Perspective of Direct Reprogramming of fibroblast to Cardiomyocyte

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ABSTRACT :

A cure for cardiovascular disease remains a major unachieved medical need. Recent investigations have started to uncover the mechanisms of mammalian heart regeneration. Adult cardiomyocytes have slight regenerative capacity following injuries and also myocardium heals by fibroblast proliferation and scar formation. Mixtures of Cardiac-specific defined factors can generate cardiomyocytes from cardiac fibroblasts. By the use of Cardiac-specific transcription factors: Gata4, Mef2c, and Tbx5 (GMT), GMT plus Hand2 (GHMT), or Mef2c, Myocd, and Tbx5 in vitro Mouse fibroblasts can be directly converted into cardiomyocyte-like cells. Human fibroblasts can be reprogrammed into differentiated cardiomyocyte-like cells by overexpressing GMT plus Myocd and Mesp1 or Gata4, Hand2, Tbx5, Myocd, miR-1, and miR-133. Cardiac reprogramming technology may be a possible approach that could regenerate diseased hearts. This article reviews the current studies in cardiac reprogramming, and discusses the possibilities and disputes of direct cardiac reprogramming towards regenerative therapy.

Keywords : Cardiovascular Diseases, Fibroblasts, Humans, Mice, Myocytes, Regeneration, Transcription Factors, Wound Healing.

INTRODUCTION

Although, trustworthy estimates of heart failure are unavailable in India because of the deficiency of a surveillance to track incidence and prevalence of heart failure. According to WHO estimates, in 2002, 16.7 million people around the globe (1/3 of all deaths globally) expire of cardiovascular diseases per year.

Also, statistics show a depressing trend worldwide in which heart disease is expected to be the leading cause of death in the world by 2020[1].

Lizards could regrow their lost tails was observed by Aristotle. Adult zebrafish heart vigorously adds cardiac cells during adult animal growth and size maintenance which is not observed in mammalian heart[2]. These are bringing inspirational perspectives into the regeneration of relatively static mammalian heart.

Cardiac fibroblasts (CF) are the most common cell type in the heart and plays important role in regulating normal myocardial function and in the remodeling that occurs with myocardial infarction, heart failure and hypertension. It supports cardiomyocytes and contributes to scar development after injury.

In recent times it had become clear that the adult mammalian heart possesses intrinsic repair and regenerative potential to some extent. CF is a possible supply of cardiomyocytes for regenerative therapy if it were feasible to directly reprogram the resident fibroblasts into cardiomyocytes.

The Recent trends of cardiovascular line of investigation are focused on (1) the use of endogenous adult stem cells, (2) the reprogramming of CF into functional cardiomyocytes, and (3) the experimental activation of dormant regenerative mechanisms promoting mammalian cardiac repair.

Detection of MyoD, a master regulator for skeletal muscle differentiation and Highly purified umbilical vein endothelial cells or adult dermal microvascular endothelial cells were transduced with the transcription factors FGRS (FOSB, GFI1, RUNX1 and SPI1) induces outgrowth of haematopoietic colonies containing cells with functional and immunophenotypic features of multipotent progenitor cells (MPPs)[3]. Transdifferentiation can provide pancreatic exocrine cells to beta-cells. Direct reprogramming of hepatocyte-like cells, and cardiomyocyte-like from fibroblasts.

Recently, a “newest generation” of cellular reprogramming has evolved without passing through a pluripotentstem cell state, the direct conversion of one adult cell type into another by miRNAs. In this Review, we will discuss several innovative findings and provide a background overview of direct reprogramming fibroblast using several combinations of defined factors.

Direct reprogramming of Cardiac Fibroblasts into functional Cardiomyocytes (in vitro)

Reprogramming of mouse postnatal CF and dermal fibroblasts directly into functional cardiomyocytes in vitro by combination of transcription GMT (Gata4, Mef2c, and Tbx5)[4]. Kohei Inagawa et al give confirmation that GMT gene transfer induces cardiomyocyte- like cells in infarcted hearts and that expression of GMT via a polycistronic vector enhances cardiac differentiation[5].

Selection for mature Cardiomyocyte-Inducing Factors

Fluorescence-activated cell sorting (FACS) could be used to analyze the generation of mature cardiomyocytes from fibroblasts[6]. Only cardiomyocytes, expressed green fluorescent protein (GFP) in the transgenic mouse hearts and in primary cultured neonatal mouse cardiac cells. Thy1 and Vimentin markers were expressed by cardiac fibroblasts[7,8] and mature cardiomyocytes expressed cardiac troponin T (cTnT). Heart tissue fragments were removed by the filtered cells by cell strainers and FASC. By this we could produce greater than twice the number of cardiac fibroblasts than by conventional fibroblast isolation techniques[8].

Selection of cardiac reprogramming factors was done by microarray analyses, to identify transcription factors and remodeling factors that cause functionally relevant changes to the genome without change in the nucleotide sequence[8]. 13 Factors that causes severe developmental cardiac defects and mesoderm–specific transcription factor were selected[9]. To express every gene in cardiac fibroblasts separate retroviruses were generated.

