At the Heart of Genome Editing and Cardiovascular Diseases

Reyna Hernandez-Benitez, Maria Llanos Martinez-Martinez, Jeronimo Lajara, Pierre Magistretti, Nuria Montserrat, Juan Carlos Izpisua Belmonte

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, 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 had 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.[1] Moreover, for CRISPR/Cas9 to truly be a viable strategy against CVD, technical limitations of this technology (eg, 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 several 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, ZFNs (zinc-finger nucleases), and TALENs (transcription activator-like effector nucleases), all of which generate double-strand breaks. 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 and nonhomologous 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. Homology-directed repair 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 knockin phenotypes.[2] In this context, the nucleases only trigger the correction. It seems that the repair mechanisms and outcome is cell-specific and a detailed understanding of how this takes place in cardiomyocytes is currently being investigated (Figure).

Studies using different nucleases had achieved success. One study introduced TALENs into human induced pluripotent stem cell–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) improved cardiac function during pressure overload or after induced myocardial infarction.[3] This reveals the different outcomes of genome editing tools in regenerative medicine, because 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 toward 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 CVDs 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 because site-specific manipulation of the genome was first demonstrated. Recently, CRISPR/Cas9 has been used in early-stage human embryos

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia (R.H.-B., P.M.); Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA (R.H.-B., J.C.I.B.); Universidad Católica San Antonio de Murcia, Spain (M.L.M.-M., J.L.); Pluripotency for organ regeneration (PR Lab), Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Spain (N.M.); and Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine, Madrid, Spain (N.M.).

Correspondence to Juan Carlos Izpisua Belmonte, PhD, Salk Institute for Biological Studies, 10010 N Torrey Pines Rd, La Jolla, CA 92037. E-mail belmonte@salk.edu

(*Circ Res*. 2018;123:221-223.
DOI: 10.1161/CIRCRESAHA.118.312676.)

