General introduction

Cardiovascular disease

Cardiovascular disorders are currently the leading cause of death in the Western world. Despite advances in pharmacotherapy, heart and blood vessel surgery and the use of vascular stents and cardiac assist devices, mortality rates as a result of these diseases are rising\(^1\). Much effort is therefore invested in the prevention and early detection of cardiovascular disorders and in limiting the amount of tissue damage after a cardiovascular event. A major problem in cardiovascular medicine is the absence of significant cardiomyoregenerative capacity in human adults. Whereas acute skeletal and smooth muscle injury typically induce a hyperplasic response resulting in the formation of new myocytes and the repair of the damaged tissue, myocardial infarctions (MIs) or other types of acute injury to the human heart usually lead to the irreversible loss of cardiac muscle cells. The lost cardiomyocytes are replaced by myocardial scar tissue composed of (myo)fibroblasts and large amounts of extracellular matrix. This does not only compromise the contractile function of the heart but may also cause arrhythmias\(^2\). Furthermore, the increased hemodynamic stress that is often experienced by surviving cardiomyocytes in damaged hearts triggers a hypertrophic response in these cells. The fibrotic and hypertrophic changes in infarcted myocardium leads to remodeling causing a further decline in cardiac performance and the development of congestive heart failure (CHF).

Although heart transplantation has been shown to be an effective therapy for CHF, it is only applied to a limited number of patients due to a large shortage of donor hearts, the complications associated with long-term immunosuppression, the risk of rejection of the transplanted organ, and the frequent development of allograft coronary vasculopathy\(^3\). Most CHF patients therefore only receive palliative treatment, which includes the administration of drugs to reduce cardiac fibrosis, hypertrophy and remodeling, left ventricle (LV) reconstruction surgery and electrical resynchronization therapy. Accordingly, there is a huge interest in the development of potentially curative therapies for the treatment of CHF patients. Especially (stem) cell therapy is currently in the spotlight of cardiovascular research as a possible means to regenerate damaged hearts.

Stem cell therapy for cardiac regeneration

The development of stem cell therapy to repair myocardial damage boomed after the demonstration by Orlic and colleagues that injection of hematopoietic stem cell (HSC)-enriched bone marrow (BM) fractions into the border zone of the infarcted myocardium of mice that had been previously subjected to permanent ligation of the left main coronary artery, resulted in the improvement of LV function and the presence of transplant-derived cardiomyocytes in their hearts\(^4\). However, although the presence of donor cells and an improvement of LV function could be reproduced by other groups, significant amounts of donor-derived myocytes were not found in these studies\(^5-8\). These data strongly suggest that mechanisms other than cardiac regeneration were responsible for the observed improvement.
of LV function in damaged hearts after stem cell treatment. The exact nature of this mechanism remains unknown, but as cytokines are likely involved it is commonly referred to as the paracrine effects of stem cell therapy. Based on these data, it may be warranted to include cell types that provide supportive functions for the myocardium in future stem cell therapy strategies. A cell type that may be an interesting candidate for such a strategy, based on their role in cardiac development during embryogenesis is the epicardium-derived cell (EPDC). During embryogenesis EPDCs migrate from the epicardial cell layer into the primitive myocardium to contribute connective tissue and smooth muscle cells to the coronary arteries and to provide essential inductive signaling for myocardial growth. In chapter 3 of this thesis, we investigate whether EPDCs can be obtained from the epicardium of human adults, and whether these adult cells still have the multilineage differentiation potential of their embryonic counterparts.

