenerative medicine including stem cells and gene therapy could provide with a solution to an increasing number of patients currently without a therapeutic option.

While most of the research of gene therapy in CVD has been for HF; it is not limited to normalize the expression of down regulated genes, it also offers the possibility of modulating inflammatory and angiogenic mediators, which have a key role in several other CVD. Ischemic heart disease continues to be one of the main causes of mortality in the developed world, and while the treatment by medical, interventional and surgical options, has greatly decreased the morbidity and mortality associated, there is still a subset of patients that cannot be successfully treated with the current therapeutic options. Thus therapeutic angiogenesis in ischemic myocardium, mediated by gene therapy may also be an option in a clinical setting. As mentioned the treatment by interventional cardiology (PTCA) and surgery (CABG), are highly effective strategies, both with some long term drawbacks, specifically intra-stent restenosis in the former, and vein graft disease in the later. In both cases gene therapy may provide a key role to enhance, already very successful therapies. For example, transient, nonintegrative gene expression has been shown to be sufficient to promote neovascularization in the case of angiogenesis. This may also apply to antiproliferative strategies for the prevention of neointima formation in patients that received PTCA, for the prevention of intra-stent restenosis, or for inhibition of

atherosclerosis in venous and arterial grafts. Because cardiovascular diseases are diverse and as such have unique traits, they require precise tailoring of gene therapy strategies for particular disease. Those features, which may vary include mode of delivery, type of vector, length of gene expression, and target tissue. The angiogenic factors that have been object of a more exhaustive study are the vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF). These cytokines can be administered in the form of recombinant protein or of the genes that they codify for these proteins. Each of these approaches presents a series of advantages and disadvantages that are being investigated in detail, in animal models and in clinical tests with humans; and may also become options in the clinical setting in a not so distant future.

Another exciting new target for gene therapy are the electrical and rhythm disturbances in the heart. While a number of tachy-arrhythmias have a clear genetic origin in channel protein mutations, or calcium modulating proteins, these monogenetic arrhythmias, may only benefit a small number of patients, but current research has shown encouraging results in the treatment of bradi- and tachy-arrhythmias of multifactorial origin, and also in atrial fibrillation.

In the last decade a significant number of molecular mechanisms have been identified as the molecular basis of HF (Yla-Herttuala & Alitalo, 2003). Likewise the possibility of perform transference of genomic material *in vivo*, has been made possible exploring this technique as an option to correct the defects present in CVD, under the premise of being a therapy able to correct the underlying defects present in the failing heart.

Although the treatment by transference of genetic material still presents many challenges, small clinical tests have already been performed, applying angiogenic factors in ischemic cardiomyopathy (Hedman et al., 2003; Kastrup et al., 2005; Stewart et al., 2006). In relation to other molecular mechanisms of the HF, as it is mentioned below, several of them are in clinical phases in different stages of proximity to a wider clinical setting. The potential that has this new therapeutic option, combined to the novelty of its mechanism of action, makes imperative that its advantages and disadvantages are known to be able to take advantage of this emergent therapeutic option.

During the late 1990s and early 2000s, gene therapy for CVD, experienced a significant expansion, searching for targets that could benefit a large number of patients; while the results where promising on experimental models, these were not translated to the clinical setting. This added to the rising of stem-cell research, resulted in an abandonment of the field, however with the increasing data identifying the molecular basis of HF, it has become clearer that a "silver bullet" is not feasible and a more combinatorial approach may be required. Thus the field of gene therapy has experienced a rebirth in the last years. Evidently an ideal gene therapy depends on the underlying cause of HF, and as such a multistep approach may be required. For example, when utilizing gene therapy for the most common cause of HF which is IHD, it may be needed to target endothelial cells to manage the atherosclerotic plaques and induce angiogenesis, providing means to inhibit apoptosis of hibernating myocardium, and long term inotropic support, reducing cardiac remodeling and reducing risk of arrhythmias. This approach clearly illustrates that a "one size fits all" approach is impossible, and that manipulation at several levels is needed, in most cases gene therapy would be needed to over-express target molecules, modifying of intracellular signaling, loss of function by the use of dominant negative molecule or introducing siRNA, correction of mutations in the genome; and also combine with cellular therapy by introducing genetically modified cells (Vinge et al., 2008). In 2010, the number of gene

therapy clinical trials for CVD in the world was 144, representing 8.5% of the total, occupying the second place after gene therapy for cancer diseases that represented 64.5% of all ongoing gene therapy trials.

Therefore, it becomes clearer that the possibility that by gene therapy, we could improve the cardiovascular function through a normalization and/or modulation of gene expression in the near future is one of the most promising and active clinical investigation goals in the 21st century. The purpose of this chapter, is to review the current status of the literature in the basic and clinical settings, and analyze future perspectives for cardiovascular gene therapy.

2. Vectors used for cardiovascular gene therapy

The initial tests to transfer genetic material into the heart were done using plasmid DNA, chosen by its easy production and security. Although the first studies showed positive results (Fortuin et al., 2003; Tsurumi et al., 1996), in randomized larger tests, where genes coding for vascular endothelial growth factor (VEGF) as well other angiogenic factors were transferred, they showed that due to its very low efficiency was not a useful technique (Kastrup et al., 2005). The use of cationic liposomes in the transference of genetic material in cells in culture, does not increase the efficiency of transference *in vivo* (Laitinen et al., 1997; Wright et al., 2001). One of the main problems with the use of plasmid DNA, is the lack of response with an increasing dosage (Rutanen et al., 2004). The use of this vector, is considered relatively safe, but it has demonstrated in animal models that it can produce fever, inflammation and infarcts in the skeletal muscle and in the myocardium (Hedman et al., 2003). DNA and RNA oligonucleotides potential to be used as decoys, were mentioned over 10 year ago, but their minimum effectiveness in tissues, also discards them for some clinical application (Alexander et al., 2005). Due to above factors the viral vectors can be a better option for cardiovascular gene therapy (Table 1).

Small interference RNA (siRNA) is a useful tool to down-regulate the expression of genes *in vitro*, but as an isolated technique has proven to be inefficient in the preclinical and clinical setting; thus it was discarded over 10 years ago, as a viable option. However in the recent years its combination with viral vectors has reignited the interest on this strategy; and it may become a key tool for inhibiting the expression of genes with deleterious effects (Poller et al., 2010; Suckau et al., 2009; Yang et al., 2010).

Among the viral vectors, the retrovirus were the initial choice, because of the limited immune response generated and the possibility of a sustained expression (years), nevertheless, the retrovirus have several disadvantages for its use on CVD, including low efficiency of infection, limited cardiac tropism because they infect mainly dividing cells; and also concerns in their safety have been mentioned due to the oncogenic potential (Laitinen et al., 1997). Because of this reasons it is a vector in which the interest has decreased. Recently, lentivirus have been used mainly to transfer genetic material to cells of the vascular wall, they have demonstrated a high efficiency of infection in smooth muscle and elicit a minimum immune response, nevertheless, the tropism for myocardium is limited and its production in great amounts is difficult, thus currently is not a viable option and requires engineering of this vector to improve its utility on a large scale, facilitating its production (Kankkonen et al., 2004). Sendaivirus and herpesvirus, have been mentioned as possible useful vectors, but sufficient data to this date does not exist on their utility (Masaki et al., 2001). Currently the adenovirus (Ad), and the adeno associated virus (AAV), are the most studied vectors in the cardiovascular gene therapy, since both have demonstrated a great effectiveness in cardiac muscle, the vascular wall and the liver (Wright et al., 2001).

