Review Article

Narrative review: application progress of *CRISPR/Cas9* gene editing in cardiovascular field

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Contributions: (I) Conception and design: Y Xu, H Liu; (II) Administrative support: Y Xu; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

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Objective: To describe and discuss the CRISPR/Cas9 application in basic cardiovascular research to facilitate screening of targets for large clinical applications.

Background: CRISPR/Cas9 is a new genetic intervention method, which has been widely used in the clinical treatment of tumors and rare diseases, but its application in basic research and clinical research on cardiovascular diseases are both limited.

Methods: This article focuses on CRISPR/Cas9 use in cardiac development and cardiovascular diseases (such as atherosclerosis, heart failure, etc.), and other related basic researches are summarized in order to extract effective information and combine their own research directions. We have summarized the concepts and advantages of gene editing that are different from other gene modification methods. And there is currently no relevant clinical application. Due to the differences in gene editing methods, cell types, and treatment methods in various fields (such as tumors, etc.), there are no relevant summaries in other fields. Only summarize cardiovascular related research, hoping to obtain relevant research targets.

Conclusions: At present, CRISPR/Cas9 has no related applications in cardiovascular, so we are looking for possible breakthroughs on the basis of summarizing the current research to promote the application of CRISPR/Cas9 in cardiovascular basic research. All the protein targets discussed in this article and treatment methods in other fields can be used for reference.

Keywords: CRISPR/Cas9; cardiovascular; basic and clinical research

Received: 09 August 2021; Accepted: 30 November 2021; Published: 30 December 2021.

doi: 10.21037/jxym-21-26

View this article at: https://dx.doi.org/10.21037/jxym-21-26

Gene therapy has the potential to treat more than 10,000 human single-gene diseases and can benefit more complex polygenic diseases. The CRISPR/Cas system is a natural immune system of prokaryotes, which exists in most bacteria and archaea. After being invaded by a virus, a prokaryote can extract a small piece of viral DNA and store it in a specific area of its own genome. We call this area the CRISPR storage space. When encountering a virus invasion again, prokaryotes can recognize the virus based on the stored DNA fragments and cut the virus's DNA to make it ineffective (1).

According to the characteristics of the CRISPR/Cas system, scientists have transformed it into the most efficient genome editing tool (1). Among them, CRISPR/Cas9 is an ancient bacterial immune defense system. It was first identified in prokaryotes in 2002 and reapplied to gene editing technology, providing researchers with a revolutionary gene therapy tool (2). In 2012, Jennifer Doudna and Emmanuelle Charpentier proved that the CRISPR/Cas9 system can cut double-stranded DNA *in vitro* (1); in 2014, Zhang *et al.* used the CRISPR/Cas9 system for the first time to edit the genome in prokaryotes

