See Article page 1479.

Commentary: Divine decree or a novel panacea in Clustered Regularly Interspaced Short Palindromic Repeats Associated 9-steered cellular reprogramming in the fate of failing heart

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The advent of stem cells unveiled the self-regeneration potential of heart tissue under physiological and pathological conditions despite its status as a terminally differentiated organ. Evidently, the heart tissue has been decorated with diverse precursor, progenitor, and stem cell populations capable of differentiating into multiple cell phenotypes of cardiac lineage. However, their exact origin and the underlying signaling leading to their activation, differentiation, and regulation are yet to be unveiled.¹ Being the primary “slayer” (with ~50% of patients dying within 5 years of prognosis), myocardial infarction (MI) remains the major global health menace. Fortunately, the recent advancements in stem cell biology have laid a robust foundation to the next-generation management strategies for MI, promising a pleasing hope to millions of people across the planet. The “Holy Grail” concept/approach of regenerative cardiology using stem cells is still in infancy, and the unavailability of ideal stem cell candidate, phenotype switch/integrity, undesired differentiation, inappropriate secretome, and teratoma formation are the potential challenges elicited by diverse stem cell populations availed for MI

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The research work of Agrawal is supported by Research Grants R01HL128063, R01HL144125, and R01HL147662 from the National Institutes of Health. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosures: The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

Received for publication May 10, 2020; revisions received May 10, 2020; accepted for publication May 11, 2020; available ahead of print May 24, 2020.

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J Thorac Cardiovasc Surg 2022;163:1491-3

0022-5223/\$36.00

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<https://doi.org/10.1016/j.jtcvs.2020.05.026>

Devendra K. Agrawal, PhD (left), and Finosh G. Thankam, PhD (right)

CENTRAL MESSAGE

Replenishment of reprogrammed CDCs, designed by the forced expression of CM-specific key regulatory genes, in the failing myocardium restores the cardiac function.

management.^{2,3} Moreover, the replenishment of cardiomyocytes (CMs), the beating component of myocardium, and the complete restoration of cardiac output using stem cells and stem cell-based technologies are yet to be achieved.

How to trounce these challenges? Theoretically, the establishment of a specific cell phenotype destined to differentiate solely to CM lineage by the transdifferentiation, dedifferentiation, or programming/reprogramming of resident cardiac cells or stem cells is ideal to overcome hurdles. This can be accomplished by forced expression of CM-specific regulatory genes, especially the cardiac-specific transcription factors including Gata4, Mef2c, Nkx2-5, connexin-43, Hand2, and Tnnt2 in the cells of interest.⁴ Thus, the success of such strategies largely depends on the choice of precursor cells and the mediators to be expressed. How is it possible? The inception of genome editing and cell reprogramming has transfigured the fundamental, applied, and translational sciences, which has reverberations in regenerative cardiology. More specifically, the discovery of Clustered Regularly Interspaced Short Palindromic Repeats– (CRISPR) associated 9 (Cas9) (CRISPR/Cas9) system has imparted a new dimension to cell-based cardiac regeneration because of its simplicity, flexibility, and versatility. Shortly after the first demonstration in 2012, the CRISPR/Cas9 technology became an ideal choice for genome editing, thereby contributing significantly to understand the molecular pathology and to explore promising

translational avenues for diverse diseases including cardiovascular disease.⁵ Inspired from the adaptive immunity of the bacterium *Streptococcus pyogenes* against viral infections, CRISPR/Cas9 technology has loomed to be a potent genomic editing tool that has uncovered immense applications in cardiovascular disease research, including energy metabolism, electrophysiology, genetics, development, programming of induced pluripotent stem cells, and the design of knockout or knockin animal models and cell lines.⁵

Taking advantage of this technology, the original research article by Sano and colleagues⁶ in this issue of the *Journal* adopted an encouraging approach to create a specific phenotype of cardiomyogenic cells by forcefully inducing the “intrinsic transcriptional activation” of a battery of key regulatory genes in cardiosphere-derived cells

(CDCs) using CRISPR/Cas9-based genome editing to address pathological impairments associated with the permanent loss of CM after MI. The findings support the successful generation of a large population of CM and adequately address the challenges, including the uncontrolled proliferation and off-target effects associated with conventional external induction systems. The authors deserve great appreciation for so brilliantly choosing the CDCs for intervention, appropriate selection of the target genes (*Gata4*, *Mef2c*, *Nkx2-5*, *Hand2*, and *Tnnt2*), and effective screening/identification of specific sequences of enhancers and promoters for their transcriptional activation using the modified CRISPR/dCas9 system fused with transcriptional activator VP64 (CRISPR-dCas9-VP64). Furthermore, the authors succeeded to effectively