Vector	Advantages	Disadvantages
Naked plasmid DNA	Easy to produce. Safe.	Temporary expression. Low transfer rate.
Adenovirus	High transfer rate. Easy to produce substantial quantities. Transfer to quiescent cells. Big size transgene (7-30 kb). Multiple cell type tropisms.	Immune response, dose dependent. Temporary expression.
Adeno associated virus (AAV-1, 2, 5, 6, 8, 9)	Long-term expression. Moderate immune response. Transfer to quiescent cells. High tropism for skeletal muscle (AAV-1, -6) and cardiac (AAV-8, -9). Do not produce diseases in humans.	Limited transgene size (5 kb). High yield production difficulties.
Retrovirus	Long-term expression. Minimal immune response.	Low transfer rate. Non-controlled integration to genome. Limited tropism. Transfer only on dividing cells.
Antisense oligonucleotides, siRNA	Easy to produce.	Limited *in vivo* efficiency. Requires high transfer rate.

Table 1. Vectors used in Cardiovascular Gene Therapy.

The first generation Ad, have a very high initial expression, reaching their maximum effect in the first days after the material transference, but the expression is diminished after approximately 2 weeks (Poliakova et al., 1999; Rissanen et al., 2003; Rutanen et al., 2004). Therefore they seem to be useful when a high expression is required for a limited amount of time, like in therapeutic angiogenesis for wounds. The repeated administration of Ad for gene therapy is not useful in great mammals, since it evokes an important immune response. Small doses of Ad produce a minimum inflammatory response in the myocardium. The second and third generation Ad, produce a smaller immune response and seem to have a prolonged expression, but its utility in gene therapy trials in humans is not yet known (Wen et al., 2004). The security of the Ad has demonstrated to be very high in clinical trials, being the fever the most important complication.

The AAV have a number of characteristics that makes them potentially useful for cardiac gene therapy, they have a natural tropism towards vascular smooth muscle, skeletal muscle and myocardium (Gruchala et al., 2004). They are able to express genes in quiescent cells like Ad, and for a long period of time, although the maximum expression is reached in days

(Su et al., 2004). Because they do not produce diseases in the human, the inflammatory response that they generate is minimum; the native AAV are able to integrate themselves to the human genome, but modified AAV vectors cannot, and maintain their long term expression by means of episomal associations with the genomic DNA, thus reducing the risk of mutagenic and oncogenic insertions, a threat that at least theoretically is present with other vectors of long term expression like the retrovirus (Schnepp et al., 2003). In murine and rodent models single stranded (ss) AAV6, self-complementary (sc) AAV6 and ssAAV9 are capable of long term expression after transference of genetic material to the cardiomyocytes, however it has not been reproduced in large animals. Recently it has been proposed that the highest levels of expression in large animals, are obtained using scAAV6 vectors, delivered through surgical methods (White et al., 2011).

3. Routes of administration

The transference of genetic material to the heart can be obtained by three different routes, by intravascular transference, *ex-vivo* transference, and by intra-organ injection, in the following sections we describe in more detail these routes.

3.1 Intravascular transference

For the intravascular transference, different systems using catheters have been developed to improve penetration of the vectors through the intima vascular layer. Nevertheless the transference in vascular athrosclerotic injuries with calcifications, deposits of cholesterol, infiltrated and continuous inflammation are a significant obstacle to achieve satisfactory transfer (Laitinen et al., 1998). The use of porous stents, in which the vectors with therapeutic genes can be eluted, has been proposed as an option to improve the transference, but currently has not been sufficiently explored (Sharif et al., 2006; Walter et al., 2004). The ideal route of administration for gene therapy is an intravenous vector, but the required amount of virus to obtain an optimal expression in great mammals, is probably very high to consider it safe for humans.

3.2 *Ex vivo* transference

The *ex vivo* administration is a combination of the cellular therapy and gene therapy, it consists of the administration of the test-tube vector, to isolated stem cells from the bone marrow, or cultures of skeletal myoblasts, with the intent of later transferring these cells transformed with the transgenes into the patient (Assmus et al., 2006; Dib et al., 2005; Haider et al., 2008; Haider et al., 2007; Nasseri et al., 2007; Sim et al., 2007; Smits, 2004; Yau et al., 2005; Ye et al., 2005). This form of administration has been studied mainly in the expression of angiogenic factors, and cytokines (Assmus et al., 2006; Haider & Ashraf, 2008; Ye et al., 2005). Among the advantages of this form of administration it is the possibility of using efficient methods for the transference of genetic material, like cationic lipids or electroporation, that are not possible to use in the whole subject, and which in addition the location of the modified cells in the heart, has a localized, continuous expression and with constant levels, without systemic effects (Haider & Ashraf, 2008; Sim et al., 2007; Smits, 2004). The cardiomyocytes are by nature resistant to the transference of genetic material, and this form of administration avoids this complication. The *ex vivo* administration, has the potential to become a very useful tool, but at present it depends on stem cell research and their differentiation to the cardiac phenotype, so that it can be explored widely and to know

its potentials and limitations. One of the major drawbacks with this route of administration is the location of the transferred cells to the patient. The intramuscular injection of *ex vivo* transformed cells to express the gene of interest has been mentioned, but the little distribution of the transferred cells has made difficult its use (Lee et al., 2000; Springer et al., 1998).

3.3 Intra-organ transference

The administration by intra-myocardial injection, at present it is considered the most efficient form to obtain the desired expression in the cardiac tissue, but it as has been reported the administration of a high volume of vector to obtain an expression, can be associated with a local injury and alterations in the structure of the myocardium wall (Kastrup et al., 2005; Rutanen et al., 2004; Vale et al., 2001). Different studies have demonstrated that the intra-arterial route is of little effectiveness unless the permeability of the endothelial lining is increased, or a high pressure gradient is used; in these cases a problem to consider is the increased tissue distribution and ectopic expression of the transferred gene (Lee et al., 2000; Rissanen et al., 2003; Wright et al., 2001). In animal models the most used techniques include the use of percutaneous devices, one is known as the antegrade epicardial coronary artery infusion, where the left main coronary artery is catheterized, simultaneously with the coronary sinus, isolating the coronary circulation by means of an oxigenator and a extracorporeal pump, where the solution with the vector is added and circulated through the coronary circulation continuously for the desired time (Kaye et al., 2007). This system is known as "V-Focus", and has shown satisfactory results in larger animal models. Another system which employs percutaneous devices, is the percutaneous antegrade myocardial gene transfer, in this system the left anterior coronary artery (LAD) and the circumflex artery are catheterized, while the great cardiac vein or the anterior descending vein are catheterized, when the vector is infused through the arterial catheters, a balloon is inflated in the venous catheter, thus creating a high pressure gradient for up to three minutes (Hayase et al., 2005). The system in which the greater effectiveness of transference has been observed, consist of perfusion of the myocardium by surgical methods, a technique known as Molecular Cardiac Surgery with Recirculating Delivery (MCARD); this consists in the establishment of conventional cardiopulmonary bypass, with isolation of the heart circulation and retrograde perfusion through the coronary sinus. MCARD allows performing surgical procedures in the heart while the transference of genetic material is occurring (Hayase et al., 2005; Katz et al., 2010; Raake et al., 2004; White et al., 2011). An option to avoid the ectopic expression of the transferred gene is to put the expression of the transferred gene under the control of a specific cardiac promoter, as is the case of the human cardiac calsequestrin gene (hCasq2) (Reyes-Juarez et al., 2007). At the moment, in our laboratory we are developing adenoviral vectors that contain the promoter of the gene hCasq2 and direct the expression of the calcium pump SERCA2a in specific form for the cardiac myocytes (unpublished results).