Aside from autologous somatic stem cells, embryonic stem cells represent a potential source of new cardiomyocytes, although ethical objections and immune rejection are two major disadvantages hampering the use of these cells their ability to differentiate into cardiomyocytes is well established. Unfortunately, recent studies now show that when embryonic stem cells are injected into either healthy or damaged hearts they do not display any specific differentiation into cardiomyocytes, and are prone to forming local teratocarcinomas. This indicates that embryonic stem cells, which are fully capable of cardiomyocyte differentiation ex vivo, do not undergo cardiomyogenesis solely in response to local stimuli from the (injured) myocardium. Nevertheless, the promising results of the early animal studies, using autologous stem cells, have led to the initiation of many small clinical studies investigating autologous (stem) cell therapy for cardiac repair (reviewed in11). These studies have overwhelmingly shown safety and feasibility. However, due to their small scale and non-randomized setup they provide only some first putative indications of efficacy. Based on these studies, it can be tentatively concluded that (stem) cell therapy can reduce the loss of cardiac function after myocardial injury, but in its present form seems unable to achieve significant cardiac regeneration. Developing methods to stimulate differentiation into desired cell types, beyond relying on spontaneous differentiation in response to local cues, would be a logical next step for achieving cardiac regeneration.

Molecular mechanisms of differentiation

During development distinct tissues arise from the same initial cell, by a process of cellular differentiation. The distinct phenotypes cells adopt during differentiation are in turn a reflection of different gene expression programs active in these cells. Currently it is thought that at least for some tissues these cell type-specific gene expression programs are initiated by the activation of a small set of “master regulator” genes. To date several master regulator genes have been identified. The first gene proposed to be a master regulator gene was MyoD. Forced expression of the MyoD gene was shown to initiate skeletal muscle differentiation in a wide variety of cells. Overexpression of pancreatic and duodenal homeobox gene 1 (Pdx1) has been shown to convert liver cells into pancreas cells, while a combination of the gene products C/EBPα and PU.1 can turn a T cell precursor
into a functional macrophage. Recently the power of using transcription factors to reprogram cells was exemplified by Takahashi et al. who described the conversion of skin fibroblasts from the tail tips of mice into pluripotent embryonic stem cells, by overexpression of the four genes encoding Oct3/4, Sox2, c-Myc, and Klf4. However, it should be stressed that the extent of (de)differentiation reported for these putative master switch genes varies, and that the number of cell types that respond to overexpression of the aforementioned factors has either not been extensively investigated, or is known to be restricted (as discussed for MyoD below). Current data do, however, illustrate the power of master regulator genes, in inducing specific differentiation in cells not normally known to do so.

An essential requirement for the use of master regulator genes is the ability to efficiently and specifically express them in the desired target cells. Both viral and non-viral vectors have been extensively studied for their safety and efficiency in gene transfer. Recent advances have led to significant improvement of vector systems, which are currently being tested in a number of clinical trials. In debt discussion of this subject is beyond the scope of this work, and interested readers are directed to other papers.

**MyoD the archetypical master switch gene**

The concept of a master switch gene was introduced after a series of subtractive hybridization experiments performed by Lassar et al. who showed that a single locus, later identified as the gene MyoD was responsible and sufficient for the transdifferentiation of 10T1/2 fibroblasts into skeletal muscle cells. Overexpression of MyoD in a wide variety of primary cells, such as dermal fibroblasts, pigment, nerve, fat and liver cells also resulted in conversion to skeletal muscle. These findings provided the first direct evidence that a single gene product can initiate a complex program of differentiation, acting as a master switch.

Skeletal muscle conversion by overexpression of MyoD has been observed in many different cell types, including fibroblasts, chondroblasts, smooth muscle cells, melanocytes, and adipocytes. After conversion these cells express a wide variety of skeletal muscle proteins, including: sarcomeric myosin heavy and light chain, alpha actinin, alpha actin, titin, nebulin, desmin, myosin light chain 1, muscle creatin kinase, acetylcholine receptor, myogenin, troponin I, tropomyosin, and myomedin. Furthermore, like regular myoblasts, these cells can fuse into multi-nucleated myofiber-like structures, called myotubes. These myotubes possess a properly organized sarcomere, and under certain conditions can contract spontaneously. Importantly, after skeletal muscle conversion of chondroblasts, melanocytes, and adipocytes, these cells loose all markers of their respective original differentiation program, indicating that the endogenous differentiation program is overruled by the new skeletal muscle gene expression program.