FIGURE 1. Schematic representation of the overall design and treatment strategies in the study by Sano and colleagues⁶ in the CRISPR-dCas9-VP64-steered cellular reprogramming to rejuvenate the failing heart. CDC, Cardiosphere-derived cell; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats associated 9; IGF-1, insulin-like growth factor 1.

extrapolate the cardiomyogenic-CDCs to the rat MI model demonstrating the translational significance of their approach. The findings revealed significant myocardial regeneration with a concomitant improvement in ejection fraction, ventricular stroke volume and overall cardiac performance, upregulation of insulin-like growth factor-1-mediated paracrine signaling, and activation of pro-healing pathways. The overall message of the study is depicted in [Figure 1](#).

A seminal study by Nam and colleagues⁷ demonstrated the successful reprogramming of mouse fibroblasts into myogenic lineage by the forced expression of CM transcription factors such as GATA4, Hand2, T-box5, and myocardin along with miR-1 and miR-133. The resultant cell progeny displayed sarcomere-like structures, calcium transients, and spontaneous contractility, despite the upregulation of multiple myocyte genes with a concomitant downregulation of nonmyogenic genes.⁷ Recently, similar findings were reported by Dal-Pra and colleagues⁸ while considering the same genes (Gata4, Mef2C, Tbx5, and Hand2). They demonstrated the reprogramming of fibroblasts into CM using a combination of cardiac reprogramming microRNAs (miR-1, miR-133, miR-208, and miR-499), which was more effective than the CRISPR/Cas9 approach.⁸ These studies debate for additional requirement/consideration of epigenetic mediators for the fruitful programming of non-myogenic cells to myogenic lineage, indicating a potential limitation of the present study by Sano and colleagues.⁶ In addition, the dearth of quantitative data on the retention of injected CDCs at the target site, lack of information regarding the gene expression profile of cardiomyogenic CDCs, electrophysiology and contractility, and inadequate secretome biology of cardiomyogenic CDCs warrant further attention. Furthermore, the major drawbacks of

CRISPR/Cas9 system, including off-target mutagenesis, lower efficiency in terminally differentiated nondividing cells such as CMs, immunogenicity, and the social/ethical issues,^{9,10} need to be considered before translating the findings into clinical arena. Evidently, the findings are felicitous but warrant careful investigation in clinically relevant large animal models before clinical trials. Nonetheless, the findings by Sano and colleagues⁶ have added a new flavor to the field of regenerative cardiology revealing a novel approach in the use of CRISPR/Cas9 system and CDCs for regenerating the failing myocardium.

References

1. Leri A, Kajstura J, Anversa P, Frishman WH. Myocardial regeneration and stem cell repair. *Curr Probl Cardiol*. 2008;33:91-153.
2. Leong YY, Ng WH, Ellison-Hughes GM, Tan JJ. Cardiac stem cells for myocardial regeneration: they are not alone. *Front Cardiovasc Med*. 2017;4:47.
3. Chandrasekhar SK, Thankam FG, Agrawal DK, Ouseph JC. Interface biology of stem cell-driven tissue engineering: concepts, concerns, and approaches. In: Sharma CP, ed. *Biointegration of Medical Implant Materials* (2nd ed.). Woodhead Publishing Series in Biomaterials. Cambridge, UK: Woodhead Publishing; 2020:19-44.
4. Finosh GT, Jayabalan M. Regenerative therapy and tissue engineering for the treatment of end-stage cardiac failure. *Biomater*. 2012;2:1-14.
5. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337:816-21.
6. Sano T, Ito T, Ishigami S, Bandaru S, Sano S. Intrinsic activation of cardiosphere-derived cells enhance myocardial repair. *J Thorac Cardiovasc Surg*. 2022;163:1479-90.e5.
7. Nam Y-J, Song K, Luo X, Daniel E, Lambeth K, West K, et al. Reprogramming of human fibroblasts toward a cardiac fate. *Proc Natl Acad Sci U S A*. 2013;110:5588-93.
8. Dal-Pra S, Hodgkinson CP, Dzau VJ. Induced cardiomyocyte maturation: cardiac transcription factors are necessary but not sufficient. *PLoS One*. 2019;14:e0223842.
9. Vermersch E, Jouve C, Hulot J-S. CRISPR/Cas9 gene-editing strategies in cardiovascular cells. *Cardiovasc Res*. 2020;116:894-907.
10. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31:822-6.