4. Heart failure: therapeutic targets

As it was mentioned briefly in the introduction, one of the main advantages of gene therapy as a therapeutic option is the possibility of modifying key molecular defects that are present in the failing heart. In the last years the knowledge of the molecular basis of HF has advanced significantly, and today multiple alterations that exist in the continuum of the

natural history of HF are known. Although there is not an integrative model that is able to ensemble the multiple alterations described in HF (Braunwald & Bristow, 2000), due to multiple etiologies, the existing data allows us to identify targets for gene therapy. In the following sections we describe the different approaches that are being explored to treat cardiovascular diseases.

4.1 Alterations in calcium handling

Without doubt the area of greater interest in the molecular mechanisms of HF, are the alterations on the regulation of the concentrations of Ca^{2+} during the process of the excitation-contraction coupling, which have been demonstrated to play a critical role in the decreased contractile properties of the myocardium. Among the involved alterations are changes in the levels of the expression of regulatory proteins that act as sensors of the intracellular Ca^{2+} concentrations (Kaprielian et al., 2002).

These alterations include decreased levels of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), with a subsequent decrease of the recapture of Ca^{2+} to the sarcoplasmic reticulum (SR) during diastole, resulting in a smaller amount of Ca^{2+} available to be released in the following contraction cycle. The decrease of the functional levels of phosphorylated phospholamban (PLB), a regulator of the activity of SERCA2a, causes the activity of SERCA2a to be decreased during HF; this decrease is not only due to a smaller expression of the SERCA2a protein, but to alterations in the level of phosphorylation of PLB as a result of an increase in the levels of the members of the family of the protein kinase C (PKC), which phosphorylates the inhibitor of phophatase-1 (PP1), activating it, which causes a dephosphorylation of PLB. The Ca^{2+} release channel of the SR (Ryanodine Receptor, RyR), has been reported in an unstable state, dissociated of the protein calstabin, which results in a higher probability of opening of the channel, and spontaneous liberations of Ca^{2+} appear during diastole (diastolic leak) which are associated with ventricular arrhythmias and more importantly with a decrease of the reserve of Ca^{2+} available for contraction (Bers et al., 2003; Braunwald & Bristow, 2000; Yla-Herttuala & Alitalo, 2003).

The existing reports have focused to the correction of these defects; the therapeutic targets include the increase of the expression of SERCA2a, or blockade the expression of their regulator PLB, the regulatory protein S100A1, and parvalbumin in order to make more efficient the cardiac relaxation. In the following pages each of these strategies is detailed.

4.1.1 The sarcoplasmic reticulum Ca^{2+}-ATPase and phospholamban

The calcium pump SERCA2a has a fundamental role in the normal myocardium function during muscular relaxation, recapturing the majority (75%) of the released Ca^{2+} from the SR for contraction, and reloading the SR maintaining an amount of Ca^{2+} that is sufficient to achieve an optimal contraction. Due to this role it is a central point of the molecular basis of HF; where its expression is regulated in a negative manner, diminishing the capacity to reload the SR with Ca^{2+} after each contraction, resulting in a deficient contraction due to lack of Ca^{2+} inside the SR (Kaprielian et al., 2002). In the last years the expression of SERCA2a, became one of the most explored pharmacological targets in basic research, and to a lesser extent in clinical investigation and, although without conclusive positive results obtained (Zarain-Herzberg & Rupp, 2002; Zarain-Herzberg et al., 1996). The use of gene therapy, to over-express SERCA2a, has the advantage of eliminating the associated effects of drugs used to increase the expression of SERCA2a (Gianni et al., 2005).

In similar way that the induction of SERCA2a expression has been extensively explored by pharmacological means, the over-expression by transference of genetic material, also has

been analyzed *in vitro* and *in vivo*. Over-expression of SERCA2a improves the contractile function and the energy consumption in animal models with HF, improving the thickness of the anterior wall, and reducing ventricular arrhythmias. Other studies have also shown a decrease of inflammatory mediators and pro-apoptotic markers after transference of viral vectors overexpressing SERCA2a in pressure overload induced HF inhibition of ventricular remodeling in ischemic and volume overload induced HF; (Beeri et al., 2010; Mariani et al., 2011; Miyamoto et al., 2000; Molina et al., 2010).

Recent studies suggest that the failing heart is not refractory to treatment as was previously believed. For example, the observation that a small percentage of subjects with left ventricular assist devices (LVADs) can be permanently weaned from the device, strongly suggests that damaged hearts are capable of recovering lost function (Baba & Wohlschlaeger, 2008; Entwistle, 2003; Kuhn et al., 2004).

Recently, in the United States a phase 2 clinical trial named CUPID (Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) of intracoronary delivery of AAV1/SERCA2a (MYDICAR®) to 39 patients with HF that started in 2007 was completed (Hajjar et al., 2008; Jaski et al., 2009). It was designed to evaluate the safety profile and biological effects of SERCA2a gene transfer; after 12 months follow-up, the patients showed an acceptable safety profile, and improvement of the functional class. There was no increase in adverse effects, disease-related events, laboratory abnormalities or arrhythmias. Currently, there are two other ongoing gene therapy clinical trials to express SERCA2a in the hearts of patients with HF using AAV6/SERCA2a; one in England and one in France. Therefore, the transfer of SERCA2a cDNA into cardiomyocytes is a promising approach to treat HF by gene therapy (Table 2).

In transgenic mice overexpressing SERCA2a subjected to ascending aortic constriction, the down-regulation of SERCA2a protein in hypertrophic hearts was prevented, however, the hearts showed no increase in inotropic response compared to the wild type mice hypertrophic hearts, suggesting that energy supply may be a limiting factor for the benefit of SERCA2a overexpression in hypertrophied hearts (Pinz et al., 2011). Therefore, it should be considered novel strategies combining energetic support with increasing SERCA2 activity might improve the therapeutic effectiveness for HF.

Closely related to SERCA2a is phospholamban (PLB), a peptide of 52 amino acids, that has a role as a regulator of the function of SERCA2a, inhibiting the pump in its native state, and dissociating from the pump when is phosphorylated by the protein kinase A (PKA), increasing the velocity of transport of Ca^{2+} by the enzyme. During HF, the SERCA2a/PLB ratio is diminished to a great extent by the diminution of SERCA2a, which causes that the amount of SERCA2a that is dissociated of PLB diminishes and with this its activity and capacity to recapture the Ca^{2+}, released during the contraction (Hoshijima et al., 2006).

