At the Heart of Genome Editing and Cardiovascular Diseases

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Cardiovascular disease (CVD) is still the leading cause of death worldwide, but the knowledge and technologies for counteracting this disease may already be in our hands. Scientific advances over the past few years, such as the isolation and differentiation of induced pluripotent stem cells (iPSCs), and the development of gene editing tools, have enabled us to model CVD, but more importantly, may represent tools for CVD early diagnosis, patient stratification, and treatment.

The emergence of CRISPR/Cas9 technology has been envisioned as a simple and technically affordable tool for treating CVD. However, the biggest health burden associated with CVD cannot be addressed via CRISPR/Cas9-mediated gene correction, as most patients suffer from atherosclerosis, and the most effective treatments for this condition currently involve changes in lifestyle. CRISPR/Cas9 technologies are also generally ineffective in treating congenital heart disorders, as we have not yet fully understood the exact role of multiple genes underlying these conditions. Moreover, for CRISPR/Cas9 to truly be a viable strategy against CVD, technical limitations of this technology (e.g., mosaicism, off-target effects, low versatility in targeting different cell types, and random genome integration of CRISPR/Cas9 machinery) must be overcome. Unfortunately, these limitations are frequently being overlooked, evidenced for example by the initiation of a number of clinical trials, leading to the impression that a therapeutic solution is available.

CRISPR/Cas9 is currently the most popular gene editing tool, but there are other gene editing technologies with additional capabilities. A more thorough understanding of DNA repair mechanisms is essential for further developing and translating to the clinic this gene-editing toolkit. DNA modifications can be generated by multiple site-directed nucleases, including the traditional meganucleases, zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), all of which generate double-strand breaks (DSBs). Briefly, these nucleases recognize a specific DNA locus and cleave it. The DNA is then repaired by cellular machineries, with the primary repair pathways being homology directed repair (HDR) and non-homologous end-joining (NHEJ). NHEJ is the most prevalent mechanism, in which exposed DNA ends are directly reconnected. Because the NHEJ pathway is error prone, the repair is often associated with an insertion or deletion that can shift the reading frame. This is the ideal mechanism for producing genetic loss-of-function phenotypes. HDR is primarily active in proliferative cells, allowing the incorporation of DNA sequences into the targeted region of the genome in the presence of an appropriate donor with homologous arms. This enables genetic knock-in phenotypes. In this context, the nucleases only trigger the correction. It appears that the repair mechanisms and outcome is cell specific and a detailed understanding of how this takes place in cardiomyocytes is currently being investigated.

Studies using different nucleases had achieved success. One study introduced TALENs into human iPSC-derived cardiomyocytes to correct a genetic mutation in the PLN gene which associates with dilated cardiomyopathy. A zinc finger protein-based transcriptional repressor has also been used to silence PLN. Administration of this system to mice in vivo (via adeno-associated viruses-AAVs) improved cardiac function during pressure overload or after induced myocardial infarction. This reveals the different outcomes of genome editing tools in regenerative medicine,
since their use would not only be restricted to permanent changes that may result in long-standing benefits in patients, but also, in gene modifications in a temporal controlled manner.

Use of these genetic tools has greatly enhanced our mechanistic understanding of the CV system and CVD, but the number of studies attempting to use these technologies towards the treatment of CV disorders is disproportionately small, probably because of technical limitations. Most studies seek to delineate disease phenotypes, rather than offer a method for treatment. Modeling CV diseases is an essential step toward developing a cure and a prerequisite for clinical trials, but in practical terms, disease correction is a long way off. Major expectations have been raised since site-specific manipulation of the genome was first demonstrated. Recently, CRISPR/Cas9 has been used in early stage human embryos to correct a mutation in the MYBPC3 gene that is associated with hypertrophic cardiomyopathy. Correction of the MYBPC3 gene in human embryos is an example of how these technologies can be used to prevent a variety of CV disorders originated from the inheritance of a single copy of a defective gene. Additional cardiomyopathies (e.g., hypoplastic left heart syndrome, Brugada syndrome, LQT-arrhythmias) caused by mutations in various genetic loci (e.g., MYH7, TNNT2, TPM1) can be potentially identified and corrected in an organism before birth.

However, the efficacy of gene editing procedures is still inadequate. For the MYBPC3 correction, the net rate of success is less than 2%, given ideal in vitro conditions. In most cases the genetic mutation was corrected, but new mutations were generated at the DNA lesion was repaired. This DNA repair mechanism is not part of the CRISPR/Cas9 system, but is instead a normal cellular process. It is important to note that the versatility of DNA repair machineries has been largely ignored when developing CRISPR/Cas9 approaches and must be better understood before CRISPR/Cas9 can be applied.

