

Regeneration Progress in Heart Attack

Baicheng Chen *

Department of Anatomy and Cell Biology, McGill University, Montreal, Canada, H3A 1A8

* Corresponding Author Email: baicheng.chen@mail.mcgill.ca

Abstract. Heart attack, also known as myocardial infarction, is a common and fatal cardiovascular disease all around the world. Not only is there the high death rate, but is there the increased risk of further heart failure, which makes the researches on heart regeneration of great importance and worthy of continuing. Although great progress has been made in recent years, current treatment of heart attack cannot perfectly eliminate the potential risks of future heart problems. In order to find an ultimate solution to the disease, scientists have been studying the heart regeneration abilities on newts, zebrafish, and neonatal mice, etc, and have understood various mechanisms of animal heart regeneration; however, heart regeneration possibility on humans is still not studied enough and the side effects on humans are the main obstacles for the regeneration techniques to be medically used, but this technology still has great potentials and might ultimately eliminate the underlying risks of heart attack.

Keywords: Dedifferentiation; regeneration; oncostatin M; nsCCN; mps1; Baf60c.

1. Introduction

Heart attack, also known as myocardial infarction, is among the diseases with the highest death rate in many countries. Approximately 805,000 Americans experience a heart attack each year, of which 605,000 are having their first one and 200,000 have already experienced one [1]. Coronary heart disease is usually the reason for heart attacks. The blockage formed by the accumulation of fat or bad cholesterol within artery walls is the main reason for the coronary heart diseases. Consequently, during a heart attack, the heart is not supplied with enough blood, which causes damage to the heart muscle tissues [2]. In adult humans, these damaged cardiomyocytes will form scar tissues.

The danger of the disease is not only its high death rate, but also the potential risk of causing further heart problems. The symptoms of heart attacks vary, from no symptom to severe symptoms like short of breathe and nausea before strike [3]. Regardless of severity of symptoms, the lack of blood supply during heart attacks cause damage to heart cells, which forms the scar tissues. The ventricular wall is maintained, but the scar tissue cells' ability to pump blood out of the body is compromised, which results in congestive heart failure [4]. In addition, soon after birth, mammalian heart muscle cells stop proliferating [5], which leads to the inability to replace the damaged heart muscle tissues; thus, to eliminate the underlying risk, scientists aim to regenerate healthy muscle cells as to replace the scar tissues.

The newts were known to be able to fully regrow their lost body parts. Researchers have discovered that newts are capable of completely healing the damaged cardiac muscle tissues caused by a heart attack. This is accomplished by dedifferentiating adult cardiomyocytes and then proliferating them [6]. Particularly, during this phase, the heavy myosin chain and different troponins, which are present in heart muscle cells, were markedly down-regulated while the cells engaged in enormous cell division, which takes about two weeks to complete [7]. Besides, scientists have also found that the protein oncostatin M (OSM) produced by white blood cells is the messenger that causes this dedifferentiation process after a heart attack [6].

Given the severity of heart failure, it is extremely important and worthy to investigate more on the possibility of human heart repair techniques. Scientists have understood several heart reconstruction mechanisms in certain animals, but the lack of information and the side effects of these molecules involved in animal heart repair in human body have become the main obstacles in pursuing medical application of heart regeneration method, and more studies needs to be done.

2. Mechanism of Heart Cell Dedifferentiation

2.1. Immune Response during Heart Attack

One of the key elements of the innate immune system that acts importantly after a heart attack is the macrophages. An inflammatory response frequently follows a heart attack in an effort to lessen the harm. Myocardial necrosis starts a sterile inflammatory process that is brought on by the release of damage-related molecular patterns (DAMPs) [8]. In addition, after blood supply is re-established, local cardiac mast cells release the mediators that start an amplification loop with nearby cells including infiltrating neutrophils and resident macrophages [9,10]. This immune cell infiltration, especially OSM-secreting macrophages, is crucial for the remodelling and regeneration of the heart.

2.2. OSM Initiates Dedifferentiation

Damaged hearts can be fully repaired throughout heart regeneration and remodelling. In order to identify the mechanism behind heart regeneration, acute myocardial infarction (MI) patients' samples were examined by Jochen Pöling and his colleagues. By examining the expression of α -smooth muscle actinin (SM-actinin), which is linked with the expression of OSM in the border zone (BZ) of infarction, they were able to determine that macrophage infiltration occurred during the course of MI. They also proved that cellular dedifferentiation of cardiomyocytes was initiated by OSM. They removed some cardiomyocytes from adult rat hearts, treated them with various cytokines and growth factors, and discovered that cardiomyocytes treated with OSM underwent the most remarkable morphological alterations [11].