The most used strategy of gene therapy that involves PLB, consists of the transference of a peptide that codifies for a pseudo-phosphorylated mutant of PLB, which imitates the conformational changes induced by phosphorylation, and competes with the native form of PLB, increasing the activity of SERCA2a (Hoshijima et al., 2002). The tests realized in animal models have used AAV vectors, and followed the evolution up to 6 months after the transference of genes. In animals with HF subjected to gene therapy with the PLB mutant, an improvement of cardiac function is observed, delaying the advance of HF, and approaching near normal levels, corroborated by hemodynamic values, besides this strategy prevents the remodeling of the cardiac wall and it even reverts the changes observed in HF, decreasing fibrosis (Iwanaga et al., 2004). Like in the case of S100A1 mentioned below, the

expressed fetal phenotype observed during HF reverts to the adult phenotype of the normal heart, thus improving its metabolism.
In the last few years, the use of siRNA molecules to down-regulate the expression of PLB, and improving the SERCA2 function has been explored with promising results in rat models in the short and medium term. As mentioned previously isolated siRNA is of little use in a clinical setting, thus it needs to be transferred with a viral vector, AAV9 was used in the experimental models with good results. PLB siRNA improves myocardial function by means of increasing the activity of SERCA2a in a pressure overload model in rats (Suckau et al., 2009; Tsuji et al., 2008). A cautionary note must be made when the choice is made to target PLB in HF, it is well documented that the knockout or continuous blocking expression of PLB results in cardiomyopathy and HF, thus the use of PLB as a target in gene therapy must be strictly controlled so that once the desired effect of restoring the heart to a correct function, the inhibition of PLB function and/or expression can be modulated, to avoid secondary effects (MacLennan & Kranias, 2003; Shanmugam et al., 2011).
Another strategy that involves PLB, is the inhibition of PP1, the enzyme responsible of dephosphorylate it, keeping SERCA2a in an activated state (Nicolaou et al., 2009). Overexpressing the inhibitor-2 gene in a dilated cardiomyopathy hamster model, significantly reduced the activity of PP1, increasing the activity of SERCA2a with an improvement in the handling of intracellular Ca^{2+} concentrations, and consequently with an improvement of the cardiac function, as well as a diminution of the ventricular remodeling diminishing fibrosis significantly (Yamada et al., 2006). The above studies indicate that gene therapy directed towards PLB or its regulation, is an area that can in the future be of importance, but requires further research with more studies in HF models, to be able to reach a phase of clinical investigation.

4.1.2 The S100A1 protein

The S100A1 protein is a member of the S100 family, binding Ca^{2+} and has an "EF-hand" domain. The members of this family are involved in several functions, among them signal transduction, control of the cellular cycle, interactions with the cytoskeleton, cellular differentiation. It has an important role in the function of the striated muscle; it is abundantly expressed in muscle tissue, particularly in cardiac muscle, where it co-localizes with SR and contractile filaments. Its expression is affected negatively during HF secondary to cardiomyopathies (Most et al., 2001; Most et al., 2004a). It has been demonstrated that S100A1 has a positive inotropic and lusitropic effect in the heart. The inotropic effects are due to the interaction with SR and its components, particularly SERCA2a, increasing its activity, thus promoting the recapture of Ca^{2+} during the relaxation of the myocardium, increasing the amount of Ca^{2+} available to release during the next contraction cycle, which is directly related to the contraction force. It also interacts with RyR, stabilizing it and diminishing the diastolic leak of Ca^{2+} (Most et al., 2001; Most et al., 2004a). These data indicate that S100A1 is involved in two of the key molecular processes of HF, added to the fact that its expression falls in HF, turns it into an objective of great value to explore.
In vitro tests have demonstrated that the over-expression of S100A1, improves the contractile function of the cardiomyocytes by means of the mechanisms before mentioned (Most et al., 2004b; Pleger et al., 2005). In animal models of HF, the S100A1 over-expression has been proven to be enough to preserve the myocardial function after an acute infarction, and to prevent the appearance of HF (Most et al., 2004b; Most et al., 2007). Recent reports using AAV have followed these models for prolonged periods (20 weeks), where it has been

observed that the S100A1 over-expression improves the myocardium function in sustained form, and in an independent and synergic effect to the treatment with β-blocker agents (Pleger et al., 2007), restoring the sensitivity of the myocardium to β-adrenergic agonists, that usually is very decreased in HF. In these models, over-expression of S100A1 is able to revert the phenotype of HF expressed by the myocardium, returning after two months to the normal pattern of the adult cardiac muscle, in similar form to that observed in patients with HF awaiting cardiac transplant, with devices of left ventricular assist, who can be weaned from the device after a prolonged period with a significant improvement of cardiac function (Entwistle, 2003; Kuhn et al., 2004). The available data, indicate that S100A1 is an important therapeutic target, but further research is needed before it can be used in a clinical or pre-clinical setting (Rohde et al., 2010).

4.1.3 Parvalbumin

Diastolic dysfunction is a component associated to HF, found mainly in patients older than 65 years; currently specific treatments for this type of dysfunction do not exist. In cardiac myocytes obtained of these patients it is observed that the required time to remove Ca^{2+} from the cytoplasm is increased; this slows down the relaxation of the heart and jeopardizes the filling of the cardiac chambers for the following beat. Parvalbumin is a soluble intracellular protein of low molecular weight, with capacity to bind Ca^{2+}, which is expressed importantly in fast-twitch skeletal muscle, but it is not expressed natively in the heart. Its main function is to accelerate the rate of decrease of the intracellular Ca^{2+} in an ATP-independent manner. The gene therapy interventions that involve parvalbumin, to this time have been limited to *in vitro* studies and to animal models followed by short terms of time (one week), these tests have demonstrated that the expression of parvalbumin in the heart, is associated with an increase of the speed of relaxation. The data can be used as the basis to express parvalbumin in the heart, by means of AAV vectors, to evaluate its effects in the long term. Although the effects that are obtained expressing parvalbumin in the heart are very similar to the observed ones with over-expression of SERCA2a, the ones obtained with parvalbumin do not require of ATP, avoiding compromising even more the energetic needs of the heart. Due to this, parvalbumin constitutes a therapeutic target that must be further explored in the future (Michele et al., 2004; Szatkowski et al., 2001).

4.2 β-Adrenergic system

Alterations of the β-adrenergic receptors during HF have been described extensively, including a decreased density of receptors in the myocardium, to desensitization to β-agonists. The desensitization of the receptors explains to a great extent the refractory response to the treatments directed to the adrenergic routes. The β-adrenergic receptors are coupled to G proteins that are regulated by protein kinases of receptors coupled to G proteins, known in the case the adrenergic receptors (Beta Receptor Adrenergic Kinase) βARK1 and βARK2, the expression of the two enzymes is up-regulated during HF and it plays a key role on the desensitization of the β-receptors (Koch, 2004). Transgenic mice with over-expression of the β1-receptors develop cardiomyopathy, whereas in animals that over-express β2-receptors contractility is increased without developing any pathology.

