Editorial

Get Your Cell K.O. in the First Round

Jean-Sébastien Hulot

Recent advances in the development of genome-editing technologies based on programmable nucleases have substantially improved our ability to make precise modifications in the genomes of eukaryotic cells. Specifically, programmable nucleases enable precise genome editing by creating DNA double-strand breaks at specific genomic loci.[1] In the absence of a repair template, the DNA lesion will be repaired through nonhomologous end joining, a mechanism that religates the 2 free double-strand break ends. Nonhomologous end joining is, however, error prone, often creating small insertion or deletion mutations bridging the break site.[2] If these insertion or deletion mutations are introduced in the coding sequence of a gene, they can potentially lead to loss-of-function mutations into the targeted gene, thus leading to its permanent inactivation. This approach can then be applied to different cell types where it will be extremely efficient to knockout a gene of interest.

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Genome-editing technologies are, thus, broadening our ability to elucidate the contribution of genetics to disease, including cardiovascular, by allowing the unprecedented and fast creation of appropriate cellular or animal models of pathological processes.[3,4] Their application is particularly appealing into pluripotent stem cells (such as human induced pluripotent stem cells [hiPSCs]) to perform functional investigations on a putative gene (or a mutation) in a genetically modified cellular model of cardiac or vascular disorders. However, with ≈25 000 annotated genes in the human genome and >3700 already linked to disease phenotypes, the overall strategy means a huge effort. Cardiovascular genetics have uncovered a plethora of genes associated with inherited cardiovascular diseases, and many new studies could benefit from genome-editing technologies to delineate the underlying molecular mechanisms.

In this issue of *Circulation Research*, Karakikes et al[5] propose to foster this strategy by developing a validated library of genome-editing tools to knockout 88 human genes that have been associated with cardiomyopathies and congenital heart diseases. The collection is made of transcription activator-like effector nucleases (TALENs), one of the major class of nucleases, that are chimeric enzymes that achieve binding to a specific DNA sequence via protein–DNA interactions and cut the DNA through a sequence-agnostic FokI nuclease.[2] TALENs are efficient nucleases that have previously been used for genome engineering of hiPSC,[6] but their use has been generally limited, as they require complex molecular cloning to be developed. In this study, Karakikes et al[5] designed TALEN pair constructs for each of the selected 88 genes. By targeting sequences located around the start codon of each gene, they developed an efficient and ready-to-use collection of tools to simplify the custom generation of knockout cell lines.

To illustrate the scientific potential of this library, the authors then performed gene knockout in hiPSCs in different situations. First, the newly developed TALENs can be used to generate complete knockout (ie, with insertion or deletion mutations on both alleles) of a gene of interest (Figure, left). The strategy consisting of mutation insertion or gene knock out in a normal cell is a faster and more efficient way to investigate the role of this gene on a cellular phenotype as it will limit the time- and effort-consuming generation of multiple patient-specific iPSC clones. Indeed, a limited number of fully characterized iPSC clones can be used instead, and the newly developed genome-editing platform could now be used for the custom generation of iPSC knockout cell lines.[7] Similarly, the team previously reported on this strategy by recapitulating long-QT syndrome by inserting the mutated genes in safe harbor sites of normal hiPSCs.[8] As exemplified by targeted manipulation of *Tbx5* in the present study,[5] combination of hiPSC, genome-editing, and deep-sequencing technologies provide unprecedented opportunities to identify and understand the molecular basis of cardiomyopathies.

In addition, in the present study, Karakikes et al[5] moves further by showing the added value of this approach in dissecting genotype-to-phenotype relationships. The authors used their newly designed TALENs to knockout cardiac troponin T (*TNNT2*) in control hiPSCs. *TNNT2* mutations have been implicated in familial hypertrophic cardiomyopathy, but other *TNNT2* mutations can lead to dilated cardiomyopathy. Furthermore, whether the pathological effect is supported by haploinsufficiency or by a dominant-negative mode was unclear. By generating and comparing both monoallelic ($TNNT2^{+/-}$) versus biallelic ($TNNT2^{-/-}$) *TNNT2* knockout iPSC lines, the authors first found evidence for the lack of haploinsufficiency to explain the pathogenesis of cardiomyopathies associated with *TNNT2* loss-of-function mutations. The lack of functional or structural abnormalities and normal levels of cTNT expression in $TNNT2^{+/-}$ hiPSC-derived cardiomyocytes further supported this hypothesis. On the contrary, $TNNT2^{-/-}$ iPSC-derived cardiomyocytes showed a complete abolition of cTNT protein expression, severe sarcomeric disarray, and impaired intracellular calcium cycling. These first results rather suggest a dominant-negative mechanism that was further demonstrated using the same TALEN

