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Potential Role of Gene Therapy Targeting Hippo Signaling with Adeno-Associated Virus Based Vectors in Heart Failure Post-Myocardial Infarction

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ABSTRACT

Heart failure is a condition that occurs when the heart cannot pump enough blood for the body's needs. This disease is caused by loss of cardiomyocytes and fibrosis, and is a leading cause of death worldwide. Current therapeutic treatments cannot generally directly replace lost cardiomyocytes in the myocardium, so the prognosis for patients with post-myocardial infarction heart failure remains poor. Based on the results of recent research, deactivating the Salvador (Sav) function in the Hippo pathway can regenerate cardiomyocyte cells. Gene therapy using Sav knockdown with adeno-associated virus (AAV) vector-based short hairpin RNA (shRNA), and restricted to cardiomyocytes by using the cTnT promoter, can specifically target cardiomyocyte cells in the infarction area so that it can become a new therapy for heart failure after myocardial infarction.

Keywords: AAV, gene therapy, hippo pathway, post-myocardial infarction heart failure

INTRODUCTION

Cardiovascular disease is still a threat in Indonesia and even in the world. Cardiovascular diseases (CVDs) are the main cause of death globally, based on data from WHO, causing around 17.9 million lives every year and more than 75% occur in countries with low to medium economies.[1] In Indonesia, based on the Sample Registration System (SRS), heart disease is the second leading cause of death after stroke.[2] According to the Centers for Disease Control and Prevention, there are more than 6 million adults in the United States experience heart failure.[3] Heart failure is a condition that occurs when the heart does not pump enough blood to meet the body's needs. This can happen when the heart cannot fill with enough blood or when the heart is too weak to pump properly. Heart failure is caused by other medical conditions such as coronary heart disease, inflammation of the heart, high blood pressure, cardiomyopathy, or an irregular heartbeat. Today, heart failure is a serious condition, but treatment such as lifestyle changes, medications, some therapies and measures can help many people have a higher quality of life.[1,2]

Heart failure caused by loss of cardiomyocytes and fibrosis is the leading cause of death worldwide. The survival rate of heart failure patients has started to increase along with advances in treatment facilities, but the long-term prognosis of this disease is still disappointing. Existing therapeutic treatments could not directly replace lost cardiomyocytes in the myocardium so that the prognosis of post-myocardial infarction heart failure patients remains poor. Although current therapies such as heart transplantation and left

ventricular assist device implantation are having promising results, new approaches are still needed. Unraveling the genetic mechanisms underlying cardiomyocyte renewal is an important step in developing new approaches to repair the heart. Recently, many studies have targeted to promote cardiomyocyte proliferation via inducing cardiomyocyte cell cycle re-entry for cardiac repair after myocardial infarction. [3,4]

Recent studies reveal that inhibition of the Hippo pathway is sufficient to promote endogenous cardiomyocyte proliferation, suggesting that manipulation of the Hippo pathway in the heart may be a promising treatment for heart failure in the future. Based on recent research results, disabling Salvador (Sav) function in the Hippo pathway is a promising approach to improve cardiac function and regenerate cardiomyocyte cells. By inhibiting the Sav gene, YAP gene activation will occur which functions to control proliferation, cytoskeletal remodeling, and protect cardiomyocyte cells from stress due to muscle contraction.[5,6] One way is to inhibit the Sav gene using RNA interference (RNAi) in combination with viral vector delivery to a suitable cell type to modify the function of the gene in a particular organ. One of the viral vectors that could be suitable for this type of therapy is Adeno-Associated Virus (AAV) based vector, which can ensure stable and long-term gene expression in cardiomyocytes. In this review article, we summarize the emerging evidence about potential role of gene therapy targeting hippo signaling with adeno-associated virus-based vector in heart failure post myocardial infarction.[7,8]

Pathophysiology of Hippo Pathway

Myocardial infarction (MI) is caused by the heart receiving reduced blood perfusion/ischemia for an extended period, leading to permanent death of heart muscle cells. The death of heart muscle cells is triggered by the activation of the Hippo pathway and the inhibition of the YAP/TAZ coactivator. [9,10]