In animal models it has been demonstrated that the overexpression of β2-receptors directed to the cardiac tissue, is associated with an increased contractility and a greater response to isoproterenol (Jones et al., 2004a; Maurice et al., 1999; Tevaearai et al., 2002). In mice that

over-express βARK1 a contractility-diminished response to isoproterenol is observed. An inhibitor of βARK1 has been developed, the transference of adenoviral vectors with βARK1ct in rabbits with acute infarct has been demonstrated to prevent the alterations in the β-adrenergic system, and improve the myocardial function and prevent the development of HF in short term basis (Koch, 2004). This same strategy was tested with AAV vectors to assess the long term effects, it improved the contractility, reversed ventricular remodeling and normalized the neuroendocrine axis, and intracellular adrenergic signaling, interestingly this work shows that addition of adrenergic antagonists, was not additive to the gene transfer, but treatment with pharmacological inhibitors yielded similar but less effective results (Rengo et al., 2009). These data suggest that manipulation of βARK1 function has an impact on cardiac function, and that is susceptible to be manipulated by gene therapy.

Another strategy used to modify the components of the β-adrenergic system by gene therapy, includes over-expression of the protein that re-uptakes norepinephrine in the heart (NE uptake-1). This protein is down-regulated during HF, which leads to an increase of extracellular catecholamine levels, resulting in a hyper-adrenergic state that it has a central role in the desensitization of the receptors. In rabbits with HF with an over-expression of the NE-uptake-1, the concentration of norepinephrine was kept near normal levels, and was associated with an improvement in cardiac function, and the cardiac hypertrophy was reverted, recovering the normal phenotype of the heart (Munch et al., 2005).

4.3 Intracellular signaling

In the pathogenesis of HF, the hyper-adrenergic state and the alterations in the intracellular concentrations of Ca^{2+} contribute to alter the activation of different intracellular signaling pathways, which turns them into potential targets for gene therapy. To this date, the adenylyl cyclase (AC), and the protein kinase C (PKC) have been explored as potential targets. The adenylyl cyclase is a key role molecule in the cardiac myocyte, particularly the type 6 (AC6) in animal models with HF is demonstrated that over-expression of the AC6 improves the cardiac function and increase the life span. In pigs with established HF (Gao et al., 1999) besides improving the hemodynamic values, the cardiac function, and the response to isoproterenol, the over-expression of the AC6 prevents the remodeling of the cardiac wall and the appearance of fibrosis (Lai et al., 2004). It is thought that in the treatment of the HF response to AC6 is not only elicited due to an increase of the levels of cAMP, but it also modifies expression of other genes favoring the expression of useful genes for the myocardial contraction (Phan et al., 2007). There is an ongoing clinical trial, in which AAV5 encoding human AC6 is being delivered by intracoronary injection to patients with congestive HF (Table 2).

There is evidence that damaged and inflamed tissues produce signals to attract stem cells to the injured tissue, and many of these signals have been identified including stromal-derived factor (SDF)-1. There has been growing evidence that the receptor of SDF-1 (CXCR4) participates in regulating myocardial repair after ischemic injury. SDF-1 has been shown to increase after myocardial ischemia, and several studies have shown that enhancing SDF-1 levels around the infarct improves myocardial remodeling after infarction. Currently, a clinical trial is exploring the effects of percutaneous injection of SDF-1 naked DNA directly into the myocardium of patients with ischemic heart disease (Agarwal et al., 2010) (Table 2).

The other target for gene therapy that has been explored in the intracellular signaling setting is the family of the PKC, which is a component of several routes of transduction associated to the cellular membrane. The PKCα isoform is the most abundant in the heart where it plays a central role regulating the cardiac contractility. The lost of the contractile function in HF goes accompanied of an increase in the levels of PKCα in the heart. On this basis, studies have been performed in animal models transferring a dominant negative of PKCα by means of an adenoviral vector to determine their viability as therapeutic option. In rats with post-infarction HF, the transference of a PKCα dominant negative improves myocardial contractility and improves the hemodynamic values (Hambleton, 2006; Palaniyandi et al., 2009). While most of the research on the role and susceptibility of PKC as a therapeutic target has been done with conventional pharmacological therapy, the data show that at the very least is an option to be explored for gene therapy.

4.4 Apoptosis

It is well described that during the dilation of heart cavities observed in HF exists a remodeling of the myocardial wall, it has been proposed as one of the mechanisms responsible for this processes, the apoptosis of the cardiac myocytes. Although the precise mechanisms by which the apoptosis contributes to the remodeling of the myocardial wall are not understood, several studies have been realized using gene therapy to block apoptotic pathways with positive results. The Bcl-2 factor is able to block apoptosis, and it has been administered by means of an adenoviral vector after a period of myocardial ischemia in rabbits and it was found to avoid deterioration in the short and long term cardiac function, and also being able to prevent the dilation of the heart cavities and the remodeling of the same (Chatterjee et al., 2002). It was also demonstrated that prevents apoptosis in the border of the injured area; although has not determined if the expression of Bcl-2 is required at the initial moment of the injury or if it is required in the long term expression (Weisleder et al., 2004).

The protein p38 is a member of mitogen-activated protein kinases (MAPK) that is a fundamental regulator of growth, life span and cellular death. It has been reported that after myocardial infarction the activity of p38 falls, contributing to the development of apoptosis in the heart, increasing the downgrading of the function and the remodeling of the myocardial wall. When post-infarcted rats are treated with an adenoviral vector to over-express p38, it prevents the deterioration of the myocardial function, has an anti-apoptotic effect, and avoids the fibrosis and remodeling of the ventricular wall (Bassi et al., 2008; Tenhunen et al., 2006).

5. Therapy for heart failure of genetic causes

Multiple causes of HF have been identified due to genetic mutations; nevertheless, it is known that these constitute a minimum percentage of the cases of HF. These causes involve several pathological mechanisms, mainly proteins of the sarcomere and the cytoskeleton (Bos et al., 2007; Chang et al., 2008; Karkkainen & Peuhkurinen, 2007; Lind et al., 2006; Ramaraj, 2008; van Spaendonck-Zwarts et al., 2008; Wiersma et al., 2007). Theoretically these causes of HF offer an immediate target for its treatment, since they are monogenic and have a direct pathological mechanism, and are isolated from the complicated networks involved in the regulation of other elements already mentioned. In this line of research, it has been demonstrated that when an exogenous sarcomeric protein is over-expressed it assembles to

Trial	Target Gene	Diseases	Route/Vector	Stage	Num. patients
CUPID	SERCA2a (cardiac sarcoplasmic reticulum Ca^{2+} ATPase)	HF, ischemic and nonischemic	Intracoronary/AAV1-SERCA2a (MYDICAR®)	Phase 2, completed	39
SERCA2a Gene Therapy in LVAD patients	SERCA2a	Advanced HF with LVAD, ischemic and nonischemic	Intracoronary/AAV6	Phase 2, enrolling	16
AGENT-HF	SERCA2a	HF, ischemic and nonischemic	Intracoronary/AAV6	Phase 2, enrolling	30
AC6 Gene transfer for CHF	AC6 (Adenylyl Cyclase-6)	HF, ischemic and nonischemic	IntracoronaryAdenovirus	Phase 1/2, enrolling	72
Study to evaluate the safety of a single dose of ACRX-100 in adults with ischemic HF	SDF-1 (Stromal-Derived Factor-1)	HF, ischemic only	Intramyocardial/naked DNA	Phase 1, enrolling	16

Table 2. Gene Therapy Clinical Trials for Heart Failure.

form organized and stoichiometric complexes in the cytoplasm. The idea of this type of therapy is to displace the endogenous protein that has the defect, when changing the stoichiometry inside the cell. This type of therapy has not been explored experimentally, although it is susceptible to be done with animal models that reproduce hereditary cardiomyopathies.

