CRISPR-Cas9: A New Frontier in the Treatment and Research of Cardiovascular Diseases

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Abstract: CRISPR-Cas9 provides accurate and effective techniques for altering genetic sequences, revolutionizing the field of genome editing, which holds significant potential for treating cardiovascular diseases (CVD) by correcting pathogenic mutations and modulating gene expression. LDLR gene mutations have been successfully corrected using CRISPR-Cas9, which is associated with familial hypercholesterolemia, thereby improving the atherosclerotic phenotype in mouse models. Additionally, CRISPR-Cas9 shows promise in treating familial cardiomyopathies by correcting mutations in genes such as RBM20 and MYH6, leading to the restoration of normal cardiac function. However, several challenges remain, including off-target effects, which leads to unintended genetic alterations, and delivery challenges that limit the precise targeting of cardiovascular tissues. The creation of innovative delivery systems, like lipid nanoparticles, is one area of future research, to enhance specificity and reduce off-target effects. Personalized medicine may benefit from the combination of CRISPR-Cas9 and next-generation sequencing, which could result in the development of CVD cures.

1 INTRODUCTION

Cardiovascular disease (CVD) continues to represent the majority of morbidity and mortality worldwide. The World Health Organization estimates that around 17.9 million people died globally in 2019，due to cardiovascular issues. These deaths accounted for 32% of the total fatalities, which places a significant financial burden on healthcare systems (Amini et al. 2021). The pathophysiology of cardiovascular disease is complex, involving multiple genetic and environmental factors, and developing effective prevention and treatment strategies is bound to be a daunting challenge (Cheng et al. 2024). In recent years, the introduction of CRISPR-Cas9 technology (Clustered Regularly Interspaced Short Palindromic Repeats) has brought about a major transformation in molecular biology. The bacterial adaptive immune system is the source of both CRISPR and the protein Cas9. This system enables bacteria to recognize foreign and cut DNA, such as DNA from invading viruses. In the lab, researchers use this natural defense mechanism for precise gene editing. A Cas9 nuclease that cuts DNA at the target location and a single conducting RNA that detects a particular DNA sequence make up the CRISPR-Cas9 mechanism. Once the DNA has been sliced, desired genetic alterations, such as gene knockouts, knockins, or point mutations, can be introduced by utilizing the cell's natural DNA repair systems. The possibilities of CRISPR-Cas9 technology within cardiovascular disease is enormous. Because many cardiovascular diseases have a genetic basis, the ability to precisely edit genes offers additional avenues for understanding disease mechanisms and developing new therapies.

Several studies have demonstrated the promise of CRISPR-Cas9 in cardiovascular research (Bharucha et al. 2022). In animal models, the researchers have successfully used CRISPR-Cas9 to modify genes involved in lipid metabolism, which is crucial in the development of atherosclerosis. By knocking out genes involved in cholesterol synthesis or uptake, it has been possible to reduce plaque formation in the arteries. In addition, in heart failure models, CRISPR-Cas9 has been used to manipulate genes involved in heart remodeling, showing potential to improve heart function. Despite these exciting advances, many challenges remain. The main problem is the off-target effect. The CRISPR-Cas9 system may cut

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DNA at locations other than the intended target, leading to unintended genetic changes that could be harmful. Another challenge is effectively delivering CRISPR-Cas9 components to target cells in the cardiovascular system. The heart and blood vessels are complex organs, which requires ensuring that the gene-editing mechanism reaches the appropriate cells without causing damage to the surrounding tissues (Bonowicz et al. 2025). This review provided a thorough summary of the state of the use of CRISPR-Cas9 in cardiovascular disease research and treatment. In this quickly developing topic, the most recent research developments, difficulties, and possible future paths were examined. The creation of safer and more efficient gene-based therapies for cardiovascular disease is facilitated by an awareness of the state of the technology today. the creation of safer and more efficient gene-based treatments for heart conditions.