***Circulation Research* is available at https://www.ahajournals.org/journal/res**
DOI: 10.1161/CIRCRESAHA.118.312676

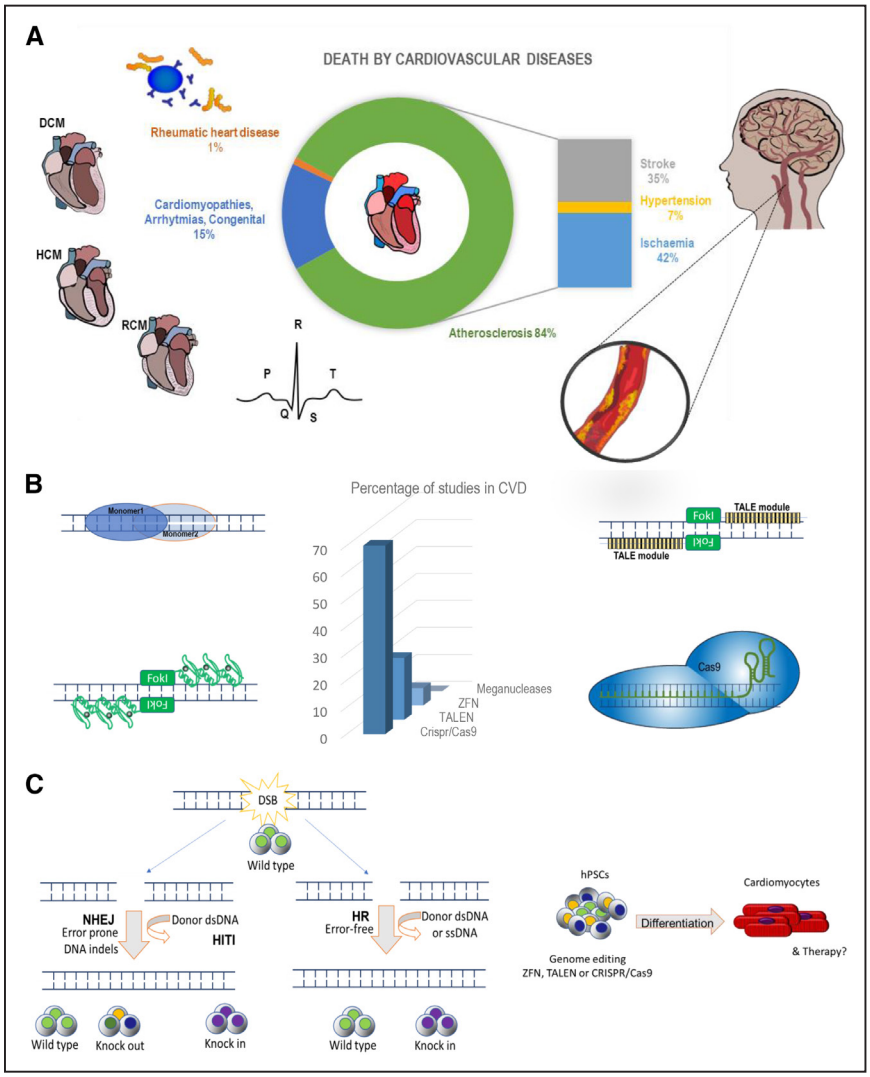

Figure. Gene editing in cardiovascular disease (CVD). A, Distribution of people affected by each specific type of CVD. Percentages were calculated based on 2015 data and statistics from the World Health Organization. **B**, Distribution of CVD studies using site-directed nucleases. Data were estimated from direct research on PubMed lists by using the terms: cardiovascular and the name of each type of nuclease. Representation of Meganucleases and ZFNs (zinc-finger nucleases) are on the **left** of the graph. Representation of TALEN (transcription activator-like effector nuclease) and CRISPR/Cas9 are on the **right**. **C**, Gene-editing mechanisms and their consequent application on cardiac cells. DCM indicates dilated cardiomyopathy; DSB, double-strand breaks; HCM, hypertrophic cardiomyopathy; HITI, homologous independent targeted integration; hPSCs, human pluripotent stem cells; HR, homologous recombination; NHEJ, nonhomologous end joining; and RCM, restrictive cardiomyopathy.

to correct a mutation in the *MYBPC3* gene that is associated with hypertrophic cardiomyopathy.[4] 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 (eg, hypoplastic left heart syndrome, Brugada syndrome, LQT-arrhythmias) caused by mutations in various genetic loci (eg, *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 <2%, given ideal in vitro conditions. In most cases, the genetic mutation was corrected, but new mutations were generated at the CRISPR/Cas9 target site when 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 represents 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 homology-directed repair machinery is most active). To overcome this limitation, some studies have suggested inducing postmitotic cells to proliferate or inhibiting the NHEJ machinery—both with the goal of increasing gene correction by the CRISPR/Cas9 system.[5,6] 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 knockin phenotypes via NHEJ, even in nonproliferating differentiated cells.[7] This system can be applied to mature cardiac cells, which usually show low levels of homology-directed repair–mediated gene correction because of their quiescent stage; they mainly use NHEJ to repair double-strand breaks. Efficacy of HITI to target postmitotic cells was initially

demonstrated in postmitotic 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 adeno-associated virus–HITI, resulting in GFP (green fluorescent protein) knockin in heart cells after 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 (eg, 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 double-strand break, potentially enabling the tunable introduction of all 4 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 transepigenetic 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 (eg, 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.

Disclosures

None.

Sources of Funding

Work in the laboratory of J.C. Izpisua Belmonte was supported by G. Harold and Leila Y. Mathers Charitable Foundation, The Leona M. and Harry B. Helmsley Charitable Trust, The Moxie Foundation, King Abdullah University of Science and Technology, and Universidad Católica San Antonio de Murcia.

References

1. Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: the glass half empty. *Circ Res*. 2013;112:707–720. doi: 10.1161/CIRCRESAHA.112.300853.
2. Chandrasegaran S, Carroll D. Origins of programmable nucleases for genome engineering. *J Mol Biol*. 2016;428:963–989. doi: 10.1016/j.jmb.2015.10.014.
3. Stillitano F, Turnbull IC, Karakikes I, Nonnenmacher M, Backeris P, Hulot JS, Kranias EG, Hajjar RJ, Costa KD. Genomic correction of familial cardiomyopathy in human engineered cardiac tissues. *Eur Heart J*. 2016;37:3282–3284. doi: 10.1093/eurheartj/ehw307.
4. Ma H, Marti-Gutierrez N, Park SW, et al. Correction of a pathogenic gene mutation in human embryos. *Nature*. 2017;548:413–419. doi: 10.1038/nature23305.
5. Chu VT, Weber T, Wefers B, Wurst W, Sander S, Rajewsky K, Kühn R. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. *Nat Biotechnol*. 2015;33:543–548. doi: 10.1038/nbt.3198.
6. Maruyama T, Dougan SK, Truttmann MC, Bilate AM, Ingram JR, Ploegh HL. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. *Nat Biotechnol*. 2015;33:538–542. doi: 10.1038/nbt.3190.
7. Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. *Nature*. 2016;540:144–149. doi: 10.1038/nature20565.
8. Gaudelli NM, Kormor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*. 2017;551:464–471. doi: 10.1038/nature24644.
9. Liao HK, Hatanaka F, Araoka T, et al. In vivo target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell*. 2017;171:1495–1507.e15. doi: 10.1016/j.cell.2017.10.025.
10. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. *Mol Ther Nucleic Acids*. 2015;4:e264. doi: 10.1038/mtna.2015.37.

Key Words: atherosclerosis ■ cardiovascular diseases ■ cause of death ■ gene editing ■ induced pluripotent stem cells