After the identification of MyoD, three other closely related basic helix-loop helix (bHLH)-family members have been identified, Myf5, myogenin and herculin, each of which is also able to induce a skeletal muscle phenotype. During embryogenesis, MyoD and Myf5 initiate the initial muscle determination. Myf5 is highly expressed in apaxial myotome, giving rise to the back, intercostal, and
abdominal muscles, while MyoD is expressed at high levels in the hypaxial myotome, giving rise to limb, tongue, and diaphragm muscles. In line with the similar roles that MyoD and Myf5 fulfill, they are to a large extent mutually redundant. Knockout of either factor results in compensatory upregulation of the other factor and almost normal muscle formation, only double knockout of both MyoD and Myf5 results in the absence of skeletal muscle development. The other two bHLH transcription factors myogenin and herculin are thought to function downstream of MyoD and Myf5, directly facilitating the activation of many muscle structural genes. Although downstream targets of MyoD and Myf5, overexpression of myogenin and herculin still can result in skeletal muscle conversion, possibly because both myogenin and herculin induce endogenous MyoD expression.

Limitations of MyoD mediated differentiation

Not all cell types are equally responsive to forced MyoD expression, and the endogenous differentiation program of cells is not always overruled. When MyoD was overexpressed in melanoma and neuroblastoma cells, in combination with cAMP to trigger neuroblast differentiation, both markers of skeletal muscle differentiation (MHC and desmin) and formation of axon-like processes indicative of neuronal differentiation were detected in the same cells. In these cases it would appear that both the endogenous differentiation program and the muscle differentiation program induced by MyoD were initiated and are able to coexist in a single cell. On the other hand when adipogenic cell lines were transfected with MyoD in combination with adipogenic induction, they displayed either an adipocyte phenotype distinguished by the presence of fat droplets, or a muscle phenotype in a mutually exclusive manner, indicating that fat cell and muscle cell differentiation programs are not compatible. NIH3T3 fibroblasts could not be forced to fuse into myotubes, but did express several muscle structural genes, upon forced expression of either MyoD, Myf5, myogenin or herculin. Interestingly, prechondrocytes and preosteoblasts were found to be responsive to MyoD mediated myogenesis, but more mature chondrocytes and osteoblasts were not. Similarly, kidney- and liver-derived cells did not display any markers of a muscle phenotype in response to MyoD transduction. This illustrates that not all cells are permissive to MyoD induced differentiation. The differences in permissiveness of various cell types to MyoD may be explained either by the presence of inhibitory factors or the absence of necessary co-stimulatory factors, the discussion of which lies outside the scope of the current work and has been reviewed previously.

MyoD gene transfer for myocardial repair

Only little data exists concerning MyoD gene transfer in vivo. The application most studied is using MyoD gene transfer to convert scar fibroblasts from the damaged myocardium into skeletal myocytes with the aim to improve cardiac function. A major problem with this strategy is, however, that when injected into the damaged myocardium, skeletal myoblasts do not differentiate into cardiomyocytes, and remain electrically isolated from the surrounding cardiomyocytes. Concomitantly, clinical trials investigating the effects of
transplantation of skeletal myoblasts into the heart have been terminated, as the risk of malignant arrhythmias was found to be increased\(^1\). A potential explanation for the electrically isolated nature of skeletal myoblasts is the absence of gap junctional coupling\(^4\). To solve this problem, Kizana et al.\(^2\) co-infected fibroblasts with self-inactivating (SIN) lentivirus vectors coding for gap junction protein α1 (Cx43) and for MYOD1. This resulted in the formation of myotubes that could propagate calcium transients and transfer a gap junction-permeable cytoplasmic fluorophore to adjacent cells. Given the differences in ion channel composition between skeletal muscle cells and cardiomyocytes\(^5\) it is however unlikely that MyoD- and Cx43-transduced fibroblasts can produce a cardiac action potential. To overcome this problem, it would be preferable to use a gene encoding a transcription factor involved in cardiomyogenesis to obtain a true cardiac muscle phenotype.