Mutations of larger proteins like dystrophin have also been identified as responsible in the genesis of muscular dystrophy of Duchenne, where several mutations have been described and it has even been proved to be caused by mutations that truncate the protein (Rodino-Klapac et al., 2007). In these cases the possibility of being treated by gene therapy presents major technical difficulties, although it has been demonstrated that the transference of a mini-gene of dystrophin can improve the symptoms of the disease (Goyenvalle et al., 2004; Townsend et al., 2007).

A phase 1 clinical trial of rAAV2.5-CMV-Mini-Dystrophin Gene Vector in Duchenne Muscular Dystrophy is in progress. This study investigates the safety and efficacy of the mini-dystrophin gene transferred to the biceps muscle for Duchenne muscular dystrophy patients, ages 5 to 12 years of age, using a recombinant AAV. The mini-dystrophin gene or a placebo agent (normal saline or empty viral capsids) is injected directly into both biceps

muscles while under conscious sedation. Following the gene transfer, patients are admitted to the hospital for 48 hours of observation followed by weekly outpatient visits. A bilateral muscle biopsy is performed following 6 weeks with long-term follow up will consisting of bi-annual visits for the next 2 years.

6. Ischemic heart disease

Atherosclerotic cardiovascular diseases continue to be the main cause of morbidity and mortality in the world. According to the American Heart Association the direct and indirect costs of coronary heart disease (CHD) for the year 2010 add up to 177.1 billion dollars. Pharmacologic therapies for CHD and HF have multiple systemic side effects and are predisposed to several adverse drug interactions since polypharmacy is frequently involved in the treatment of such patients. Importantly, pharmacologic therapies aim to reduce symptoms and halt progression of disease but do not necessarily reverse the pathophysiology associated with CHD and HF. While revascularization procedures have a significant role in treating CHD, there are problems with this therapies further explained later, and frequently patients remain symptomatic despite maximal anti-anginal therapies and may require repeat revascularization procedures. While the obvious target for gene therapy in CHD is angiogenesis, one must keep in mind that there is a wide spectrum of mechanisms in its natural history, such as those of myocardial reperfusion injury during and after myocardial infarction (MI) and cell survival and apoptosis after MI, and during ventricular remodeling (Lavu et al., 2010).

A significant part of the research in CHD has been focused on the administration of angiogenic growth factors, in the form of recombinant protein or by gene transfer, to promote the development of additional collateral blood vessels that would act like endogenous conduits of bypass around the occluded native arteries; a strategy known as therapeutic angiogenesis. This strategy has demonstrated to increase the tissue perfusion by means of neovascularization in a considerable number of preclinical tests of ischemia. In patients with critical ischemia of the lower extremities or with coronary terminal arterial disease, the clinical tests have demonstrated a symptomatic improvement and have contributed new objective evidence of improvement in perfusion, suggesting that this strategy can constitute an alternative method for treatment in patients in whom the therapies available at the moment have failed or they are not viable. The next targets of the research in the field of angiogenesis are going to be to determine the optimal dose, the formulation, the route of administration and the combination of growth factors; to determine the needs of endothelial progenitor cells or the supplementation with stem cells; to provide an effective and safe therapeutic angiogenesis, as well as to adapt the angiogenesis to the individual needs of the patients.

Among the strategies to induce angiogenesis various growth factors have been used including the vascular endothelial growth factor (VEGF), the angiopoietines, the fibroblastic growth factor (FGF), the hypoxia-inducible factor-1α (HIF-1α), the monocyte chemotactic protein-1 of (MCP-1), the hepatic growth factor (HGF), and the stimulating factor of colonies of granulocytes and macrophages (GM-CSF). The strategy consists of transferring the genes that codify for some of the above proteins to the target tissue, favoring the development of neovascularization in the ischemic myocardium. In preclinical animal models this approach has demonstrated to be effective promoting the growth and generation of new capillaries, although the relevance for the myocardial tissue has not been evaluated (Shen & Vatner,

1995; Wijns et al., 1998). It has been shown that with an expression for more than 4 weeks the newly formed vessels undergo a tissue remodeling that allows them to remain even though the stimulus is no longer present.

There is increasing evidence that VEGF is the master regulator of angiogenesis and thus is the prototype of angiogenic growth factors for gene therapy (Yla-Herttuala & Alitalo, 2003; Yla-Herttuala et al., 2007). In animal models with HF secondary to myocardial ischemia, administration of gene therapy for angiogenesis using VEGF has been associated with an improvement of the myocardial perfusion after 3 weeks of administration, and the myocardial function was also improved. The angiogenesis induced by gene therapy, is one of the more explored strategies at present, to treat CVD in humans, up to date with more than 15 different clinical trials of phase 1 already completed and many clinical trials of phase 2/3 are ongoing using adenoviral gene therapy in patients with coronary artery disease, with different goals, including therapeutic angiogenesis to improve the myocardial perfusion, the prevention of re-stenosis of stents, the prevention of the fault of venous grafts in revascularization, however, the results obtained are difficult to interpret, mainly due to the variation among the used vectors, the therapeutic routes of administration and the targets (Zachary & Morgan, 2011). Besides, one of the problems to consider is that the efficiency of the transference of genes is reciprocal to the size of the guest, partly by the limited diffusion of the vector in a greater amount of tissue. For this reason although several studies are promising in animal models, the results could be difficult to reproduce in humans.

Most of the cardiovascular gene therapy trials have been designed to study therapeutic blood vessel growth. The use of therapies using recombinant VEGF, FGF-2 and granulocyte macrophage colony-stimulating factor proteins has been researched in peripheral and myocardial ischemia, but there were no clear improvements in the clinical outcome of the patients (Henry et al., 2003; Lederman et al., 2002; Lederman et al., 2001). Several VEGF gene therapies using naked DNA have been tested by intramyocardial injection or percutaneously into the ischemic myocardium with the NOGA catheter or via thoracotomy alleviated angina and reduced the area of ischemic myocardium (Reilly et al., 2005; Vale et al., 2001).

Some gene therapy clinical trials using FGF have been reported. In the phase 1/2 AGENT-1 trial, intracoronary administration of AdFGF-4 improved exercise time at 4 weeks in stable angina pectoris patients (Grines et al., 2002). Similarly, in the phase 2 AGENT-2 trial, was observed a reduction of the myocardial ischemic area (Grines et al., 2003). Nevertheless, the results obtained from the gene therapy clinical trials performed to date verify that gene therapy is a strategy with viability to treat patients who are not susceptible to be treated with more conventional alternatives.