The Hippo pathway regulates the proliferation, differentiation, growth, and death of various cell types, including heart muscle cells. The Hippo pathway comprises several important protein components, with Sav being one of them. Activation of the Hippo pathway will phosphorylate the YAP/TAZ coactivator, inhibiting the translocation of the YAP/TAZ coactivator into the nucleus for gene expression in regeneration and cell proliferation. [11,12]

In heart failure following MI, there is an increase in signaling from the Hippo pathway, causing several compensatory increases in the repair processes of dead heart muscle cells through three stages: inflammation, fibrosis, and remodeling. In the inflammation stage, there is activation of inflammatory cells consisting of neutrophils, macrophages, and lymphocytes, leading to Matrix Metalloproteinase (MMP) activation and degradation of necrotic heart muscle cells. Inflammatory cells also activate various cytokines, growth factors, and hormones to stimulate fibroblast activation, resulting in an increase in the concentration of fibroblasts. [13]

In addition to inflammatory cells, Hippo pathway activation also causes increased proliferation and differentiation of fibroblast cells.[14] This leads to an increase in collagen fibers replacing the necrotic zone of heart muscle cells. The scar tissue interferes with heart function by reducing the contractility of the left ventricle in pumping blood throughout the body. This results in a decrease in the ejection fraction, leading to post-myocardial infarction heart failure. [15]

Gene Therapy Targeting Hippo Signaling in Heart Failure

Treatment for heart failure has reached a plateau phase due to the difficulty in finding a specific drug that targets intracellular activities in the pathogenesis of heart failure. Therefore, gene therapy is an alternative breakthrough in the management of heart failure by reducing pathological

regulation or counteracting harmful molecular processes. One part of the molecular process related to heart failure is the Hippo pathway. The Hippo pathway is a molecular process that regulates the size and proliferation of an organ. The Hippo pathway has four main components: Ste20-like kinase HIPPO (HPO), Salvador protein (SALV), nuclear Dbf2-related (NDR) family protein warts, and Mob-as-tumor-suppressor protein (MATS).[16,17]

In mice, the MST1/2 kinase (orthologous to HPO) and Salv1 as its regulatory protein form an activated complex. This complex will phosphorylate large tumor suppressor homolog kinase (LATS) 1/2 and MOB1 (orthologous to MATS) as its regulatory protein. Phosphorylated LATS1/2 will phosphorylate the YAP coactivator, preventing its translocation to the nucleus and inhibiting heart muscle regeneration. Gene therapy targeting some of these proteins has the potential for heart muscle regeneration in heart failure.[17]

Gene therapy targeting Salv by reducing or eliminating this protein can lead to increased proliferation and decreased fibrosis levels in the heart. In addition, research involving mice with cardiac-specific deletion of Salv found no disruption in the apoptosis process of heart muscle. It can be concluded that Salv is specific in regulating the proliferation process in heart muscle regeneration. Gene therapy targeting the YAP protein by increasing its levels can lead to heart muscle regeneration through the regulation of the apoptosis process. Anti-apoptotic activity due to increased expression of YAP protein has been found to improve heart function and reduce scar tissue size. The overall process will ultimately inhibit the Hippo pathway, leading to heart muscle regeneration.[17-19]

Role of Adenovirus Based Vector in Cardiac Gene Therapy

Adeno-associated virus (AAV) is a nonenveloped ssDNA virus in the *parvoviridae* family. AAV is found in human and non-human primates (NHPs) and does not cause human disease. The basic structure of the AAV DNA genome is 4-6 kbs with icosahedral protein capsid size 23-28 nm. In 1984, AAV development for gene therapy began. A trans-complementing system produces high-quality recombinant AAV (rAAV) for in vitro and in vivo gene delivery.[20,21] AAV vector is widely used because of its benefits, such as ability to infect host cells – including muscle cells – and low immunogenicity and cytotoxicity.[22]

The capsid sequence and structure of wild-type AAVs (wtAAVs) are the composition of rAAVs and the construction of AAV vector depends on the aim (for example, cardiac disorders, muscular dystrophies, and neurological disorders).[20,21] The main sequences of the viral origin are the Inverted Terminal Repeat (ITR), which plays a role in the life cycle of AAV to induce genome replication and vector packaging. Besides the ITR, each AAV protein-coding sequences have cassettes designed to generate specific therapeutic gene expression.[21,23] The effectiveness of rAAV is determined by capsid and target cell receptors interaction which affecting gene delivery's potency. The molecular interactions rely on the serotypes with different pathways in each serum proteins.[21]