2 AN OVERVIEW OF CRISPR-Cas9 TECHNOLOGY

2.1 Mechanisms of CRISPR-Cas9 Technology

As a groundbreaking technology in molecular biology, the CRISPR-Cas9 gene-editing system is engineered based on the natural immune defense mechanisms of bacteria. This system comprises two core functional modules: **(1) Cas9 Nuclease**: Functions as "molecular scissors" for site-specific double-strand DNA cleavage. **(2) Single-Guide RNA (sgRNA)**: Serves as a navigation system that enables precise genomic targeting through base complementary pairing. The mechanism of action follows a refined three-stage workflow: **(1) Target Recognition**: sgRNA binds specifically to the target DNA sequence. **(2) Nuclease Activation**: Cas9 protein executes precise DNA cleavage at predetermined genomic coordinates. **(3) Genetic Reprogramming**: Cellular autonomous repair mechanisms (primarily via non-homologous end joining [NHEJ] or homology-directed repair [HDR] pathways) mediate genomic modifications, ultimately achieving precise edits including gene inactivation, sequence insertion, and single-base substitutions.

The CRISPR-Cas9 system demonstrates remarkable utility in functional genomics research. In genome-wide functional screening applications, this technology enables comprehensive analysis of gene functions. Taking gene knockout as an example, by specifically targeting and inactivating genomic loci through coordinated delivery of large-scale sgRNA libraries and Cas9 protein, researchers can systematically identify key genes involved in cellular processes and drug resistance mechanisms, establishing a novel research paradigm for elucidating genetic disease etiology (Shalem et al. 2015). Particularly noteworthy is the system's capability for precise gene expression modulation. By fusing transcriptional regulatory elements with catalytically inactive dCas9 protein, researchers can upregulate or downregulate target gene expression without altering DNA sequences (Lee et al. 2024). This innovative approach has pioneered new avenues for gene function studies.

2.2 Advantages of CRISPR-Cas9 Technology

The CRISPR-Cas9 system's simplicity and efficiency have positioned it as a preferred tool for genome editing. CRISPR-Cas9 is easier to design and perform when compared with older gene-editing technologies such as zinc-finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs) because it relies on RNA-DNA interactions rather than intricate protein engineering. This system can be easily reprogrammed by altering the sgRNA sequence to target different genomic loci, allowing for high-throughput and multiplexed gene editing (Shojaei Baghini et al. 2022).

2.3 Applications of CRISPR-Cas9 Technology in High-Throughput Functional Genomics

CRISPR-Cas9 has been instrumental in genome-scale screening, enabling researchers to identify critical genes for various cellular functions and responses. By delivering Cas9 and sgRNA libraries into cells, scientists can perform knockout screens to determine gene essentiality and function. For instance, Shalem et al. demonstrated the use of CRISPR-Cas9 for genome-wide screens, identifying genes involved in cellular viability and drug resistance (Shalem et al. 2015). This approach has been crucial for understanding the genetic basis of diseases and mechanisms of drug resistance.

2.4 Transcriptional Modulations of CRISPR-Cas9 Technology

In addition to gene deletion, CRISPR-Cas9 has the ability to alter gene expression. By combining transcriptional activator or repressor domains with catalytically inactive Cas9 (dCas9), researchers can precisely regulate gene expression without altering the DNA sequence. Studying gene function and creating treatment plans for illnesses where gene control is involved have benefited greatly from this.

2.5 Challenges and Future Directions of CRISPR-Cas9 Technology

CRISPR-Cas9 has several challenges despite the many benefits it offers. Off-target effects, in which unwanted parts of the genome are cleaved and may result in dangerous mutations, are a major problem. Researchers are creating methods to lessen this, like refining sgRNA design and employing high-fidelity Cas9 variations. Additionally, delivering the CRISPR components efficiently and specifically to target cells remains a hurdle, especially for in vivo applications. Future research will focus on enhancing the specificity and efficiency of CRISPR-Cas9, exploring alternative Cas proteins, and developing novel delivery systems. CRISPR-Cas9 has transformed the landscape of molecular biology and holds immense potential for advancing our understanding and treatment of genetic diseases. Its applications in genome editing, transcriptional regulation, and high-throughput screening have already yielded significant insights. As researchers continue to refine technology and address its challenges, CRISPR-Cas9 is positioned to play a pivotal role in the future of genetic medicine.