Another potential target for gene therapy in CHD is the injury generated by oxidative stress, mainly observed in myocardial infarction and ischemia-reperfusion injury. The central point is the generation of reactive oxygen species, which produce lipid and protein oxidation, and also interact with several calcium handling proteins, leading to an augmented entry of calcium into the cell and a secondary elevation of intracellular concentration, which leads to cellular injury (Cantor et al., 2003; Dhalla et al., 2000; Maddika et al., 2009). Among the targets to prevent oxidative stress, is the over expression of the extracellular superoxide dismutase (Ec SOD), which has a central antioxidant role, has been tested pre-emptively in animals later subjected to myocardial infarction and it was observed that mimicking myocardial ischemic preconditioning, it leads to decreased infarct size (Agrawal et al., 2004;

Li et al., 2001). The Thioredoxins (Trx) are proteins that have a potent antioxidant role and protect against oxidative stress by decreasing the superoxide anion generation and interacting with intracellular signalling pathways as p38MAPK (Tao et al., 2006). In mice models of CHD and myocardial infarction, the over-expression of the Trx-1 gene has an angiogenic effect, decreases apoptosis and reduces the ventricular remodelling (Adluri et al., 2011; Samuel et al., 2010), thus the use of Trx as a potential target is exciting and guarantees further research. Finally, it has been demonstrated that nitric oxide has a protective role in myocardial infarction minimizing oxidative stress, in mice the transfer of adenoviral delivered nitric oxide synthase previous to the generation of an infarction, results in reduced infarct size, improved contractility and ventricular function. It also decreases the inflammation in the infracted area and decreases phosphorylation of the MAPK overall improving the outcome of the treated mice (Chen et al., 2010; Jones et al., 2004b).

As mentioned previously, cell survival and apoptosis plays a key role in the chronic evolution of CHD, after a myocardial infarction or chronic ischemia, some surviving cells will start apoptosis and lead to ventricular remodeling and deterioration of the function, we have previously mentioned the role and potential target of the MAPK pathway and Bcl-2. The use of heat shock proteins, specifically HSP72 over-expression, in rats subjected to ischemia-reperfusion injury, resulted in decreased levels of apoptosis, increased activity of intracellular antioxidant systems, increased levels of Bcl-2, thus showing a cardioprotective effect (Suzuki et al., 2002). Also the use of the troponin I type 3 interacting kinase (TNNI3K) a member of the MAP kinase family with angiogenic and myogenic roles, has been explored combined with cellular therapy, over expressing it on pluripotent progenitor cells, which are then transferred into infarcted mice hearts, the results show a decrease infarction injury, decreased remodeling, induction of myogenesis and inhibition of p38-JNK mediated apoptosis (Lai et al., 2008).

7. Arrhythmias

Cardiac arrhythmias constitute a significant cause of morbidity, and sudden cardiac death primary o secondary to other cardiac diseases constitutes the main cause of death in CVD. While most are susceptible to pharmacological treatment, these therapies have a global effect in the heart, meaning that while it has the desired effect on the affected region of the heart, it also modifies the behavior of other regions and may even have a pro-arrhythmic effect. It must also be mentioned that a number of cardiac arrhythmias are caused by genetic causes, and as previously mentioned these constitute the most obvious targets for gene therapy, but only a small number of arrhythmias have a monogenic origin.

While the vectors used in arrhythmia treatment follow the same principles as in other CVD, delivery constitutes a significant problem. Direct myocardial injection is limited due to the size, location and thickness of the atria. Intracoronary perfusion is suboptimal since the atria vasculature is not as well defined as that of the ventricles. At this time the most effective method of gene delivery in an experimental setting is known as epicardial gene panting, where a solution containing the vector, a polymerization compound and a diluted protease are applied over the surface of both atria, obtaining full wall thickness transfer (Kikuchi et al., 2005).

Atrial fibrillation (AF) is the most common arrhythmia encountered. In the United States only, AF affects 2-5 million people and it has an increasing incidence. AF may be of primary origin, or secondary to structural disease; while in the former case it is due to electrical

imbalances, in the latter is mainly to atrial remodeling with fibrosis of the atria and remodeling (Benjamin et al., 1998). Thus it is mainly the primary AF which has been explored as a potential target for gene therapy. Therapeutic strategies in AF are to controlling cardiac rate or restoring sinus rhythm. Primary AF is an abbreviation of action potential duration and, therefore, refractory period. This provides the substrate for abbreviated reentrant wavelengths, which serve to stabilize the chaotic atrial electrical activity.

One approach that has been explored for restoring sinus rhythm is the use of a dominant negative mutant potassium channel KCNH2-G628S that blocks the ion channel pore region. The transfer of an adenoviral vector by epicardial painting in a pig model of AF, a significant prolongation of the monophasic action potential was observed, with a reversal of AF observed (Amit et al., 2010). These results constitute a significant pathway to be explored. A second approach researched mimics the rate control; a mutated constitutively active inhibitory G protein in a viral vector is transferred to pigs with induced AF, and a 20% decrease on the maximal ventricular rate is observed (Bauer et al., 2004; Donahue et al., 2000).

The ventricular arrhythmias (VT) constitute a significant cause of death in several CVD, they have been mainly attributed to the beat-to-beat variation of the action potential. The proposed mechanisms underlying these variations are alterations of ionic currents or of the calcium cycling, with a slop clearing of calcium during the relaxation. In hearts where a viral vector expressing SERCA2 is transferred, the susceptibility of mice hearts to ventricular arrhythmias after rapid pacing is decreased (Cutler et al., 2009). One of the most relevant groups in the VT spectrum are those secondary to myocardial ischemia and infarction. One of the probable mechanisms of this type of VT is the slow conduction due to altered gap junction expression and reduced velocity of the action potential. One of the proposed mechanisms for velocity is a more positive resting membrane potential in scar border myocytes, secondary to an inactivation of some of the sodium current carried by the Nav1.5 (SCN5A) channel. To correct these alterations, the transfer of a skeletal muscle sodium channel has been tested in a canine post-MI model, observing a significant reduction of induced VT (Lau et al., 2009). MicroRNAs have also been explored as targets for the treatment of VT, in post-MI hearts miR-1 is up-regulated, with a subsequent inhibition of the expression of ion channels, when antagomirs are transferred, a decrease of the arrhythmic potential is observed (Yang et al., 2007).

An increasing trend observed in an ageing population is the increasing incidence of bradi-arrhythmias. An abnormal generation of the electrical impulse in the atria (sinus node dysfunction) or its propagation to the ventricles (atrioventricular [AV] block) may result in the development of abnormally slow heart rate, and in most of the cases the only satisfactory option is the implantation of a definitive pacemaker. Pacemaker implantation lacks sufficient physiological feedback parameters to grant greater freedom to the recipient. Other disadvantages are the mechanical nature of the treatment and associated risks. The experimental approaches for bradi-arrhythmias are focused on altering the function of existing cardiomyocytes.