(*E. coli*) (3). But at present, there is no relevant clinical level application (4,5). The natural CRISPR-Cas9 system consists of three parts: SpCas9 (hereinafter referred to as Cas9), crRNA, and tracrRNA. Among them, crRNA and tracrRNA form gRNA (guide RNA) through local base pairing, and the gRNA binds to the Cas9 protein to guide the Cas9 protein to recognize and cut the target DNA sequence (Schematic representation of the Streptococcus pyogenes Cas9 nuclease (green) targeted to genomic DNA by a single-guide RNA (sgRNA) consisting of an ~20-nt guide sequence (blue) and a scaffold (red). The guide sequence is directly upstream of the protospacer adjacent motif (PAM), NGG (orange circles). Cas9 mediates a double-strand DNA break (DSB) ~3 bp upstream of the PAM (red triangles). The break is repaired by 1 of 2 mechanisms: nonhomologous end joining (NHEJ) that creates random insertions or deletions at the target site or homology-directed repair (HDR). Two types of template can be used for HDR: small single-stranded DNA (ssDNA) oligonucleotide donor with short 60–70-bp homology arms and a linear or circular dsDNA plasmid with long homology arms of 1 to 3 kb (6). For Cas9 protein to successfully recognize the target sequence, two conditions must be met: (I) the base pairing between the 20 nt of the 5'end of the sgRNA and the target DNA; (II) the appropriate PAM sequence at the 3'end of the target DNA. CRISPR/Cas9 cuts the target DNA and produces DSB (double-strand break) (7). The most basic technology of CRISPR/Cas9 is gene knockout, which is to design a guide RNA (guide RNA1, guide RNA2) upstream and downstream of the gene, and transfer it into the cell together with the plasmid containing the Cas9 protein coding gene. The guide RNA passes Base complementary pairing can target the target sequence near PAM, and the Cas9 protein will cause DNA double-strand breaks in the upstream and downstream of the gene to achieve the knock-out of the target gene in the cell (NHEJ repair) (8). In addition, on the basis of this NHEJ repair, the repair template plasmid (donor DNA molecule) will be introduced into the cell, which will introduce fragment insertion or site-directed mutations to achieve gene replacement or mutation (for HDR repair), such as gene editing of fertilized egg cells, and introducing it into the surrogate mother can realize the construction of gene-edited animal models (9). At present, CRISPR/Cas technology has been widely used in gene activation, disease model construction, even gene therapy and other related fields. In the past, the efficiency of gene editing and precise cell editing was usually very low (10), while CRISPR/Cas9 provided targetable Target specific precise sites (11), and can interfere with targeting multiple genes at the same time (12), providing new possibilities for the study of complex polygenic diseases, especially suitable for different *in vitro* and *in vivo* research and has been used in many studies such as evaluating gene function, disease modeling, transcription regulation and testing new treatment methods (13). At present, CRISPR/Cas9 is widely used in the treatment of tumors and genetic diseases, such as small cell lung cancer, gastric cancer, and colorectal cancer. And rare disease treatment (14-16). Most ongoing clinical treatment trials for cancer use CRISPR/Cas9 to create chimeric antigen receptor T cell gene editing (CAR-T) for cancer immunotherapy (17,18). The CRISPR system is also used for non-gene editing purposes, such as activating and inhibiting gene expression, and for fluorescently positioning and labeling chromosomal regions and individual mRNAs (19). In addition, CRISPR/Cas9 gene editing has successfully constructed a variety of model animals, such as transgenic sheep and knockout pigs (20,21). That is, in the past ten years, the development of genome editing technology has fundamentally changed biomedical research. It has been widely used in tumor scientific research and clinical fields, but related research in the cardiovascular field is worthy of summary and discussion.

Cardiovascular disease is one of the main causes of morbidity and death among the elderly in our country, and is considered to be a major public health problem. At present, genome editing tools have been used to explore the mechanism of cardiovascular-related diseases such as cardiomyopathy, arrhythmia and lipid metabolism (13). And previous studies have shown that the specific expression of Cas9 in cardiomyocytes does not affect cardiac function or related gene expression. Cas9-specific knock-in mice have been used to edit cardiac genes such as *Myh6*, *Sav1*, and *Tbx2061*. However, the current research on therapeutic genome editing in the cardiovascular field is limited (22). The following will describe the related research progress of the two in more detail.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/jxym-21-26).

Heart development

Before the cardiovascular system starts to operate, the embryo mainly obtains nutrients and oxygen through diffusion. However, as the embryo develops, the

heart development will play an important role in the follow-up (23). Studies have shown that CRISPR/Cas9 constructs a zebrafish heart development model by interfering with the microporous in heart regeneration, the migration of cardiac progenitor cells in heart development, and Wnt/β-Catenin signal transduction (23). In addition to constructing animal models, CRISPR/Cas9 can also participate in heart development through germline genome editing, and be developed as a treatment tool for hereditary heart disease. For example, using CRISPR/Cas9 to correct the *MYBPC3* mutation that causes hypertrophic cardiomyopathy in human germ cells (24). Using the CRISPR/Cas9 system to generate *gtpbp3* gene knockout zebrafish, it was found that *gtpbp3* knockout zebrafish mitochondrial tRNA metabolism is abnormal. Abnormal mitochondrial tRNA metabolism damages mitochondrial translation, produces protease inhibitory stress, changes the activity of respiratory chain complexes, and leads to embryonic heart Developmental changes, and reduce the shortening of the mutant zebrafish ventricles, and the ventricular cardiomyocytes of the zebrafish that knock out the *gtpbp3* gene are hypertrophy and myocardial fiber disorders (25). It suggests that CRISPR/Cas9 participates in heart development by intervening in related gene editing.