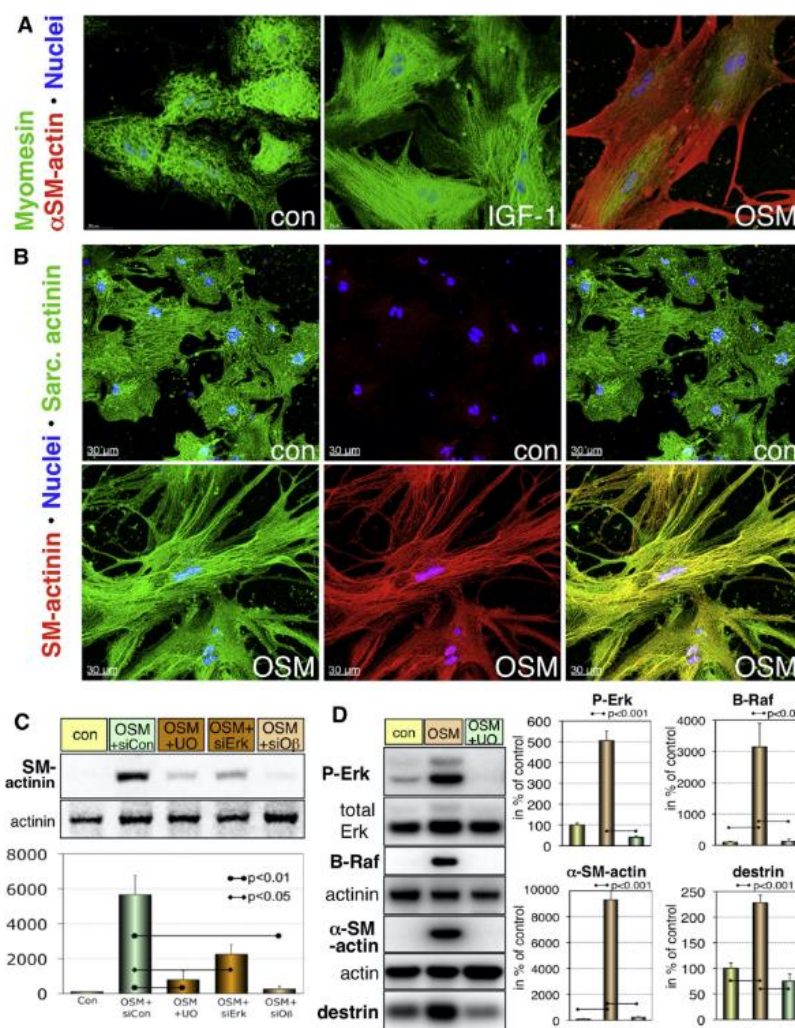


Figure 1. OSM Stimulates Ras/Raf/MEK/Erk Cascade Via OSM Receptor to Induce Cardiomyocyte Dedifferentiation in Vitro (Oβ) [11].

By inducing MI in O β deleted mice through ligating the descending coronary artery (LAD) at a distal site, Pöling et al. also showed that when OSM signalling is suppressed, a substantial reduction in left ventricular performance is seen [11]. This demonstrates that OSM is crucial to heart regeneration and is likely to be able to promote cardiac dedifferentiation. The Max Planck scientists also presented their findings on the heart regeneration process following a heart attack, demonstrating that OSM transmits a signal for cardiac cells to go through dedifferentiation. The heart regenerates because regression cells divide and a small number of cardiac stem cells migrate in.

