Chapter 1

Introduction and aims
INTRODUCTION

Heart failure is a syndrome that is diagnosed when the heart is unable to sufficiently pump blood through the body. Heart failure is a major global public health care burden with a prevalence of 1-2% and a lifetime risk for developing heart failure of 1 in 5 for both men and women. Furthermore, heart failure patients have a five-year survival rate of 50%. In an aging population, heart failure prevalence as well as the economic and health care burden will increase drastically. Consequently, there is an unmet need to discover new treatment strategies in order to improve prognosis.

Various risk factors for the development of heart failure are well known, but poorly understood. Clear examples of this are iron deficiency and cardiac hypertrophy. Iron deficiency is a clinically relevant co-morbidity for heart failure and is observed in patients with or without anemia, and causes impaired exercise tolerance, reduced quality of life and worse prognosis. Other than its major role in general oxygen transport as part of hemoglobin, iron also plays essential roles in cellular mechanisms related to redox cycling, electron transport, and as an enzymatic cofactor. Cellular iron deficiency impairs functional status in heart failure patients independently of hemoglobin levels and intravenous supplementation with iron reverses adverse effects. The direct effects of iron deficiency on cardiomyocytes are unknown and identifying relevant mechanisms may lead to improved therapies to combat heart failure.

Another major co-morbidity for heart failure is (pathological) cardiac hypertrophy, which is primarily caused by hemodynamic stress or ventricular wall stress in patients. The first coping mechanism is the activation of a transcriptional hypertrophic response program that is reversible at first, but persistent wall stress will lead to pathological hypertrophy before the onset of maladaptive cardiac remodeling. Preclinical studies demonstrated that this hypertrophic response is generally detrimental to cardiac function, but the underlying molecular mechanisms are poorly understood. Therefore, identifying these mechanisms and preventing hypertrophy may lead to novel therapeutic interventions.

Studying molecular mechanisms of pathophysiology in the human heart is challenging as there are major ethical concerns with respect to obtaining healthy cardiac tissue from humans. Samples used for molecular profiling are often obtained during the end-stage of the disease or post-mortem. Moreover, the heart consists of non-proliferative cells that cannot be expanded in vitro. Therefore, relatively large cardiac biopsies are required to acquire the minimally sufficient amounts for basic assays. In turn, researchers have elected to employ animal models to great extent, but with varying degrees of success. Mice genetically resemble human to great extent and can be used in a wide variety of experiments. However, animal models have produced unreproducible results that could not be applied to human heart failure. Therefore, novel approaches to model human pathophysiology were needed. In recent years, human pluripotent stem cells have become
a popular tool to obtain a virtually unlimited number of functional human cardiomyocytes for *in vitro* studies. Robust human cardiac differentiation protocols were developed and human cardiomyocytes could be studied in great detail\textsuperscript{15-17}. Hence, cardiac-specific effects could be assessed at an early stage in detail, preventing previously unexpected side-effects of drugs during clinical trials. Notably, the introduction of induced pluripotent stem cells provided more accessible means for studying diseases *in vitro*, especially in a patient-specific fashion\textsuperscript{18}. Seminal studies demonstrated that pathological cellular mechanisms could be recreated *in vitro*\textsuperscript{19,20}. Consequently, human cardiomyocytes have become a common platform for *in vitro* disease modeling, but also for screening of currently available drugs as well as novel drugs\textsuperscript{21-23}. In order to improve *in vitro* models, research is currently being conducted to improve tissue engineering and consequently provide optimal models for the human heart.

These developments pave the way to accurately studying complex cardiovascular disease. Peripartum cardiomyopathy is an interesting example of such a disease. Peripartum cardiomyopathy is a severe form of heart failure that occurs in women during the last trimester of pregnancy or in the first six months after childbirth. Disease severity increases with every pregnancy, but patients generally recover when treated adequately. Diagnosis is established according to specific guidelines and is mostly based on exclusion criteria\textsuperscript{24}. Recent studies have identified cathepsin D as a pivotal mediator in molecular pathophysiology\textsuperscript{25}. Hilfiker-Kleiner et al. have demonstrated that cathepsin D secreted from cardiomyocytes cleaves the nursing hormone prolactin into antiangiogenic fragments that induce apoptosis in endothelial cells. As a result, endothelial cells secrete microRNA-146a-loaded exomes that are taken up by adjacent cardiomyocytes. MicroRNA-146a inhibits specific metabolic pathways and is thought to ultimately induce heart failure\textsuperscript{26}. Notably, it remains unknown what causes the secretion of cathepsin D into the circulation. Nonetheless, several studies aimed to intervene at various levels of the recently identified pathological mechanism, with varying degrees of success. Therefore, more research needs to be done in order to elucidate the cause of peripartum cardiomyopathy.