3 THE USE OF CRISPR-Cas9 IN THE TREATMENT OF CARDIOVASCULAR DISEASES

3.1 Repairing Gene Mutations

Cardiovascular disease (CVD) has a genetic basis, and CRISPR-Cas9 offers great potential for correcting pathogenic mutations (Lloyd-Jones et al. 2006). LDLR gene mutations are the classic cause of familial hypercholesterolemia (FH). In a mouse model with the LdlrE208X mutation, Zhao et al. showed that CRISPR-Cas9 delivered by AAV8 improved the atherosclerotic phenotype and largely restored LDL receptor expression. Treated mice had reduced cholesterol levels and smaller atherosclerotic plaques. There is also hope that CRISPR-Cas9 can be used to treat familial cardiomyopathies. In dilated cardiomyopathy (DCM), the ABEmax-VRQR-SpCas9 system was used to correct mutations in the RBM20 gene in induced pluripotent stem cells (iPSCs) and mouse heart tissue. As a result, the dilatation of the heart was reversed, and the heart's normal function was restored (Nammi et al. 2024). For hypertrophic cardiomyopathy (HCM), AAV9-targeted delivery of sgRNA to the MYH6 locus in cardiomyocytes inactivated the pathogenic allele in more than 70% of ventricular myocytes, preventing the development of structural and functional features of HCM in treated mice (Smolderen et al. 2024). In addition to these applications, CRISPR-Cas9 has been used to target other cardiovascular conditions. For instance, Ding et al. showed that CRISPR-Cas9 could knock out the Pcsk9 gene in mice, leading to a significant reduction in plasma cholesterol levels. This approach has the potential to prevent atherosclerosis and related cardiovascular diseases. In a similar vein, Wang et al. showed that human hepatocytes with mutations in the ApoB gene, linked to familial hypercholesterolemia, could be corrected using CRISPR-Cas9. These investigations demonstrate the adaptability and promise of CRISPR-Cas9 in the management of various cardiovascular disorders.

Despite these promising results, several challenges remain. Negative genetic changes may result from off-target impacts, in which the CRISPR-Cas9 mechanism cleaves DNA at unwanted locations. High-fidelity Cas9 variants, such SpCas9-HF1, have been created by researchers to solve this problem by lowering off-target activity. Furthermore, the creation of base editors has demonstrated promise in lowering off-target effects. These editors combine nucleotide deaminases and Cas9 nickases to accomplish single nucleotide conversion without causing double-strand breaks (DSBs). Delivering the components of CRISPR-Cas9 to the intended tissues presents another difficulty. Viral vectors, such as AAV, are commonly used but can result in permanent expression of nuclease proteins, leading to undesired DNA cleavage. Non-viral delivery methods, such as lipid nanoparticles and synthetic polymers, offer alternatives that can reduce immunogenicity and toxicity. For example, polysaccharide-based delivery systems have shown potential in overcoming delivery challenges while advancing the pharmaceutical

applications of CRISPR-Cas9 gene therapy. By repairing harmful mutations, CRISPR-Cas9 technology has enormous potential for treating cardiovascular disorders. Realizing the full therapeutic potential of CRISPR-Cas9 requires ongoing research into enhancing its precision, creating high-fidelity variations, and streamlining delivery systems.

3.2 Regulating Protein Expression

CRISPR-Cas9 can cure cardiovascular illnesses by modifying protein expression in addition to correcting mutations. Targeting genes linked to protein overexpression has showed potential in hypertension. For instance, in adult rats with spontaneous hypertension, systolic and diastolic blood pressure were considerably lowered when exon 2 of the angiotensinogen (AGT) gene was disrupted in hepatocytes using AAV8-Cas9-AGT gRNA. Partial suppression of AGT expression was adequate to avoid hypertension without altering cardiovascular stress responses (Okobi et al. 2024). In atherosclerosis, overexpression of proteins such as APOC3 and PCSK9 contributes to disease progression. When CRISPR-Cas9 disrupted APOC3 exon 2 in rabbits and hamsters, plasma triglycerides decreased, HDL-C levels rose, and aortic plaque formation decreased (Morgan et al. 2024). In a mouse model, CRISPR-Cas9 genome editing of the Pcsk9 gene effectively reduced plasma cholesterol levels by disrupting the gene, showing its potential to improve cholesterol metabolism and reduce the risk of cardiovascular disease (Shalem et al. 2015).

