Adipose derived stromal cells in cardiovascular regenerative medicine
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INTRODUCTION
AND AIM OF THE THESIS
Cardiovascular diseases, such as Myocardial Infarction (MI) are the main cause of mortality leading to approximately 30% of all annual global deaths (www.heartstats.org, www.who.org). Myocardial Infarction occurs as a consequence of occlusion of the branches of the coronary artery system, which results in myocardial ischemia and damage to the surrounding tissues. In the early post-MI phase, the loss of efficient perfusion (hypoxia) coincides with massive death of cardiomyocytes and the vasculature. Together these events activate the innate immune system and cause a rapid influx of inflammatory cells [1, 2]. The course of inflammation in a hypoxic post-MI microenvironment dictates the subsequent remodeling and repair of the cardiac wall. In the events following MI, the debris of cardiac mass is cleared by the infiltrated inflammatory cells and the wound healing process is activated. The adult heart is a highly plastic organ with variable renewal rate: absent or low for cardiomyocytes (CM) but higher for fibroblasts and vascular cells [3]. The adult CM or existing cardiac progenitor cells do proliferate in adults and lead to total replacement of up to 50% of the total heart tissue during healthy life span [4]. Remarkably the regenerative programs of damaged heart are even further accelerated compared to physiologically aged cardiac tissue [5]. Yet, the replicative potential of adult cardiomyocytes is not sufficient to repair the severely damaged heart such as after acute MI. In response to that, myofibroblasts are activated to help maintain the structural integrity of the heart by formation of collagenous scar. In the subsequent remodeling phase, the remaining cardiomyocytes compensate for the loss of contractile mass through hypertrophic growth. Eventually, the imbalance of the cardiac contraction i.e. arrhythmia and cardiac dilatation hamper the cardiac function, which results in heart failure. Most published post-MI intervention studies aim to improve perfusion, reduce apoptosis and acute inflammatory responses. These approaches often involve the administration of pharmacological agents, yet do not prevent the ongoing tissue damage nor induce the regenerative processes. To compensate for the loss of cardiac mass it is essential to provide a new source of cardiomyocytes with proper perfusion and tissue stiffness. Unfortunately, despite early intervention and the advances in the treatment of cardiovascular diseases such as myocardial infarction there is still no cure to regenerate the heart tissue after the injury, thus the developments of alternative cardiac therapies are required.

Mesenchymal Stem Cells/Adipose Derived Stromal Cells therapy

Currently, stem cells emerged as an additional treatment of patients after myocardial infarction [6]. To achieve cardiac repair it is necessary to modulate and tune the post-MI microenvironment. The post-MI microenvironment consists of cells, mediators and extracellular matrix in constant interactions under pro-inflammatory and hypoxic conditions. The orchestration of those interactions by stem cells might reduce adverse remodeling such as cardiomyocyte hypertrophy, scar formation, cardiac dilatation, appearance of arrhythmia and heart failure. Several clinical trials for cardiac stem cell therapy proved that the most promising cells for the cardiac repair are of mesenchymal origin, which is actually similar to the origin of a cardiac tissue itself [7]. Mesenchymal Stem/Stromal Cells (MSC) are defined as highly regenerative, fibroblast-like cells that adhere to tissue culture polystyrene. MSC occur in all organs and can differentiate into various cell types such as osteoblasts, adipocytes, chondrocytes, neurons, cardiomyocytes among others [8-10]. MSC can be easily isolated and immediately used in the autologous applications [11]. The advantage of use of MSC is their secretion of a plethora of immunoregulatory, mitogenic and pro-angiogenic cytokines, growth factors and the extracellular matrix components [12, 13]. Furthermore, the regenerative potential of MSC is
promoted by hypoxic and inflammatory stimuli, which render them as the preferential cells for cardiac repair [14, 15]. Bone marrow is the major reservoir of MSC but their harvest is cumbersome and associated with the donor site morbidity. Currently, adipose tissue gained interest as an available source of therapeutic stem and multipotent progenitor cells [11]. Adipose tissue is highly vascularized and at least part of the heterogeneous population of Derived Stem/Stromal Cells (ADSC) is derived from the perivascular cells known as Stromal Vascular Fraction [16-19]. The isolation of therapeutic cells through lipospiration procedure is less invasive and less painful compared to the bone marrow aspiration. Additionally, cells can be obtained in the clinically relevant numbers or rapidly expanded in vitro. ADSC isolated from patients that suffered MI can be used for autologous cell transplantation into the site of injury to drive cardiac repair (Figure 1). Recent reports from experimental clinical trials with intracoronary infusion of ADSC indicate that the procedure is safe and lead to the improved cardiac function mainly through enhanced perfusion and reduced scar size [20]. Although clinical and pre-clinical studies on safety and efficiency of ADSC therapy for cardiac repair are consistent, the mechanisms by which ADSC repair cardiac damage are still not fully understood [21, 22]. Randomized clinical trials with intramyocardial injection of ADSC after aMI suggest that therapeutic potential of ADSC is mainly due to their pro-angiogenic, anti-apoptotic and immunomodulatory properties [20-22].