**Genetic control of muscle development**

In *C. elegans* muscle differentiation is controlled by three functionally redundant genes, being the MyoD homologue hlh-1, the SRF/MEF2 homologue unc-120, and HAND homologue hnd-1\(^4\). When individually overexpressed each of these genes can induce muscle differentiation, and only knockout of all three results in complete absence of muscle\(^4\). Interestingly, members of the MyoD-family regulate only skeletal muscle development in vertebrates, as demonstrated by experiments showing that double knockout of two bHlH-family members, MyoD and Myf5, is sufficient to completely block skeletal muscle development in mice\(^3\), while knockout of hlh-1, representing all MyoD-family members in *C. elegans* does not affect muscle development in *C. elegans*. In vertebrates, HAND family members have clearly documented functions only in cardiac muscle, whereas the MEF2 and SRF family of transcription factors have established functions in all three muscle lineages. This suggests that the genes which regulated muscle differentiation in the ancestral muscle cell have adopted more specific functions in the differentiation of the three muscle lineages of vertebrate organisms\(^4\). Furthermore, although overexpression of MyoD can induce skeletal muscle differentiation in mammalian cells, overexpression of HAND family members, SRF or MEF2, does not. SRF and MEF2, which both belong to the MADS box family of transcription factors are essential for the development of all three muscle lineages\(^5\). However, these factors are expressed throughout the organism and also function in more generalized processes, such as control of cellular growth. Specific activation of muscle or growth factor induced genes, is therefore thought to be dependent on tissue specific co-factors\(^5\).

**Myocardin: a cardiovascular master switch gene**

Many genes have been described which are essential for the proper development of the heart\(^6\). However, none of these alone or in combinations have been found to initiate even a partial cardiomyocyte specific gene expression program in somatic cells. With the identification of the heart and smooth muscle-restricted transcription factor myocardin a key regulator of cardiac and smooth muscle
differentiation has now been identified. Myocardin was first described in Xenopus, and is expressed at the earliest stages of cardiac and smooth muscle development. Myocardin activates genes in a complex with SRF and Mef2C. A dominant negative mutant of myocardin can prevent cardiac gene expression in Xenopus embryos, however mice lacking myocardin do not exhibit a discernable cardiac phenotype which may indicate redundancy with other myocardin-family members. Nonetheless, overexpression of myocardin in various Xenopus, human and rat (unpublished results) cells leads to the activation of an extensive cardiomyocyte gene expression program. Overexpression of myocardin also leads to the activation of smooth muscle specific genes, and in chapter 2 we will show that both cardiac and smooth muscle genes are expressed at once in the same cells. Interestingly, recent data suggest that cardiac and smooth muscle cells can arise from a common precursor cell, which also temporarily expresses both cardiac and smooth muscle specific genes. Furthermore, many cardiac lineage tracing studies have shown that developing cardiomyocytes temporarily express numerous smooth muscle structural genes. These findings underscore the close evolutionary relationship between smooth muscle cells and cardiac muscle cells and are compatible with the hypothesis that single regulator genes exist that initiate early differentiation of both heart and smooth muscle cells.

MicroRNAs in muscle differentiation

Post transcriptional regulation at the RNA level by so called micro RNAs (miRNAs), has been recently recognized as an important regulatory mechanism of gene expression. The importance of miRNAs in the regulation of embryonic development has been established by experiments which employed complete disruption of all miRNAs, by knockout of the enzyme Dicer which is critical for miRNA processing, which resulted in embryonic lethality due to developmental defects at the early gastrulation phase. Two miRNAs have been identified with essential functions in muscle development, miRNA1 and miRNA133. Expression of miRNA1 and miRNA133 is found specifically in the somites and the heart at the looping stage, and becomes more abundant with the further development of skeletal and cardiac muscle in mouse, Drosophila and Xenopus. Overexpression of miRNA1 results in increased levels of structural muscle gene expression and reduced myoblasts proliferation. Mice overexpressing miRNA1 die at embryonic day 13.5 as a result of heart failure due to underdeveloped thin walled ventricles, again showing that miRNA1 reduces myoblast proliferation. This cardiac phenotype is comparable to that found in Hand2 knockout mice, and indeed Hand2 mRNA was shown to be a target for miRNA1 mediated knockdown. In the reciprocal experiment knockout or inhibition of miRNA1 was shown to result in reduced expression of heart and skeletal muscle marker genes, and increased myoblast proliferation. The function of miRNA133 is the inverse of miRNA1. Overexpression of miRNA133 represses expression of structural muscle genes and promotes myoblast proliferation. Intriguingly, expression of these miRNAs in skeletal muscle is regulated by MyoD in combination with MEF2C, and expression in cardiac muscle is regulated by SRF and myocardin. Combined these findings suggest that miRNA1 and miRNA133 are essential
regulators of the striated muscle phenotype, but that in the establishment of muscle lineages they function downstream of the myocardin and SRF in the heart and MyoD and MEF2C in skeletal muscle.