Atherosclerosis/heart failure

CRISPR/Cas9 gene editing to regulate the progress of cardiovascular-related diseases is currently a research hotspot. For example, knocking out the *ApoE/ApoC3* gene by CRISPR/Cas9 can construct a rat model of atherosclerosis (26,27); *in vivo* AAV-CRISPR/Cas9-mediated *LDLR* gene editing can partially rescue LDLR expression and effectively improve *LDLR* mutations Body-induced atherosclerosis phenotype9 (28,29). Transfect Cas9 with lentiviral vector, and guide RNA to introduce Tet2 and Dnmt3a inactivating mutations into lineage-negative bone marrow cells, implant the cells into chemotherapy mice, and continue to inject Ang II (angiotensin II), Tet2 or Dnmt3a Mice with inactivating mutations showed greater myocardial hypertrophy, weakened heart function, and more severe heart and kidney fibrosis (30). The gRNA vector expressed by CRISPR/Cas9 targeting Mef2d can effectively construct the myocardial hypertrophy phenotype (31). Combining CRISPR/Cas9 with short template oligonucleotides produces ATP-sensitive potassium channels for human cardiovascular diseases that cause human cardiovascular diseases in zebrafish homologous genes and are related to Cantu syndrome (CS) (*Kir6.1*, *KCNJ8*, *SUR2*, *ABCC9*) gene mutations in the subunits, resulting in significantly enlarged ventricles, increased cardiac output and contractile function, and significantly dilated cerebral blood vessels (32). The above shows that gene editing plays an important role in inducing cardiovascular diseases, and it also plays an equal role in basic research on the treatment of cardiovascular diseases, such as: CRISPR/Cas9-mediated *FKBP5* gene deletion enhances the effect of FKBP5 on NF-κB through a positive feedback loop Response, has the effect of treating acute myocardial infarction (33). CRISPR/Cas9 knock-in MSCs expressing dual chemokines GCP-2 and SDF-1α may be an alternative treatment option for ischemic vascular disease (34). That is, CRISPR/Cas9 provides a new methodology for the research of cardiovascular related diseases.

Cardiovascular preclinical gene therapy research

At the current clinical level, two types of cardiovascular diseases can be treated or prevented through genome editing. The first type is cardiovascular diseases associated with inheritance, such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), long QT syndrome (LQTS) and muscular dystrophy that cause cardiac dysfunction, and the second type It is diseases such as Marfan syndrome and familial pulmonary hypertension (1,10,22). In addition, with the development of human induced pluripotent stem cells (hiPSC), the generation of human cell lines with CRISPR/Cas9 knockout and knock-in specific genes can significantly increase the possibility of treating cardiovascular diseases (6). For example, using CRISPR/Cas9 technology to knock the *TALEN* gene into cardiomyocytes derived from human-induced pluripotent stem cells to correct gene mutations related to dilated cardiomyopathy in the PLN gene, and *in vivo* (via adeno-associated virus) to mice The administration of this system has been shown to improve heart function during pressure overload or after induced myocardial infarction (22,28). Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene plays an important role in regulating cholesterol homeostasis. Gain-of-function mutations in *PCSK9* gene can lead to hypercholesterolemia and related in arteriosclerosis, CRISPR-Cas9 knocking out the *PCSK9* gene will increase the expression of low-density lipoprotein receptor (LDLR) in adult mice and achieve clinical therapeutic effects (23). It suggests that CRISPR/Cas9 plays an effective role in basic research and treatment

of cardiovascular diseases, but there is no direct clinical application in the field of cardiovascular disease.

In summary, CRISPR/Cas9 represents a breakthrough in genome editing technology, opening up a new way to manipulate genomes *in vitro* and *in vivo*. Although research on small animal models has been stable so far, there are still some off-target effects of genes, which will be prolonged. Basic research and clinical treatment time, it is currently not possible to carry out gene correction in human heart. The application in cardiovascular is mainly focused on direct therapeutic intervention and basic research in preclinical animal models of hereditary heart disease (35), so it can be combined with related Subject related research, after discovering new key targets for diseases, gene editing technology is used to intervene related genes to realize possible programs for gene therapy of cardiovascular diseases. All the protein targets discussed in this article and treatment methods in other fields can be used for reference.

Acknowledgments

The authors would like to thank the laboratory colleagues of Department of Cardiology of Ten Hospital, especially Mr. Yu, who ordered reagents and consumables, etc.
Funding: None.

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://dx.doi.org/10.21037/jxym-21-26

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/jxym-21-26). There's no conflict of interest between authors and unit, excluding the support of the page fee for this article provided by the Department of Cardiology, Shanghai Tenth People's Hospital. Both authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/jxym-21-26
Cite this article as: Liu H, Xu Y. Narrative review: application progress of *CRISPR/Cas9* gene editing in cardiovascular field. J Xiangya Med 2021;6:33.