This thesis aims to further dissect the regenerative potential of ADSC under pro-inflammatory and hypoxic conditions such as occurring after aMI. Furthermore, we studied the ADSC guided stimulation of cardiomyocyte proliferation, organization and maturation (part I). In part II we elaborate on ADSC mediated vascular network formation, acquisition of the pericyte function and vascular stabilization (Figure 2).

Figure 1. ADSC mediated therapy.
Our focus within ADSC-guided cardiac repair is the modulation of the early post-MI cardiac microenvironment. The recipient environment influences the functioning of the stem cells, their retention, incorporation, survival, proliferation, differentiation and secretion of the regenerative mediators. The post-MI repair mechanism is initiated by the inflammatory response followed by activation of a wound healing phase in the infarcted area. At that moment injected therapeutic cells should function optimally to modulate adverse tissue remodeling i.e. formation of the stiff scar tissue, while maintaining the structural integration. To obtain optimal repair, initiation of the regenerative processes must occur in an early post-MI inflammatory phase i.e. shortly after MI and before appearance of scar tissue [23]. This leaves a small window of opportunity for intervention with stem cells. The local infarcted microenvironment might hamper the functioning of stem cells. Therefore, the aim of this thesis is to understand how the hypoxic and inflammatory stimuli found in the post myocardial infarction microenvironment effect the therapeutic potential of Adipose Derived Stem/Stromal Cells (ADSC) in the process of cardiac repair i.e. induction of cardiomyocyte proliferation, organization and maturation (PART I, Chapter 2-5) together with vascular structures formation and tissue remodeling (PART II, Chapter 6-9). Indicating how ADSC can be used to improve regenerative outcome of post-MI repair (Figure 2). Therefore, an extensive overview of the state in the field of cardiac regeneration by application of Mesenchymal Stem Cells i.e. ADSC is given in Chapter 2. We discuss the novel concepts and findings that are prerequisite of stem cell therapy for cardiac repair. Furthermore, we focused on the recent data obtained from the ongoing randomized clinical trials for prevention of heart failure by MSC or ADSC application. In Chapter 3 and Chapter 4 we identified that the inflammatory and hypoxic host post-MI microenvironment enhances regenerative potential of ADSC to promote cardiomyocyte proliferation. We identified that ADSC promote cardiomyocyte proliferation by paracrine stimulation of CM by secretion of IL-6 (Chapter 3) and Heparin-binding EGF-like growth factor (HB-EGF) (Chapter 4).
Introduction and aim of the thesis