Thy1+/GFP- neonatal mouse were transduced with a combination of retroviruses expressing 14 factors and Ds-Red control[10]. Ds-Red retrovirus infection failed to show any GFP+ cells in cardiac fibroblasts (CF's). While , transduction of all 14 factors into fibroblasts showed the generation of 1.7 % GFP+ cells, demonstrating the successful activation of the cardiac-enriched a myosin heavy chain aMHC gene in some cells .

Further, it was observed that four factors (Gata4, Mef2c, Mesp1, and Tbx5) were adequate for efficient GFP+ cell induction from cardiac fibroblasts. The expression of cTnT was examined by fluorescence-activated cell sorting FACS. It was found that Mesp1 was not essential for cTnT expression and cTnT+ or GFP+ cells were not observed when either Mef2c or Tbx5 was removed. Gata4 elimination did not considerably affect the number of GFP+ cells, but cTnT expression was abolished, indicative of Gata4 was also essential.

Adding a basic helix loop helix transcription factor Hand 2 to GMT converted adult cardiomyocytes into functional cardiomyocyte like cells more efficiently.

Using MicroRNAs (miRNAs) in Cardiac Reprogramming

Chemically synthesized miRNA mimics are synthetic oligonucleotides can be easily administered to cells via lipid-based transfection and reveal low toxicity in animal models11. A single miRNA may target multiple pathways simultaneously.

MicroRNAs (miRNAs) has important roles in signaling pathways, transcription factors, epigenetic regulation, and cell fate decisions. Also, the small size of a single miRNA increases reprogramming efficiency and functional homogeneity of reprogrammed cells[12]. MiRNAs binds to the 3'-untranslated region (UTR) of target mRNAs and suppress the expression of hundreds of genes.

Muscle specific miRNAs (miR-1, 133, 208, 499) reprogrammed neonatal mouse CFs into cardiomyocyte-like cells[13]. With cardiac reporter aMHC-CFP (cyan fluorescent protein) transgenic mice they established that in vitro transfection of mature miR-1, miR-133, miR-208, and miR-499 mimics induced 5% of aMHC-CFP+ cells in CFs.

They also found that induced cardiomyocytes (iCMs) generated by miRNAs expressed several cardiac specific proteins, sarcomeric structures, and additional treatment with a Janus tyrosine kinase (JAK) inhibitor improved reprogramming efficiency and quality, results in spontaneous cell contractions in 1-2% of the starting cells. It demonstrates the powerful effect of this group of small RNAs at mediating cellular reprogramming. In regenerative medicine to produce clinically useful cells from an adult patient's own common tissue had remained an eye-catching approach[14]. This method can generate immunologically matched tissues and avoid immunogenicity. By forced expression of three or four core cardiac transcription factors or Muscle-specific miRNAs mouse fibroblasts can be converted into functional cardiac-like[15,16,17,18,19]. Also, direct delivery of these transcription factors into the myocardium of mice following MI reduce scar formation and reduced worsening of cardiac function[16,17].

These are functionally immature, as indicated by their morphology, comparatively rare spontaneous contractility and low-amplitude calcium transient. Also, the human induced cardiac like myocytes (iCLMs) produced in the study were diverse, contains cells with varying levels of expression of cardiac and noncardiac genes. Diversity of iCLMs probably reflect variations in the stoichiometry and levels of expression of reprogramming factors in individual cells.

Additionally, Diversity of human fibroblast from various ages and genetic background probably gives different % of cardiac marker expressing cells. Human fibroblasts whose epigenetic stabilities vary depending on their origins are likely to have a wide spectrum of receptiveness to reprogramming. Furthermore, the differences that are present in each viral preparation also contribute to inconsistency in reprogramming efficiency.

Snail is a key molecular barrier during cardiac reprogramming, also other molecules important for cardiac reprogramming remain uncertain[20]. Moreover secreted

proteins, electrical and mechanical stimulation, and cell-to-cell contact may promote cardiac reprogramming, according to the co-culture experiments in human iCMs.

For clinical applications the safety issue associated with viral delivery of reprogramming factors need to be evaluated. Future studies investigating whether fibroblasts can be reprogrammed into functional iCMs without viral integration is vital. It will also be a concern to research on the possible role of pharmacologic agents in regeneration and to generate specialized cells in cardiac conduction as a strategy for cardiac contractility.

Moreover, optimization could be done by the use of small molecules and epigenetic enzymes to deliver the reprogramming factors. Conducting trials in large animals will be important to refine the technology and asses its safety and efficacy particularly regarding arrhythmias.

Even if, many hurdles remain in this emerging research field, accepting the molecular mechanisms of direct cardiac reprogramming and translational studies using human cells and large animals, may further develop and advance clinical potential of this technology. Since the demand is high for new heart regenerative therapies the opportunities for the potential benefits of this direct cardiac reprogramming approach are large in future clinical uses. This unique, innovative approach can be used to switch cell fate and induce regeneration in the heart (without the use of cell transplantation methods used heretofore).

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