**Differentiation between cardiac and smooth muscle gene expression programs**

The co-expression of both cardiac and smooth muscle structural genes in single cells after forced expression of myocardin does not correspond to the normal expression profile of mature cardiomyocytes and smooth muscle cells. Therefore other mechanisms must exist which further restrict the gene expression program to that of only smooth or cardiac muscle cells. Although no definitive answer to this question has been found, several additional mechanisms of regulation for myocardin mediated gene expression have been discovered.

The SRF-myocardin complex has been found to associate with the chromatin remodeling histone acetyltransferase p300 and with class II histone deacetylases. Histone acetylation which is catalyzed by histone acetyltransferases (HATs) promotes gene transcription by destabilizing chromatin structure, facilitating access of transcriptional complexes to their target genes. Conversely histone deacetylases (HDACs) catalyze the deacetylation of histones, which leads to chromatin condensation and transcriptional silencing. This suggests that myocardin through alternate association with different HATs and HDACs can in one situation activate cardiac and repress smooth muscle gene expression and in another do the reverse. The mechanism regulating which association prevails and therefore which genes are activated and which are repressed remains to be elucidated.

Recently it has been discovered that myocardin is differentially spliced in cardiac and smooth muscle tissue. The smooth muscle-enriched isoform of myocardin (SM-MYOCD) lacks the N-terminal 79 amino acids, which are present in the cardiac-enriched isoform (CM-MYOCD) and contains a MEF2 binding domain. SRF and MEF2 regulate distinct but overlapping sets of muscle genes. Therefore, the CM-MYOCD can regulate both SRF and MEF2 target genes, while SM-MYOCD is restricted to SRF target genes.

A third mechanism for differential regulation of myocardin activity on cardiac versus smooth muscle genes involves post transcriptional modification of myocardin by small ubiquitin-like modifiers (SUMOs). Post translational modification of proteins by SUMOs can alter transcription target activity by altering DNA binding activity, subcellular localization and protein stability. Murine myocardin was found to be a target for SUMO modification at the lysine residue at position 445, and this selectively enhanced cardiac gene expression.

All of the above mechanisms hold important clues as to how myocardin mediated transcription activation can be restricted to either cardiac or smooth muscle cells; however the factor(s) or signal(s) that regulate this fate-decision remain to be elucidated (summarized in Figure 1).
Roles for myocardin in cardiovascular remodeling

The role of myocardin is not limited to establishing a cardiac or smooth muscle phenotype, the activity of myocardin has a direct effect on cardiovascular remodeling. Increased levels of myocardin are associated with dilated cardiomyopathy\(^\text{64}\) and accordingly overexpression of myocardin in cardiomyocytes induces a hypertrophic phenotype, with accompanying re-expression of fetal cardiac proteins\(^\text{65, 66}\). The important negative regulator of cardiomyocyte hypertrophy glycogen synthase kinase 3β (GSK3β), was found to phosphorylate and thereby inhibit myocardin’s transcriptional activity\(^\text{66}\). This suggests that at least part of the hypertrophy inhibiting effect of GSK3β is mediated by modulation of the transcriptional activity of myocardin. BMP2 and TGFβ1, both members of the transforming growth factor super family, regulate growth and differentiation in development and also play important parts in the pathogenesis of cardiac and smooth muscle cells. In cardiomyocytes BMP2 signaling leads to smad1-mediated synergistic activation
of myocardin-dependent cardiac gene expression. In smooth muscle TGFβ1 signaling through direct interaction of smad3 with myocardin induces myocardin-target genes.