An important feature that enabled correction of the MYBPC3 mutation in human embryos was the early stage of development. Correction of the MYBPC3 locus represent a case of ‘curing the disease before it develops’. During development, most cells are highly proliferative, but this proliferation progressively decreases after birth. Differentiated cardiac cells are no longer capable of cell division, which constrains the ability of CRISPR/Cas9 to edit their genome (CRISPR/Cas9 is most effective at the S and G2 phases of cell cycle when the HDR machinery is most active). To overcome this limitation, some studies have suggested inducing post-mitotic cells to proliferate or inhibiting the NHEJ machinery – both with the goal of increasing gene correction by the CRISPR/Cas9 system. However, the artificial induction of proliferation may functionally compromise the differentiated cells, and therefore limit the therapeutic value of the CRISPR/Cas9 correction.

Recently, a new system has been developed called homologous independent targeted integration (HITI), which can generate knock-in phenotypes via NHEJ, even in non-proliferating differentiated cells. This system can be applied to mature cardiac cells, which usually show low levels of HDR-mediated gene correction due to their quiescent stage; they mainly use NHEJ to repair DSBs. Efficacy of HITI to target post-mitotic cells was initially demonstrated in post-mitotic neurons, and proof of concept for the targeted in vivo gene editing of cardiac cells has also been attained. This
was achieved by the systemic delivery of AAV-HITI, resulting in GFP knock-in in heart cells following DNA cutting by CRISPR/Cas9. It is essential to discover the full potential for using this HITI technology to modify the genomes of mature cardiac cells. For congenital cardiac anomalies associated with reduced levels of a particular protein, HITI strategies could be used to rescue the expression of the disease-related protein (e.g., by repairing the frame shift of a mutated gene in mature cells). This may provide a viable treatment option, particularly for dominant inherited conditions.

Although the CRISPR/Cas9 system is genuinely powerful, it is generally thought that we are still far from guaranteeing the absence of off-target effects when editing the human genome. Thus, researchers are searching for alternative methodologies that can provide scar-less and mistake-free repair – a system that can be applied in the clinic. Base-editing systems represent the most recent technologies to edit the genome in the absence of a DSB, potentially enabling the tunable introduction of all four transition mutations in human cells. Indeed, it has been recently shown that base-editors induce less off-target genome modification than Cas9 8, and thus, may provide additional avenues for targeting human disease, including CVD. Another recent alternative is represented by the trans-epigenetic activation mediated by CRISPR/Cas9 9: this strategy allows for the recruitment of transcriptional activation complexes in a targeted way to modulate gene expression without cutting DNA. This technology could be of help for congenital heart disorders where the expression of a gene is reduced (e.g. Holt Oram syndrome).

Improving target specificity of the CRISPR/Cas9 system will allow for genotype–phenotype correlations, enhancing diagnosis in a clinical setting 10. Current technical limitations of these systems require the scientific community to work collaboratively and in a regulated research framework. At this time, there is an unreal expectation that gene editing, particularly CRISPR/Cas9, can do everything. The truth is, however, that CRISPR/Cas9 can be feasibly implemented in many, but not all contexts. More basic science is required so that we achieve complete mechanistic understanding of the gene editing process, and can guarantee a successful edit, especially for in vivo applications. Even without these guarantees in place, clinical trials using CRISPR/Cas9 are already forging ahead. A societal debate concerning the immediate applications and risks of this technology is urgently needed, a debate that could help guide us toward a future of improved human health.

REFERENCES


Figure legend

**Gene editing in cardiovascular disease.**

A. Distribution of people affected by each specific type of cardiovascular disease. Percentages were calculated based on 2015 data and statistics from the World Health Organization. DCM, Dilated Cardiomyopathy; HCM, Hypertrophic Cardiomyopathy; RCM, Restrictive Cardiomyopathy. B. Distribution of cardiovascular disease studies using site-directed nucleases. Data were estimated from direct research on PubMed lists by using the terms: cardiovascular and the name of each type of nuclease. Representation of Meganucleases and Zinc-Finger nucleases are on the left of the graph. Representation of TALEN and CRISPR/Cas9 are on the right. C. Gene editing mechanisms and their consequent application on cardiac cells. DSB, Double Strand Breaks; NHEJ, Non-Homologous End Joining; HITI, Homologous Independent Targeted Integration; HR, Homologous Recombination.