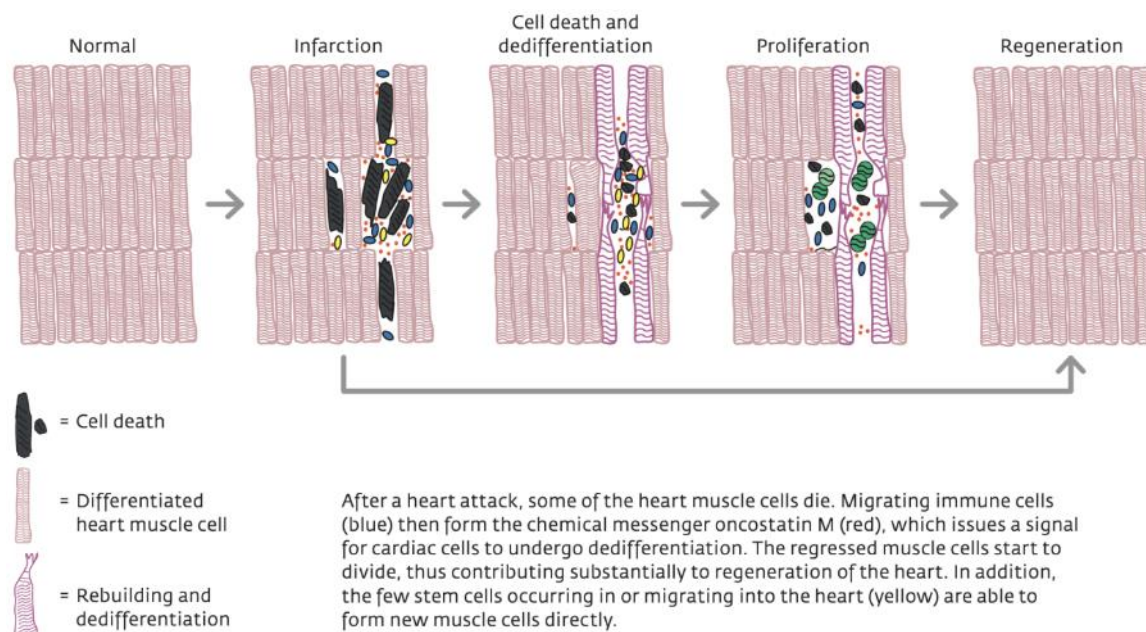


Figure 2. Immune Cell Activities during Post-injury Heart Regeneration [6].

3. Heart Regeneration Abilities of Animals

The majority of human cardiac stem cells turn off shortly after the heart is developed while the remaining ones continue to create new heart muscle cells throughout an individual's life. For an adult, within one and a half years, only approximately 5% of heart muscle cells are expected to be replaced. Consequently, human heart is not able to heal itself after a heart attack, while some animals have the ability to fully heal their damaged myocardium. Three types of animals with this ability that are being studied are newts, newborn mice, and zebrafish.

3.1. Heart Regeneration Ability of Newts

The remarkable capacity of newts to completely regenerate their missing limbs is well known, but they are also capable of repairing their damaged hearts without functional impairment after suffering a heart attack. Singh et al.'s ventricular apical resection investigations on adult newts showed full regeneration of the heart and complete function restoration of the cutted heart (25% to 30%) 60 days after the injury. Mitotic cardiomyocytes were also observed. Additionally, they also performed a 5-Ethynyl-2'-deoxyuridine (EdU) incorporation experiment, in which they labelled multiplying heart muscle (desmin⁺-EdU⁺) cells in the newt heart that is repairing after damage [12]. This shows that newts have very strong ability to globally repair their damaged heart tissues after a heart attack.

The mechanism of newt heart regeneration is a relatively new topic, and the use of newts as exotic laboratory animals has presented several challenges to researchers, including lack of treating information and the unfinished genome sequencing. In order to solve these concerns, scientists at the Max Planck Institute extracted messenger RNA (mRNA) from larval and damaged tissues from adult newts in order to pinpoint the proteins that may have been involved in heart regeneration. One of the proteins found, newt-specific cysteine-rich growth regulator, abbreviated as nsCCN, may play a role

in reverse development of newt cells [6]. nsCCN belongs to the CCN proteins. CCN proteins are associated with cell proliferation and damage repair [13]. The nsCCN is also unique, since it does not cluster with any other CCN protein on CCN family phylogenetic tree, meaning that a gene split at a very early time [14].