AAV capsid mostly composed by rAAV with capsid proteins of serotypes 1 to 9. Some in vivo studies have shown that AAV1, AAV6, and AAV9 are the most promising AAVs for cardiac gene transfer (for example, in inherited cardiomyopathies and heart failure). In HF with large animal models, AAVs play a role in some pathways, such as calcium handling, beta-adrenergic pathway, angiogenesis, and inflammation. Compared to those three serotypes, AAV9 has a long circulation time – while AAV1 almost fully cleared from the circulation in an hour – that may allow AAV9 to access cardiac capillary endothelium. The other point of view in clinical trial using AAV1 vectors carrying SERCA2a which targeting calcium

upregulation by percutaneous administration found that AAV1 is ineffectively for delivering SERCA2a to cardiomyocytes. [20,24]

AAV9 serotype is a non-pathogenic vector and it has a robust gene expression with fast onset – if compared with others – in myocardial infarction and it is potential to prevent heart failure events. Myocardial infarction rapidly developed edema and caused ischemic along with inflammation mediator releasing, which escalated capillary permeability. This condition enhances AAV9 potency to circulate in blood flow and increase the transduction effect. Moreover, DNA damage by myocardial infarction conducts DNA complex repair (MRN complex) to the damaged location and builds up a nuclear condition. The impact of that condition is AAV9 gets a conducive environment to trigger the transduction efficiency of AAV9. Some research found that AAV9 injection does not promote tumor formation and does not have toxicity effect on the lungs and liver, seen from histopathological examination.[25,26]

Knockdown of Sav Protein with Adenovirus Based Vector as Future Therapy for HF Post MI

Based on several studies, knockdown of Sav protein in the Hippo pathway has been shown to improve cardiac function after myocardial infarction and treat heart failure through genetic improvement programs. A study by Leach et al (2017) tested the effects of Sav CKO (conditional knockout) in a mouse model of ischemic heart failure after myocardial infarction. Three weeks after myocardial infarction, the mice were given Sav CKO and examined periodically for up to six weeks. From this study, an increase in cardiac function was obtained, namely the ejection fraction (left ventricular systolic function) which was significant compared to the control group (SavCKO 59% ± 13%, control 38 ± 9%, P = 0.001) and almost the same as the ejection fraction in the healthy group (65%± 8%). In hearts that were given SavCKO, there were smaller areas of fibrosis and more left ventricular cardiomyocytes than the control group (fibrosis area: control 56 ± 12%, SavCKO 36 ± 15%; cardiomyocyte cell count: control 1 × 105 ± 8 × 104, SavCKO 6 × 105 ± 2 × 105), and there was also an increase in vascularity as indicated by the finding of a three-fold increase in capillary density at the wound margins and an increase in vasculogenesis genes such as angiopoietin, fibroblast growth factors, and vascular endothelial growth factors in cardiomyocytes. These findings indicate that knockdown of Sav protein in the Hippo pathway can be a therapy for heart failure via heart regeneration.[27]

Sav knockdown in cardiomyocytes may employ RNA interference (RNAi) mediated by double-stranded RNA (dsRNA), which is a natural cellular process related to gene regulation. RNAi employs specific and highly potent gene silencing mechanisms. 13 There are various ways to induce RNAi in gene knockdown, but the two most commonly used methods are chemically synthesized small interfering RNA (siRNA) and vector-based short hairpin RNA (shRNA). Compared with chemically synthesized siRNAs, vector-based expression of shRNAs achieves a more sustained gene-knockdown effect. ShRNA has a different mechanism from siRNA, which can be assimilated by endogenous miRNAs and continuously synthesized by host cells. This mechanism causes shRNA to provide a more efficient and longer lasting effect than siRNA. To increase the efficacy and stability of shRNA, the RNAi agent can be combined with various plasmids and viral vectors, one of which is the adeno-associated virus (AAV). This vector has beneficial biological properties because it can infect various types of host cells, including muscle cells, is able to pass through the endothelium, and has a low level of immunogenicity.[15,26] AAV9 is one of the serotypes of AAV which has been shown to be selective against cardiomyocyte cells and does not affect other organs.[27] A

study by Liu et al (2021) using AAV9-Sav-shRNA treatment of cardiomyocyte cells in pig hearts induced myocardial infarction, found that there was an improvement in the ejection fraction of 14.3%, an increase in capillary density, and a decrease in wound size compared to the control group which only received AAV9-green fluorescent protein (GFP) treatment, and there was also division of cardiomyocyte cells. This indicates tissue repair and increased cardiac function after myocardial infarction.[28]