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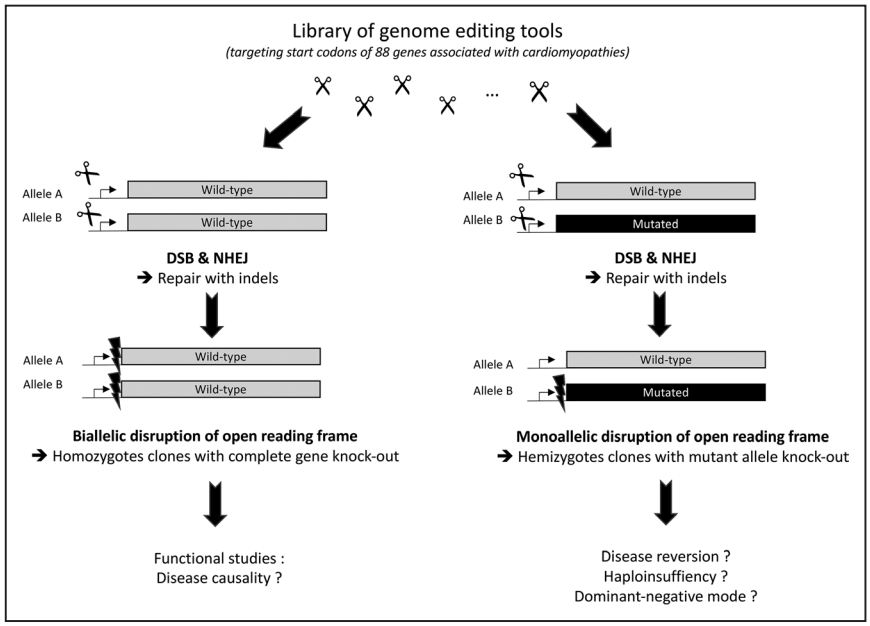

Figure. Strategies that can be developed using a prevalidated library of transcription activator-like effector nucleases targeting the start codon (or regions immediately around) of 88 genes associated with cardiomyopathy or congenital heart disease. DSB indicates double-strand breaks; Indels, insertion or deletion mutations; and NHEJ, nonhomologous end joining.

strategy but in a hiPSC line generated from a patient with a missense mutation in *TNNT2* gene (with a heterozygous genotype). Indeed, TALEN-mediated deletion of the mutated allele creates a synthetic hemizygote cell line where only the remaining wild-type allele is expressed (Figure, right). Different cellular measurements then demonstrated the phenotypic rescue in these hemizygote cell lines, thus supporting a dominant-negative mechanism for TNNT2. On the basis of this example, the novel TALENs library will, thus, help in getting faster information of influence of mutations in iPSC lines from patients carrying mutations in 1 of the 88 targeted genes. However, this approach will not address the question of genetic correction where the sequence around the mutation is targeted by a programmable nuclease and a genetic template is provided to perform homology-directed repair and correct the mutation.[2]

This study will represent an important step in the development of new strategies using genome-editing techniques to better understand the pathogenesis of inherited cardiomyopathies or congenital heart diseases. CRISPR/Cas9 is another class of programmable nucleases that uses RNA–DNA interactions to bind and cut the targeted DNA sequence.[9–11] The Cas9 protein possesses a nuclease function and is targeted to DNA sequence by a short RNA guide molecule that base pair directly the target DNA. Because the Cas9 protein is invariant, it can be easily retargeted by simply changing short RNA guide molecules. Compared with other programmable nucleases including TALEN, the ease of using Cas9 explains its rising popularity in the academic and industrial worlds. However, CRISPR/Cas9 technology also have limits including a higher risk of off-target mutagenesis and specific targeting constraints linked to the need of a 2- to 6-bp motif (so-called PAM [protospacer adjacent motif]) immediately after the targeted DNA sequence.[2] Therefore, the development of a prevalidated library of genome-editing tools is a critical step in helping cardiovascular researchers to apply this technology to their current models.

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