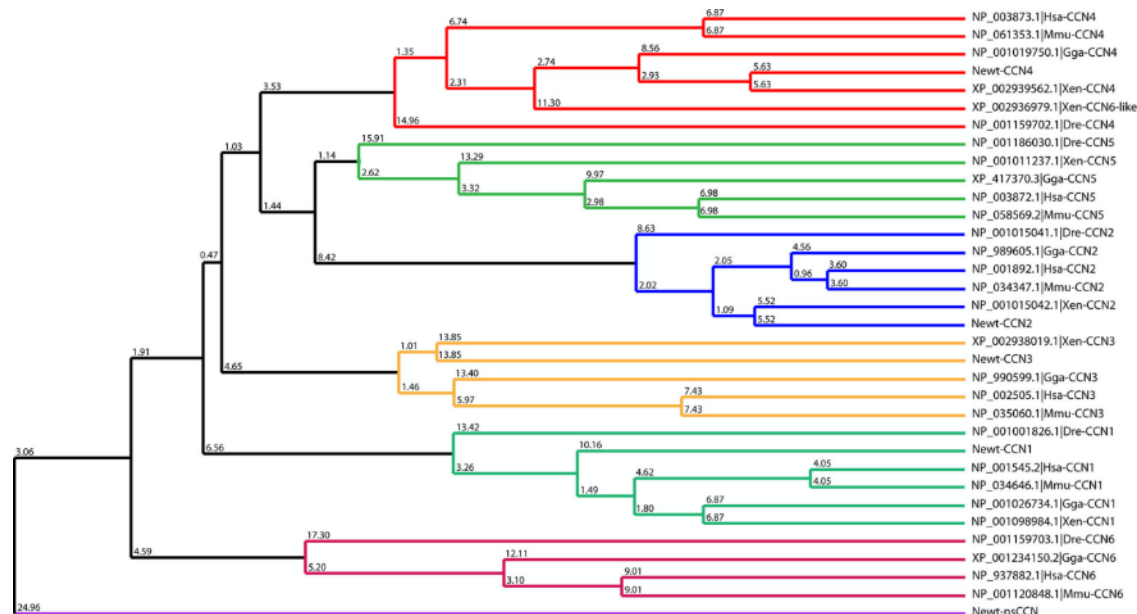


Figure 3. The CCN Family's Phylogenetic Tree and the Location of the nsCCN Gene [14].

Moreover, in situ hybridization study of the expression of nsCCN, CCN1 (=CYR61), CCN2 (=CTGF) in healthy hearts showed that baseline levels of CCN1 and CCN2 were high, but levels of nsCCN were low in heart muscle cells. Besides, following heart damage, nsCCN was up-regulated only in the endocardial cells in injured parts, indicating nsCCN is a crucial part of the regulatory system that controls this process [14].

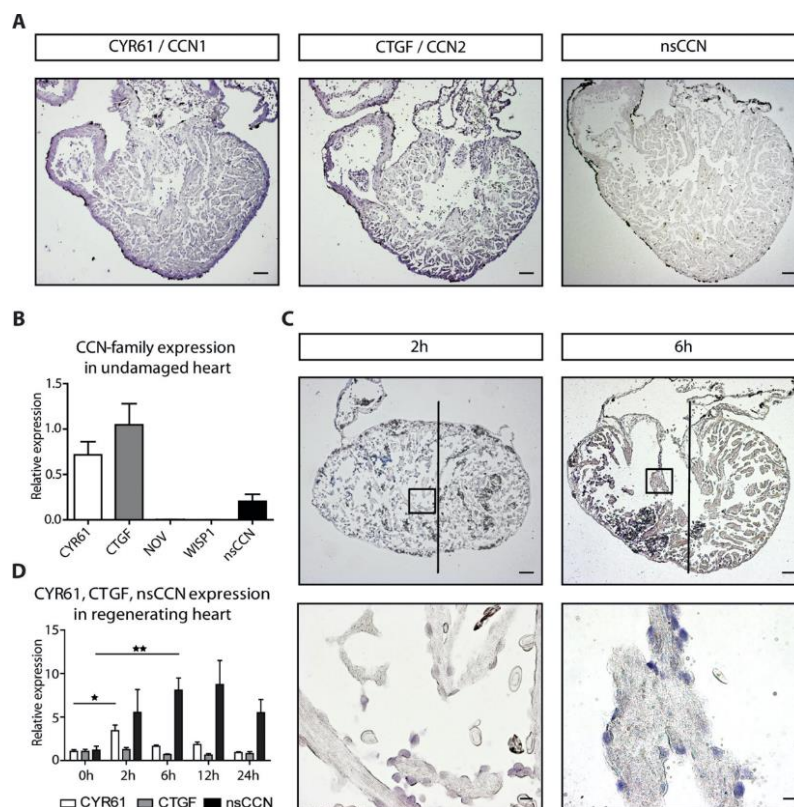


Figure 4. Following mechanical damage, an analysis on the expression of several CCN proteins in healthy and reconstructing hearts was done [14].