3.3 Targeting Mitochondrial Dysfunction

Mitochondrial dysfunction is an important cause of CVD. Mitochondrial DNA (mtDNA) mutations impair energy metabolism and lead to diseases such as hypertrophic cardiomyopathy, ischemic heart disease, and heart failure. Sukhorukov et al. used hybrid cell lines to study the m.15059G > A mutation in the MT-CYB gene. This mutation disrupts mitophagy and results in a persistent inflammatory state. The researchers were able to eradicate the mutated mtDNA copies, restore mitophagy, and lower proinflammatory reactions by using CRISPR-Cas9-based mitochondrial genome editing. However, due to the bilayer mitochondrial membrane, it is still difficult to deliver gRNA to the mitochondria. Enhancing mitochondrial import through gRNA modification with mitochondrial localization signals (MLS) or the addition of stem-loop motifs is the aim of current research, but more work is needed to develop reliable delivery mechanisms (Yang et al. 2023).

4 CHALLENGES OF CRISPR-Cas9 IN THE TREATMENT OF CARDIOVASCULAR DISEASES

4.1 Off-Target Impacts

A significant concern in the clinical use of CRISPR-Cas9 is off-target effects. The CRISPR-Cas9 system may cleave DNA at locations other than the intended target, leading to unintended genetic alterations. These off-target events may have harmful consequences, such as activation of oncogenes and disruption of normal gene function. Studies have shown that the frequency of off-target effects can be relatively high (Shalem et al. 2015). Researchers have created a number of tactics to deal with this problem, one of which is creating high-fidelity Cas9 variations like SpCas9-HF1. The purpose of these variations is to lessen off-target activity. Another approach to minimizing off-target effects is through the optimization of sgRNA design. Studies have shown that extending or truncating sgRNAs can enhance genome editing fidelity. For instance, truncating sgRNAs by 2-3 bp at the 5' end or putting two 5' end guanine nucleotides (referred to as 5'-GGX20) can decrease off-target effects while preserving on-target efficiency. Lastly, off-target effects may also be influenced by the way CRISPR-Cas9 components are delivered. It has been demonstrated that lipid nanoparticle (LNP) delivery and ribonucleoprotein (RNP) electroporation produce fewer off-target mutations and more on-target editing efficiency than alternative techniques. In order to prevent prolonged editor expression, which may result in off-target consequences, these delivery approaches seek to induce brief peak expression of CRISPR-Cas9 followed by rapid turnover.

4.2 Delivery Challenges

Another important drawback is the inability to precisely transport CRISPR-Cas9 components to the intended tissues in the cardiovascular system. Lipid nanoparticles that carry mRNA expressing Cas9 and gRNA are not very effective at delivering these

molecules, and they tend to build up in the liver and spleen instead of targeting cardiac or muscle cells. There are no specific markers for effective absorption in cardiomyocytes. Although promising, AAV-based methods for cardiac gene delivery are limited by pre-existing immunity, immune responses, nonspecific tissue transduction, high production costs, and limited packaging capabilities. Dual AAV systems require simultaneous infection of the same cell, which reduces efficiency, and high doses raise safety concerns.

4.3 Genome Stability and Immune Responses

Unintentional genomic alterations brought on by CRISPR-Cas9, like important deletions, intricate rearrangements, and off-target effects, can be harmful, particularly in cells that are undergoing mitosis. Extensive DNA damage and loss of heterozygosity can also have long-term transcriptional effects that could activate carcinogenic pathways (Bharucha et al. 2022). Furthermore, a lot of people already have adaptive immunity to S. aureus and S. pyogenes Cas9 orthologs. These immunological reactions raise the possibility of immune-mediated adverse consequences and reduce the effectiveness of gene editing. Using immunoorthogonal Cas9 variations, designing proteins with lower immunogenicity, and using temporary immunosuppressive regimens are some ways to deal with these problems (Bonowicz et al. 2025). Furthermore, Cas9 can be temporarily expressed by self-destruction or nonviral mRNA or protein delivery, which can reduce the amount of time needed for immune suppression. In summary, while CRISPR-Cas9 technology holds great promise for treating cardiovascular diseases, addressing delivery challenges and ensuring genome stability and immune compatibility are crucial steps towards its clinical application. Ongoing research in these areas is essential for CRISPR-Cas9 to reach its full therapeutic potential.