among others. IL-6 and HB-EGF has been described as respectively a pleiotrophic cytokine and growth factor that exerts mitogenic activities on mesenchymal cells such as muscles or cardiomyocytes [24-26]. The treatment of cardiomyocytes with conditioned medium of ADSC activated mitogenic signaling pathways i.e. JAK-STAT and MAPK resulting in enhancement of cardiomyocyte proliferation rate. In Chapter 5 we focused on the role of ADSC derived extracellular matrix on cardiomyocyte proliferation, organization or maturation. Due to the loss of cardiac mass after the MI, the existing fibroblasts/myofibroblasts are activated to produce scar and prevent the cardiac rupture. In the heart, the extracellular matrix components act as a cardiac scaffold that maintains tissue homeostasis. In the post-MI addition of exogenous i.e. ADSC-derived elastic extracellular matrix and matricellular components would provide a new cardiac niche for the activation of cardiac repair mechanism i.e. induction of cardiomyocyte proliferation and reduced scar size. During the wound healing phase after aMI the accompanied vascularization is initiated, albeit insufficient. It suffices to “feed” inflammation, yet falls short to reperfuse the myocardium. Hence, the primary standard treatment of MI aims on the revascularization of the ischemic area. The commonly used methods are coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA) or VEGF therapy. Unfortunately, this treatment does not prevent the ongoing damage. Stem cells such as BM-MSC or endothelial progenitor cells (EPC) have been shown to augment the vascular perfusion and reduce ongoing damage i.e. reduced apoptosis of cardiomyocytes and improved cardiac function [27-29]. Thus in PART II, we focused on the role of ADSC in the vascular network formation and tissue remodeling. Essential cells to accomplish the microvascular homeostasis are pericytes. These cells orchestrate endothelial cells proliferation, survival, vascular tone and permeability [30]. Pericytes are present in the vasculature of all organs including retina, brain, skeletal muscle or adipose tissue among others. Pericytes originate from neuroectoderm or embryonic mesenchyme, thus sharing a mesenchymal phenotype with ADSC. Adipose tissue is a highly vascularized organ with abundant number of Stromal Vascular Fraction (SVF) cells. SVF reside in the perivascular i.e. pericyte-like position and in culture are equivalent to ADSC. Thus, in Chapter 6 we focused on the pericyte overview in their ontogeny, function and regenerative prospective of ADSC as pericytes. In Chapter 7 we identified that ADSC acquired pericyte-like function in vitro and in vivo. ADSC, when in direct contact with endothelial cells, induced vascular network formation. In vitro and in vivo ADSC incorporated into the existing vasculature to promote vessel normalization. Additionally, in the model of oxygen induced retinopathy ADSC replaced the loosed pericytes and promoted vascular normalization by acquisition of pericyte function as well as by modulation of the damaged microenvironment to drive the vasculature homeostasis. To achieve the higher therapeutic potential of stem/progenitor cells it is possible to instruct the cells in vitro before their administration in vivo. The understanding of stem/progenitor plasticity in the presence or absence of post-damage microenvironmental factors will help to develop and improve therapies. Recently, the concept of “of the shelf” stem cell therapy might be achieved by use of the secretors derived from stem cells. In Chapter 8 we identified that the conditioned medium of ADSC promote vascular network formation in vitro. Furthermore, ADSC have higher proangiogenic potential compared to other fibroblasts like cells. Upon stimuli such as hypoxia and inflammation ADSC increased their expression of inflammatory and pro-angiogenic cytokines and growth factors, which render them as preferential cells for revascularization of the ischemic cardiac tissue. One of the risks in stem cell guided cardiac repair by MSC is accelerated fibrosis and scar formation. The major advance in the cardiac clinical trials with ADSC application indicated reduced infarct
size and scarring. Thus in Chapter 9, we focused on the impact of ADSC in tissue remodeling in vitro. We identified the conditioned medium of ADSC as inhibitor of TGF-β induced differentiation and maturation of human dermal fibroblasts (HDF). This might have an impact on limited scar formation. In Chapter 10, the observations described in this thesis are summarized and future perspectives of MSC/ADSC mediated cardiac regeneration are discussed.

References