Additionally, the Max Planck Institute researchers found that newt heart tissue renewal did not occur from newt stem cells but rather from differentiated newt heart cells that disassembled their supporting skeleton and dedifferentiated to a precursor stage. New mature heart muscle cells are created by continued proliferation and maturation, and the newt heart is renewed [6].

3.2. Heart Regeneration Ability of Zebrafish

An important animal in heart development and repair research is definitely zebrafish. Compared with newts, the zebrafish model has many advantages in human heart regeneration studies. It has a shorter reproductive cycle and produces hundreds of progen. Additionally, zebrafish embryos are almost transparent and simple to study. More importantly, as a species that has been studied for a long time, its genome has been completely and accurately sequenced [15].

The ability of various organ regeneration is special on zebrafish. Different organs can regenerate in adult zebrafish, but the processes that govern regeneration appear to be organ-specific [15]. Specifically, zebrafish heart regeneration takes place through a gradient of proliferation that is strongest in epicardial myocytes, following scattered cardiomyocyte proliferation in the damaged region to rebuild the ventricular wall. Mps1 the kinase is needed for the checkpoint in mitosis. The proximal regeneration blastema, a collection of cells that typically multiply rapidly during outgrowth, was the specific place where mps1 expression was stimulated. During a cardiac injury response experiment on zebrafish with temperature-sensitive mutation in mps1, the damaged hearts maintained fibrin deposits and formed significant connective tissue scars instead of the ventricular wall being repaired. Thus, it is concluded that heart muscle cell proliferation in the wounded zebrafish heart produces a new ventricular wall and needs the cell-cycle regulator mps1 [16].

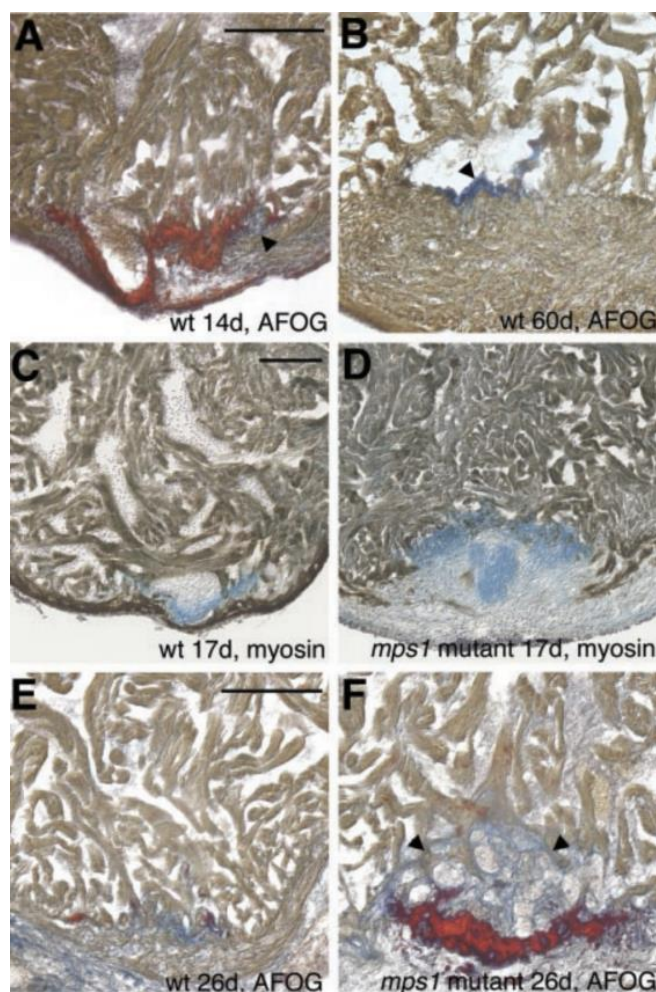


Figure 5. Mps1 mutant heart showed no evidence of new muscle formation and formed scar tissue [16].