The basis of the treatment of bradi-arrhythmias, has been focused on reducing the diastolic repolarization currents by inhibiting diastolic currents, the approach described suppresses the diastolic entrance of potassium by transferring a mutant inactive channel of Kir2.1, afterwards a new ventricular activity is observed (Miake et al., 2002). Another alternative researched is the combination on gene and cell therapy, where mesenchymal stem cells were

electroporated with plasmid DNA of HCN2, and latter injected into the anterior wall of dogs who had previously been subjected to ablation of the AV node; after 10 days spontaneous ventricular activity was observed (Plotnikov et al., 2007).
Thus as we have briefly exposed, gene therapy for arrhythmias is an exciting field which has shown encouraging and promising results, but it is also true that is probably one of the fields in CVD, that is most distanced form a translational setting. With he current pace of research it is certainly a field to keep a close watch on the next years.

8. Vein graft disease

Myocardial revascularization either by coronary artery bypass graft surgery (CABG) or percutaneous transluminal coronary angioplasty (PTCA) has became the standard of care for ischemic heart disease; both techniques are highly successful and have significantly improved its prognosis. However both have significant long-term issues with their durability, in the case of CABG, it is due to the patency of graft used, while arterial grafts have an excellent long term patency, saphenous vein graft (SVG) are still widely used due to technical considerations. SVG are a useful and valid tool in CABG but their long term patency is impaired by what is known as vein graft disease (VGD), where a proliferation of smooth cells and endothelial cells lead to intimal hyperplasia, which gradually decreases the patent lumen of the graft. Conventional treatments can help to prevent VGD, but still constitutes a significant problem in surgically revascularized patients with coronary artery disease (Parang & Arora, 2009).
Thus VGD is another entity which may be susceptible to be treated by gene therapy, one of the main advantages when using gene therapy in this disease is the possibility of delivering the therapy ex vivo, after the graft has been harvested. The pathogenesis of it, results from the graft's adaptation to high pressure and the loss of inhibition of the endothelial layer, there is a proliferation and migration to the intima of smooth muscle cells. These cells release cytokines and degrade the surrounding matrix, creating an inflammatory environment, highly atherogenic. In the hyperplasia of the intima the E2F transcription factors have been identified as key in initiating it. A decoy oligonucleotide for E2F has been designed and tested successfully in animal models, a phase 3 clinical trial was designed to test its efficacy in preventing vein graft failure, denominated as the PREVENT IV trial. The results did not show a significant decrease in the rate of vein graft failure in the treated group; while this specific approach was not successful it has proven that gene therapy is a possible approach to VGD (Alexander et al., 2005).
Another potential target which has only been tested experimentally, is the transfer of the fibromodullin gene. As previously mentioned the remodelling of the extracellular matrix plays a key role in the genesis on intimal hyperplasia, here widely known proteins have been identified, one of them is TGF-β1, fibromodullin and decorin are leucine rich-proteoglycans, which possess TGF-β1 antagonist activity *in vitro* and *in vivo*. Adenoviral transfer of the fibromodullin gene to harvested human saphenous veins, revealed significantly reduced neointimal thickness (Ranjzad et al., 2009). This proves that while clinical trials have been unsuccessful in the past, there are still a number of target that can be researched in the future and may result in an effective gene therapy option for VGD.

8.1 Intra-stent restenosis

As mentioned PTCA is widely used in the treatment of coronary artery disease, briefly an atherosclerotic obstruction of the coronary vessel is identified and dilated with a high

pressure balloon, afterwards a metal stent is deployed to prevent new obstructions. As in CABG its main drawback it's the long-term patency of treated lesions, a similar phenomena is observed where a neointimal proliferation results in the restenosis of the stent. Currently the most used option to prevent this restenosis is the use of drug eluting stents, which are covered in different drugs that inhibit the neointimal proliferation. However there is still a high number of stents that will develop restenosis. This particular field presents another option for the delivery of gene therapy, by deploying gene-delivering stents.

Intrastent restenosis has been researched in animal models, one of the strategies involves the inhibition of cellular proliferation using the CREB binding protein, which is known as a regulator of cell proliferation and apoptosis in vascular endothelial and smooth muscle cells. Rats were subjected to endothelial injury and denudation of the carotid artery, afterwards the injured artery was treated with lentivirus expressing siRNA for CREB binding protein; after 4 weeks it significantly decreased the neointimal formation. Thus providing a new target to be researched in the future (Yang et al., 2010). Another researched strategy is the use of stents delivering the gene of the endothelial nitric oxide synthase (eNOS); it is well describe that NO is a critical molecule in the vessel wall as it is responsible for a variety functions including inhibition of platelet adhesion and aggregation, inhibition of leukocyte chemotaxis, inhibition of smooth muscle cell growth and migration, vasodilatation, and re-endothelialization, thus making it a very attractive target to prevent neointimal hyperplasia. In a rabbit model of intrastent restenosis gene-eluting stents which delivered eNOS had significantly less neointimal formation, also it is important to mention, that in this study the gene was delivered by a lipid based transfection instead of a viral vector.

9. Conclusions

Despite of the therapeutic advances CVD continue to be the main cause of morbidity and mortality in the world; gene therapy appears to be a promissory alternative, having the capacity to correct the observed fundamental defects in several CVD. Currently gene therapy is an area of basic and clinical research of great significance that should produce positive results for HF in the next few years. An increase in the knowledge of the underlying molecular mechanisms of HF, along with advances in gene therapy technology has led to important efforts in *in vitro* studies and pre-clinical testing of a number of gene targets and recently in the successful completion of the first phase 2 gene therapy trial for HF. Moreover, the safety of AAV vectors has been demonstrated for the treatment of HF combined with the efficacy of the use of SERCA2a in the treatment of HF, thus opening the field for testing new targets to modulate with more advanced vector systems for gene therapy. As we have briefly exposed, myocardial ischemia along with HF constitutes one of the most researched fields in the CVD spectrum, and probably is the area where gene therapy has advanced further, and it even has several pre-clinical and clinical studies, and while the results are still non conclusive and even in some cases discouraging, it only shows the many difficulties and the need for further research, before gene therapy can be established as an everyday available clinical tool. It must also be said that there is a need to diversify the efforts in research, since in the case of IHD most of it has focused on angiogenesis, while the other pathways as ischemia reperfusion injury and apoptosis have been only scratched in the basic setting, thus more research is needed in areas which can offer new and exciting promising targets for gene therapy. One of the majors obstacles that presents the basic research and clinical application of this and other therapeutic options, is the complexity of

the underlying mechanisms, and as such it is required that these along with the indications are known and understood so that we can be able to explode to the maximum the new capacities that this therapeutic option offers, reason why is necessary to closely follow the advances in this field in the near future.

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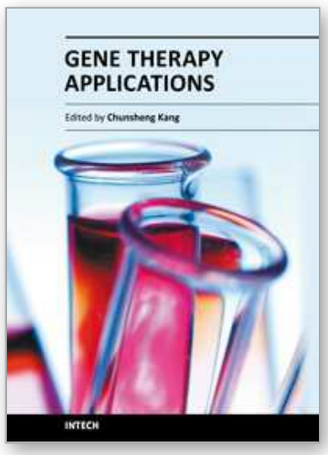

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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

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