Unlike cardiomyocytes, smooth muscle cells can readily switch between a proliferative and synthetic (contractile) phenotype in response to extracellular cues. The proliferative and synthetic phenotypes of smooth muscle cells are correlated with low and high levels of myocardin activity, respectively. General mitogenic signals such as present in serum from fetal origin were found to promote binding of the Ets-factors Elk1 to SRF, while causing displacement of myocardin from SRF, resulting in reduced smooth muscle specific contractile gene expression. Likewise Kruppel-like factor 4 (KLF4) was identified as a strong inhibitor of myocardin, and KLF4 was shown to be rapidly upregulated in the presence of the mitogen platelet derived growth factor-BB (PDGF-BB). The recently identified protein PRDM6 has four Kruppel-like zinc fingers and also represses myocardin activity. The transcriptional repressor Herp1 (HES-related repressor protein 1) also inhibits SRF/myocardin-dependent smooth muscle cell gene expression. Herp1 is a known target gene of Notch, thereby linking Notch mediated signaling to myocardin activity.

Conversely, the anti-proliferative effects of several hormonal signals have also been linked to myocardin activity in smooth muscle cells. The induction of smooth muscle contractile protein encoding genes by myocardin was shown to be greatly enhanced by a direct interaction with steroid receptor coactivator 3 (SRC3), a key factor in oestrogen receptor mediated signaling. Angiotensin II signaling stimulates myocardin expression and activity, in part by acting through the angiotensin II responsive gene paired related homeobox gene 1 (prx1). Finally, the forkhead transcription factor foxo4 inhibits smooth muscle gene expression by binding to myocardin, thereby blocking myocardin’s transcriptional activity. Foxo4 was found to be shuttled out of the nucleus (thereby releasing its repressive effects on myocardin) in response to phosphorylation of the gene Akt, which is a downstream target of insulin and insulin like growth factor 1 signaling.

Mechanical signals such as muscle contraction also affect smooth muscle contractile gene expression. A novel mechanism for this differential gene expression was shown to act though activation of the RhoA/Rho kinase signaling cascade in response to Ca2+ signaling, resulting in induction of myocardin expression, and SRF-myocardin mediated gene expression.

These findings all support a central role for myocardin in the maintenance and control of the contractile phenotype of cardiac and smooth muscle cells (summarized in Figure 1).

Other myocardin family members

Three other myocardin related transcription factors (MRTFs) have been described. The first one MAL is also called MRTF-A, MKL1 and BSAC, and the second factor is called MRTF-B, MKL2 or MAL16. Unlike myocardin, MAL and MRTF-B are expressed in a wide variety of cells. Although myocardin and the MRTFs are highly homologous, and therefore probably can functionally substitute for each other to a great extent, distinct knockout phenotypes have been described. MRTF-
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B knockout results in a failure of neural crest cell development\textsuperscript{78, 79} and vascular defects and liver hemorrhage due to defective smooth muscle gene expression in the liver sinusoids, vitelline veins, and yolk sac\textsuperscript{80}. MAL mutant mice display a defect in myoepithelial differentiation and function, required for the maintenance of lactation\textsuperscript{81}. The activity of the MRTFs is regulated by the state of the cytoskeleton and sarcomeric integrity. Both MRTFs contain a series of RPEL motifs near their N-terminus, which interacts with monomeric G-actin causing their sequestering in the cytoplasm, relaxing sarcomeric gene expression\textsuperscript{82, 83}. The most recently identified myocardin family member is named myoskeletin. Myoskeletin is highly expressed in skeletal muscle, while no expressing was found in heart and smooth muscle tissues. Furthermore, knockdown studies in Xenopus demonstrated that myoskeletin expression is essential for the formation of hypaxial muscle, while overexpression of myoskeletin in explants of non-muscle cells results in the activation of skeletal, smooth and cardiac muscle genes. The later finding suggests that myoskeletin, together with the other myocardin family members can functionally substitute for each other\textsuperscript{84}. Finally, the recently described gene MASTR shares a high degree of homology with the myocardin family of transcription factors. Like myocardin MASTR is a SAP domain containing transcription factor, and it can co-activate MEF2 like the cardiac isoform of myocardin. However, unlike all other myocardin family members MASTR does not interact with SRF and does not induce gene expression from SRF target genes. MASTR is expressed in skeletal muscle, placenta, aorta, lung and brain, and was shown to enhance skeletal muscle gene expression, when overexpressed in muscle cells\textsuperscript{49}. These findings combine to paint a picture where myocardin family members in interaction with SRF and MEF2 function as central regulators of the muscle gene expression program in all three muscle lineages.

