Gene Editing and Gene-Based Therapeutics for Cardiomyopathies

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KEYWORDS

• Genetic mutations • Genetic correction • Gene editing • Antisense oligonucleotides
• Cardiomyopathy • Heart failure • Muscular dystrophy

KEY POINTS

- Genetic editing targeting DNA sequences can correct underlying genetic mutations.
- Viral vectors are used to deliver gene editing machinery to the heart.
- Antisense oligonucleotides target RNA to modulate splicing, and this approach requires repeated dosing.

INTRODUCTION

Cardiomyopathy and heart failure are under genetic influence. Genetic correction technologies are rapidly emerging, providing the tools to correct underlying genetic defects responsible for human disease, including cardiomyopathy. Gene-editing strategies like clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) act on DNA. There are also RNA-targeted approaches, including antisense oligonucleotides or even gene editing, which are used to suppress mutations or promote expression of functional molecules. Genetic correction strategies are designed to reverse specific mutations responsible for disease, or these same methods can also be applied to modulate normal sequences in order to improve heart function. Most genetic correction approaches are gene and mutation specific, and therefore require knowledge of the underlying genetic mutations responsible for cardiomyopathy and heart failure, further underscoring the importance of genetic diagnosis.

TECHNICAL ADVANCES ENABLING GENETIC EDITING IN DNA

Tools for genetic engineering remain at the crux of scientific discovery and are poised for therapeutic application in heart failure. The Human Genome Project, and the subsequent efforts to define human genetic variation using larger scale efforts, have revolutionized the way in which heart failure and cardiomyopathy are approached.[1–3] Simultaneous with these efforts, the Xenopus-derived zinc finger nuclease (ZFN) emerged as an early technology to change genome sequences.[4] This DNA-binding motif was designed to recognize DNA sequences with high specificity,[5] but required engineering protein motifs to recognize DNA sequences of interest. Despite the broad application of these engineered nucleases for gene correction, expansion of ZNF use was limited by an inability to target complex sequences as well as issues related to sequence specificity. Yet, ZNF served as a gateway for the development of new and improved gene-editing technologies.

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Transcription activator-like effector nucleases (TALENs) represented an advance for genomic engineering.[6,7] These nucleases are engineered constructs that contain a DNA-binding domain and a nonspecific nuclease domain.[8] TALENs generate double strand breaks (DSBs) at specific sites of interest within a given DNA sequence, which are subsequently repaired by nonhomologous end joining (NHEJ). This process induces the formation of insertion and deletion mutations. Unlike ZFNs, TALENs' comparatively fast and easy construction allowed for a more precise and efficient method for genomic targeting.[9] The emergence of TALENs advanced pre-existing methods for gene editing, and these nucleases served as a guide for the onset of innovative tools for gene-based therapies.

Found in both bacteria and archaea, CRISPR/Cas9 emerged as the next generation in genome editing as a system that modifies DNA by generating site-specific cleavage events, which may then be followed by a template-driven repair process.[4,10] With a template-driven repair process, specific sequences can be changed, or new sequences can be inserted or deleted. Similar to previous mechanisms, CRISPR/Cas9 functions by creating DSBs at precise sites within the DNA. The site specificity is driven by guide RNAs, which use this homology to carry the Cas9 nuclease to specific sites. After cleavage by Cas9, DSBs can be repaired by NHEJ, which most commonly results in deletions of varying size and length. Alternatively, in the presence of a template, homology-directed repair (HDR) occurs, in which the DSB is repaired to resemble the template. HDR can be exploited to yield site-specific precise genetic correction, for example, from a mutation to a normal allele. HDR can also be exploited to add sequences of interest or more precisely delete selected regions.[11] In contrast to NHEJ, an error-prone system that fuses together blunt ends of DNA without the use of a repair template, HDR is more accurate by involving the recombination of a homologous template strand. However, HDR is a less efficient method of DSB repair compared with NHEJ. CRISPR/Cas9 was first described in 2012 and then adapted for its use in mammalian cells.[12–14] Since this discovery, CRISPR/Cas9 has been making headways in genomic engineering, providing useful tools in the laboratory setting and also in its development for therapeutic genetic correction.

DNA GENE EDITING USING CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS/CAS9

In bacteria, the CRISPR/Cas9 system is an endonuclease important for cleaving viral DNA. The adaptation of this endonuclease system ultimately relies on guide RNAs to direct the endonuclease, Cas9, to specific sequence sites that are then cleaved. Single-guide RNA sequences (sgRNAs) are short synthetic RNA molecules that direct the Cas9 endonuclease protein to generate DSBs near the site of interest (**Fig. 1**). The precision of this cleavage depends on the accuracy of the sgRNAs. The region of target homology in sgRNAs is approximately 20 base pairs, and even single base pair mismatch reduces target engagement. It is possible to utilize more than a single sgRNA (eg, to create more than one cleavage). However, a drawback of using more than a single sgRNA is decreased efficiency, because guides must find their cognate target, and an increased number of potential off-target effects may result.[15] An additional limitation of this technology is the long-term expression of Cas9 nuclease that can increase toxicity through multiple mechanisms including off-target mutations. Modifications to guide RNAs or using Cas9 nickases, which generate single-strand DNA breaks instead of double-strand DNA breaks, can reduce off-target mutations.[16] Furthermore, destabilizing domains can be added to Cas9 to provide an additional level of regulation, whereby the Cas9 can be inactivated to reduce off-target effects by limiting the lifespan of Cas9 activity.[17]

The small size of Cas9 contributes to the scalable nature of its application, making gene delivery by viral vectors possible. Cas9 endonucleases differ in size and functionality.[18] *Staphylococcus aureus* Cas9 (called SaCas9) is 1 kb smaller than Cas9 from *Streptococcus pyrogenes* Cas9,[19] providing a more compact Cas9. Additionally, the longer CRISPR RNA spacer sequence of *Neisseria meningitidis* Cas9 (referred to as NmCas9) limits its targeting range, but decreases off-target activity.[20] *Streptococcus thermophiles* (StCas9) has proven to be a more specific form of Cas9, albeit less efficient than SpCas9.[21] In tandem with the findings of new Cas9 species, ongoing studies are exploring delivery channels as a means for better transportation of these molecules intracellularly.[22]

APPLICATION OF GENE EDITING IN CARDIOMYOPATHY

There are many contemplated applications of gene editing for treating heart failure. One use of gene editing is aimed at correcting underlying genetic mutations responsible for causing cardiomyopathy. Cardiomyopathies are often attributed to genetic mutations resulting in familial or inherited forms of cardiomyopathy. Hypertrophic cardiomyopathy (HCM) is often linked to sarcomere

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