**AIMS OF THE THESIS**

In this thesis, we aim to study molecular mechanisms underlying key aspects of failing cardiomyocytes in an *in vitro* setting. To this end, we differentiate human embryonic stem cells or patient-derived induced pluripotent stem cells towards cardiomyocytes, and introduce specific conditions that mimic clinical settings in order to reproduce a diseased state of patients, including iron deficiency and mechanical stretch. Furthermore, we use *in vitro* disease modeling to unravel pathophysiological changes on a cellular level in a specific form of heart failure (peripartum cardiomyopathy). Ultimately, we hope to contribute
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to the advancement of targeted and personalized medicine with the use of adequate in vitro disease modeling.

Part I focuses on two in vitro models for cardiomyocyte-specific processes that can exacerbate or lead to heart failure. We highlight the current state of in vitro disease modeling. We study the functional effects of cellular iron deficiency on stem cell-derived cardiomyocytes. Various functional aspects of cardiomyocyte function are studied in order to unravel which mechanisms will fail due to iron deficiency. Furthermore, we apply cyclic mechanical stretch on stem cell-derived cardiomyocytes to induce pathological hypertrophy. RNA sequencing is employed to identify and target pathways involved in the onset of hypertrophy. Chapter 2 provides an overview of the current state of in vitro cardiac disease modeling with human (induced) pluripotent stem cells, focusing on tissue engineering, heritable cardiomyopathies and how diseases might be modeled when the causative mutations are unknown. In chapter 3, we investigate the effects of cellular iron deficiency on human cardiomyocyte function. We also establish which cellular mechanisms are impaired during iron deficiency and to what extent these effects are reversible by iron supplementation. In chapter 4, we introduce cyclic equiaxial stretch as an in vitro model for mechanical stretch leading to cardiomyocyte hypertrophy. Following validation of the model, we set out to determine key pathways that regulate the onset of hypertrophy by RNA sequencing. Identified pathways are inhibited in an attempt to block the pathological response to mechanical stretch.

Part II uses in vitro disease modeling to unravel the pathophysiology of peripartum cardiomyopathy, which is characterized by a specific disease onset in the last trimester or in the first six months following childbirth. Seminal studies have demonstrated that the interaction between circulating cathepsin D and prolactin results in antiangiogenic effects that may lead to peripartum cardiomyopathy. Chapter 5 reviews the currently known mechanisms involved in the pathophysiology of peripartum cardiomyopathy and possible underlying genetic background that are associated with an increased risk to develop peripartum cardiomyopathy. In chapter 6, we investigate whether cathepsin D secretion from cardiomyocytes is an event exclusive to peripartum cardiomyopathy pathophysiology or whether its secretion is common in other cardiac disease as well. Furthermore, we will assess the effects of reduced CSTD levels in human cardiomyocytes. In chapter 7, we study peripartum cardiomyopathy more in-depth. We have sequenced the transcriptomic profile of cardiomyocytes derived from a patient and a familial age-matched healthy control in order to discover putative genetic transcripts that may be causal for disease development. Since the cause for PPCM is unknown, we have designed the experiment in this specific familial patient-control setup by including a healthy sister. Consequently, the genetic background is minimalized, resulting in more reliable data in this iPSC-based disease model. This approach allows for the distinction between stretch-related effects and
PPCM effects. Therefore, we will perform thorough pathway, gene ontology enrichment, and transcription factor analysis on the obtained list of differentially expressed genes.

Finally, chapter 8 provides general discussion of the major findings and reflects upon future perspectives.
REFERENCES


PART I