Aims and outline of the thesis

Significant myocardial regeneration cannot be achieved through currently available technology. We hypothesized that overexpression of genes involved in the regulation of cardiac development might induce differentiation of target cells into cardiomyocytes. The main aims of this thesis were to test whether and to what extent the newly identified transcription factor myocardin could induce a cardiac or smooth muscle phenotype when brought to expression in clinically relevant cells and whether this leads to improved cardiac function in a mouse model of myocardial infarction.

In chapter 2 we establish that forced expression of recombinant full length human myocardin is able to induce a wide variety of cardiac and smooth muscle genes in human mesenchymal stem cells (hMSCs). Furthermore, we show that adenovirus vectors based on serotype 50 are vastly superior to common serotype 5 adenovirus vectors for gene-transfer to hMSCs.

To achieve cardiac regeneration, blood flow through the damaged area must first be restored. Although neovascularization is readily observed in the infarct area after myocardial infarction, blood flow remains much reduced and is likely insufficient for the maintenance of cardiac muscle cells. A requirement for the further development of capillary vessels, are vascular smooth muscle cells. During
embryonic development the coronary smooth muscle cells derive from the cells of the epicardium. In chapter 3 we investigate whether epicardial cells from human adults can still differentiate into smooth muscle cells in vitro, and therefore are an interesting target for autologous cell transfer studies aimed at myocardial regeneration.

After myocardial infarction a fibrous scar is formed, which contributes to deleterious remodeling and increased mechanical stiffness of the heart. This will result in diminished systolic and diastolic cardiac function and may ultimately lead to heart failure. In addition, the excessive deposition of extracellular matrix components during cardiac fibrosis reduces the electrical coupling between cardiomyocytes and impairs the electrical impulse propagation across the myocardium. In chapters 4 and 5 we focus on the electrophysiological aspect of myocardin transduced cells. To this end we examined whether we can endow isolated fibroblasts from a myocardial infarction scar with the electrical properties of cardiomyocytes using myocardin gene transfer.

In chapter 6 we use a mouse model of myocardial infarction to investigate whether injection of hMSCs transduced with an adenovirus encoding myocardin can acquire a cardiac phenotype in vivo and whether they can restore cardiac function to a greater extent than mock transduced hMSCs.

Finally, to establish the extent to which myocardin alone can activate a bonafide cardiomyocyte expression program and to determine whether the cardiac and smooth muscle enriched splice variants of myocardin display distinct heart and smooth muscle gene induction, we performed microarray analysis to compare the gene expression programs activated by both myocardin splice variants, to that of cardiomyocytes derived in vitro from human cardiac progenitor cells in chapter 7.
References


9. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noisieux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med.* 2005;11(4):367-368.


47. Creemers EE, Sutherland LB, Oh J, Barbosa AC, Olson EN. Coactivation of MEF2 by the SAP domain proteins myocardin and MASTR. *Mol Cell.* 2006;23(1):83-96.


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79. Oh J, Richardson JA, Olson EN. Requirement of myocardin-related transcription factor B for remodeling of branchial arch arteries and smooth