ShRNA-Sav expression can be restricted to cardiomyocytes by using the cTnT promoter. CTnT itself is one of the troponin subunits found in cardiac muscle filaments. Based on the study of Konkalmatt et al (2013), the expression of a gene by the AAV9 vector that is targeted to the heart and administered intravenously in experimental animals can be increased using the cardiac troponin T (cTnT) promoter. It was found that there was an increase in luciferase gene expression in heart cells up to 213 times after three days post myocardial ischemia and the strongest cTnT-eGFP gene expression in cardiomyocyte cells was found at the border of the infarction area. A decrease in left ventricular end systolic and end diastolic volume was also found on days 14 and 18 in the AAV9-cTnT-EcSOD treatment group compared to the control group. The results of the study indicate that cTnT can be used to increase the expression and targeting of certain genes such as shRNA Sav to cardiomyocyte cells in the infarct area.[5,25]

Current and Future Challenges

Based on Glybera's review, the most expensive drug in the world is AAV-based gene therapy. The thing that costs the most is the manufacturing process, for example is the production of research-grade and clinical-grade vectors that need adherent eukaryotic cells – made by triple transfection of plasmid DNA. The reality is that the three-plasmid transfection system is not enough for some conditions because not all cells receive optimal ratios of the plasmids required for efficient packaging, so in some cases, more eukaryotic cells are needed. Besides that, AAV purification and lack of standardization due to vector capsid variants contribute to high production costs. This barrier discourage investigators from transitioning preclinical studies to clinical trials. [21]

After AAV-shRNA intravenous administration, immune response can react to bring up neutralizing antibodies (NAbs) against AAV capsids. It is mostly found out due to natural AAV infections and caused a disruption to gene delivery. While the transduced cells also can be eliminated by the humoral response, NAb production, and cytotoxic T lymphocyte (CTL) response. Furthermore, innate immune responses triggered by the AAV capsid and genome, through Toll-like receptors, contribute to inflammation. Some strategies have been tried to overcome these issues. Plasmapheresis and the use of empty capsids as decoys are being explored as methods to evade NAb interactions; however, their clinical efficacy and study are still limited. Other strategies are transient B cell depletion, immune tolerance induction with agents like rapamycin, and employing regulatory T cells (Tregs) to mitigate CTL responses. To get over the inflammation caused by innate immune, include modifying the AAV-shRNA genome to reduce CpG motifs or incorporating TLR9-inhibitory sequences like TTAGGG. Continued research and clinical trials are essential to refine these approaches and improve the effectiveness and safety of AAV-based gene therapies.[21,24]

CONCLUSION

Heart failure caused by loss of cardiomyocytes and fibrosis is the leading cause of death worldwide. The death of heart muscle cells is triggered by the activation of the Hippo pathway and the inhibition of the YAP/TAZ coactivator. Recent studies reveal that inhibition of the Hippo pathway is sufficient to promote

endogenous cardiomyocyte proliferation. Gene therapy targeting Salv by reducing this protein and YAP protein by increasing its levels can lead to increased proliferation and decreased fibrosis levels in the heart. Sav knockdown in cardiomyocytes may employ RNA interference (RNAi) mediated by double-stranded RNA (dsRNA). Two most commonly used methods to induce RNAi in gene knockdown are chemically synthesized small interfering RNA (siRNA) and vector-based short hairpin RNA (shRNA). ShRNA can be assimilated by endogenous miRNAs and continuously synthesized by host cells. To increase the efficacy and stability of shRNA, the RNAi agent can be combined with adeno-associated virus (AAV). This vector can infect various types of host cells, able to pass through the endothelium, and has a low level of immunogenicity. ShRNA-Sav expression can be restricted to cardiomyocytes by using the cTnT promoter.

Declaration by Authors

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