CHAPTER I

INTRODUCTION

1.1 Background

Over the last few decades, various diagnosis methods and treatments have been developed to treat cardiovascular diseases (CVDs). However, CVDs remains to be the dominant cause of morbidity and mortality in all countries. It is estimated that 17.7 million people died from CVDs in 2015, which represents 31% of global death. Furthermore, in Southeast Asian countries the prevalence of symptomatic heart failure (HF) appears to be higher than the rest of the world due to the prevalence of risk factors such as hypertension (>24% in Cambodia and Laos), tobacco smoking (>36% in Indonesia) , and physical inactivity (>50% in Malaysia) (Lam, 2015). These data indicate that there is still a critical need for novel biomarker and treatment to decrease the morbidity and mortality rate of the disease. Out of all the types of CVDs, Ischemic Heart Disease (IHD) has the highest mortality rate (WHO, 2017). Reduced blood supply causes IHD due to blockage of the coronary arteries in conditions like coronary atherosclerosis.

Due to the high oxygen requirement of the heart, blockage of coronary arteries can result in myocardial infarction (MI), commonly known as heart attack. MI results in tissue damage that causes massive cardiomyocyte death. Due to the limited proliferation ability of cardiomyocytes, the damaged tissue is then replaced by fibrotic scar tissue primarily composed of fibroblasts. This scar tissue cannot replace the function of the damaged cardiac tissue, which compromises the heart's ability to contract. To compensate for this, pathological changes of the heart's structure may occur, which includes cardiac hypertrophy and chamber dilation (Travers, Kamal, Robbins, Yutzey, & Blaxall, 2016). These changes usually lead to progression to heart failure.

There are some readily available interventions for MI. The immediate treatment at the early hours of MI involves primary angioplasty, coronary bypass grafting, and thrombolysis. The primary goal of these treatments is to salvage the remaining function of the heart by maintaining reperfusion of the ischemic myocardium and limit infarct size (Maxwell, 1999). However, none of these treatments can repair the permanent damage caused to the heart (Aso et al., 2011).

The limitation of the current MI treatment has driven the cardiac regeneration research. Stem cell biology has been long considered as the leading field for regenerative therapy. Embryonic stem cells (ESCs) and the recently developed induced pluripotent stem cells (iPSCs) have the potential to provide an unlimited supply of human cardiomyocytes. However, the usage of stem cells is still surrounded by controversies such as ethical concerns and potential tumor formation (Laflamme & Murry, 2011). These have prompted research interest towards alternative methods to regenerate cardiomyocytes. One of these methods is transdifferentiation.

Transdifferentiation is defined as an irreversible conversion of one differentiated cell type to another differentiated cell type. Since the damaged cardiac tissues after MI are mostly composed of fibroblasts, transdifferentiation can potentially be applied to these cells to convert them into mature and functional cardiomyocytes (Figure 1). This approach provides dual advantages of inducing regeneration of healthy cardiomyocytes while reducing scar tissue (Vaseghi, Liu, & Qian, 2017). The use of somatic cells during cardiac transdifferentiation will also remove the ethical and technical challenges of using ESCs or iPSCs.

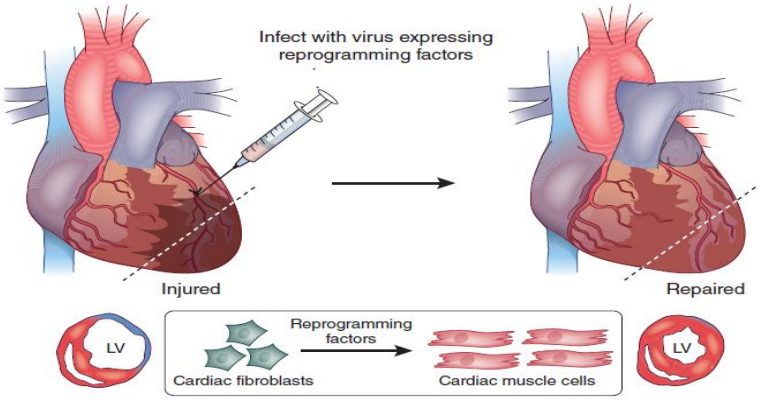

Figure 1. Mechanism of heart damage repair by in-vivo reprogramming of cardiac fibroblasts into induced cardiomyocytes. Viral cocktail containing cardiac transcription factors is injected into the infarcted area. (Young Jae Nam et al, 2013)

While several published studies have demonstrated successful transdifferentiation of murine fibroblast *in vitro* (Ieda et al., 2010)(Nam, Song, & Olson, 2013), there are still many limitations surrounding the technique that has to be addressed prior to clinical application. One such limitation is that the majority of the transdifferentiated fibroblast using the current techniques shows poor or partial maturation as compared to adult cardiomyocytes (Nam et al., 2013). The most glaring drawback of the study is, however, the low conversion efficiency of transdifferentiation. Three recent studies have been conducted to improve transdifferentiation of fibroblast to induced-cardiomyocyte like cells (iCMs). However the efficiency of the transdifferentiation in all three studies was still very low (≤25%) (Christoforou et al., 2017; J.-D. Fu et al., 2013; Nam et al., 2013; Song et al., 2012; Wada et al., 2013)

High-throughput chemical library screening was carried out to improve the efficiency of the current protocol. The screening result identified an inorganic chemical compound, labeled as "compound A," which showed to be able to enhance cardiac transdifferentiation. The main objective of this study is to identify whether this small molecule can significantly enhance the efficiency of the current cardiac transdifferentiation protocol. On top of that, it is also crucial to identify and understand the underlying molecular signaling events that are affected by Compound A. These will not only deepen our understanding of cardiac transdifferentiation but also allow us to design more potent drugs to enhance cardiac transdifferentiation.

1.2 Objectives of the Study

- To validate and characterize the effect of Compound A generated from the high-throughput chemical library screening
- To validate genes that are differentially expressed after administering Compound A to the transdifferentiation system
- To understand the underlying key signaling pathways that are involved in transdifferentiation

1.3 Limitations of the Study

- Insufficient time to perform more in-depth study.
- Insufficient time to perform the experiments in triplicates.
- The data is only collected from one transdifferentiation time point (day 14).