5 FUTURE OUTLOOK AND RESEARCH DIRECTIONS

5.1 Personalized Medicine

The combination of CRISPR-Cas9 and next-generation sequencing holds great promise for personalized medicine in the treatment of CVD. It is possible to create curative treatments by precisely altering pathogenic mutations in a patient's genome. For instance, the paradigm of treatment for hypertrophic cardiomyopathy might be completely changed by fixing a single nucleotide mutation linked to the condition. But there are issues with the healing procedures that need to be resolved, such balancing the error-prone NHEJ and HDR. In addition, issues such as mosaicism, off-target effects, and ethical concerns must be addressed before this approach can be translated into clinical-scale treatments (Bharucha et al. 2022).

5.2 Novel Delivery Systems

The widespread use of CRISPR-Cas9 in the treatment of CVD depends on the creation of novel delivery systems. Current research is focused on creating lipid nanoparticles that target cardiovascular tissues, which can enhance specificity and reduce off-target effects. For example, the PuPGEA non-viral nanosystem has demonstrated potential in delivering pCas9-Pcsk9 plasmids to hepatocytes, effectively knocking out the Pcsk9 gene and reducing cholesterol levels. This approach not only improves the efficiency of gene delivery but also minimizes the risk of off-target modifications and immune responses. To enhance these delivery systems and guarantee their efficacy and safety, more research is required. Using DNA nanoclews, which are yarn-like DNA nanoparticles produced by rolling circle amplification, is one promising tactic. The Cas9 protein and single-guide RNA (sgRNA) complexes can be encapsulated by these nanoclews and delivered to human cell nuclei while preserving cell viability. Another approach is the development of cationic lipid-based delivery systems, which have shown high transfection efficiency in both vitro and vivo studies. These systems can protect the CRISPR components from degradation and facilitate their entry into cells, thereby enhancing the overall efficacy of gene editing.

In addition to these advancements, future research should also focus on combining CRISPR-Cas9 with other therapeutic modalities. For example, integrating CRISPR technology with chimeric antigen receptor (CAR)-T cell therapies could enhance the efficacy of immunotherapy for CVD. Moreover, the use of CRISPR-Cas9 for in vivo editing of cardiovascular tissues could provide a powerful tool for treating genetic heart diseases. In conclusion, despite notable advancements in the

development of CRISPR-Cas9 technology for the treatment of CVD, ongoing research is crucial to address the remaining challenges. By improving the precision of CRISPR-Cas9 and developing more efficient and targeted delivery systems, we can bring new hope to patients with inherited cardiovascular diseases, potentially leading to more effective and personalized treatments. As technology advances, it is essential to ensure that it is used in a way that maximizes benefits and minimizes risks, both for individuals and society.

6 CONCLUSION

CRISPR-Cas9 technology has made significant progress in the treatment and research of cardiovascular diseases. It has shown great potential in correcting genetic mutations, regulating protein expression, and targeting mitochondrial dysfunction associated with CVD. In preclinical studies, it has proven effective in treating various cardiovascular diseases, such as familial hypercholesterolemia, cardiomyopathy, and atherosclerosis. However, challenges remain before CRISPR-Cas9 can be widely implemented in clinical practice. Off-target effects, delivery issues, genome stability concerns, and immune responses are significant barriers. Further research is essential to improve the precision of CRISPR-Cas9 and develop more efficient and targeted delivery systems. Overcoming these obstacles will be crucial to ensuring both the safety and effectiveness of CRISPR- Cas 9 based therapies. In spite of these difficulties, the potential with CRISPR-Cas9 to revolutionize cardiovascular treatment is undeniable. With continued research and development, it can bring new hope to patients with inherited cardiovascular diseases, potentially leading to more effective and personalized treatments. As the technology develops, it is important to ensure that it is used in a way that maximizes benefits and minimizes risks, both for individuals and society.

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