3.3. Heart Regeneration Ability of Mice

Mice is one of the most common laboratory animals. These mammals when newly born have the ability to regenerate their hearts after a heart attack, but this ability is lost during P7 [17], and the damaged heart of an adult mice forms mainly scar tissue.

In order to know more about the regenerative potential on neonatal mice, Nakumra et al. investigated on the epigenetic factors, and they found that in the early stages, the ATP-dependent chromatin-remodeling complex, Baf60c was highly expressed in the heart globally, but gradually this expression steadily decreased. Baf60c expression significantly increased during heart regeneration in newborn mice following resection. Moreover, neonatal mouse cardiomyocyte proliferation was suppressed by Baf60c breakdown. This further strengthens the possibility that in early heart formation, Baf60c is crucial to the differentiation of cardiomyocytes [18].

Additionally, it has been demonstrated that cholinergic neurons is a significant part in organ regeneration of mice. Vagotomy was performed on mice to study the function of nervous system in heart regeneration. Scarring developed 21 days after the cardiac apical excision in newborn mice which had cardiac va-gotomy, and the mortality rate of the mice rose [19]. Significant scarring appeared 21 days following cardiac apical excision in newborn mice whose sympathetic nerves were chemically inhibited [20]. This proves that the nervous system was also involved in neonatal mouse heart reconstruction.

4. Limitations on Humans

Although the heart regeneration technique has got significant progress in recent years, there are still so many problems preventing human heart regeneration to be treated with this technique. In terms of the OSM which induces heart reconstruction in newts, it has been found that OSM also bound to other receptors in humans, which causes a variety of effects, including a greater propensity for blood clotting. In addition, researchers discovered the mechanism of OSM inducing clotting risks when examining the impact of OSM on the procoagulant activity (PCA) of smooth muscle cells, abbreviated as SMC. Tissue factor (TF), and tissue factor pathway inhibitor (TFPI) are used by SMC to increase and decrease the propensity to cause thrombus of atherosclerotic plaque respectively, and at the cell membrane and in SMC lysates, OSM was observed to boost the production of TF, which in turn caused a significant PCA that was concentration-dependent. Besides, the OSM associated cytokines, interleukin 6 (IL-6) and leukaemia inhibition factor (LIF) decreased TFPI secretion as well [21]. These observations determined that OSM poses clotting dangers to humans, especially to those who have damaged heart tissues after a heart attack, which is one of the main obstacles in using OSM as a treatment. Furthermore, it was also shown that the myocardium's ability to function declines when OSM is produced for a longer period of time and the cells begin to dedifferentiate in OSM treated mice, and the regeneration ability shown in these OSM treated mice was due in part to the dedifferentiated cells' superior ability to withstand an oxygen shortage.

The nsCCN protein found in newts, cell cycle regulator kinase mps1 in vertebrates [22], and one of the BAF complexes' subunits, Baf60c, are insufficiently studied. As nsCCN is a newt specific protein, and it is believed to present a gene that separated early in evolution [14], the potential of it to be used in human organ regeneration is still unknown. mps1 is mainly known as a kinetochore-associated kinase which is essential for setting up and maintaining the mitotic checkpoint, important for studying vertebrate mitosis [22]. Baf60c is known as a part of chromatin remodelling complex, offering a new method for ensuring transcriptional control throughout organogenesis [23]. Overall, there is great potential in heart regeneration technique as to treating human cardiomyocyte damage, but that the obstacles like undesirable side effect and the lack of further studies in the effects in human body of molecules that are involved in animal organ regeneration remain.

5. Conclusion

Lots of newborn mammals can repair their hearts after a heart attack, but developmental processes that cause post-mitotic cardiomyocytes swiftly stop it. Newts and zebrafish, even in adulthood were found to be able to regenerate their organs, including hearts, and they achieve this through cell dedifferentiation. OSM and nsCCN were involved in newt heart regeneration, while mps was involved in zebrafish organ reconstruction. Newborn mice lose their regeneration ability during P7, and the neonatal mice regenerate their hearts with the help of Baf60c. Although different mechanisms of regeneration were found in animals, there are still challenges, including unfavourable side effects and a dearth of research on how chemicals involved in animal organ regeneration affect humans; however, heart regeneration still possesses great potential as important progress in comprehending important processes and pathways were made during these years, and the effort on these studies are worthy considering the severity of heart failure, so more researches on this topic should still be done.

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