Can Engineered Bacteria Bring Gene-edited Human Stem Cells to the Clinic?

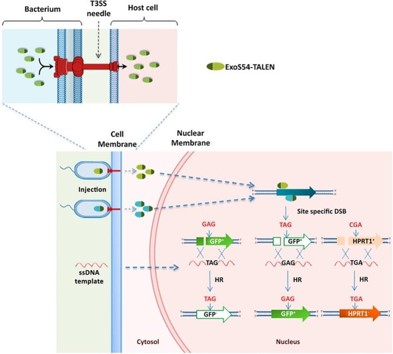
August 6, 2015 | ESCs/iPSCs

Transcription activator-like effector nucleases (TALEN) are artificial restriction enzymes used for targeted genome editing whose delivery into the cell can involve viral DNA/RNA, plasmid DNA, or mRNA. These approaches may, however, not be clinically relevant due to the presence of foreign genetic material, the relative inefficiency, and/or the risk of insertional mutagenesis. To overcome these problems, researchers from the laboratories of [Shouguang Jin](http://mgm.ufl.edu/faculty/faculty-home-pages/jin-shouguang/), [Naohiro Terada](http://teradalab.pathology.ufl.edu/), and [Weihui Wu](http://www.researchgate.net/profile/Weihui_Wu) have developed a cunning strategy: let bacteria do the work for you. Specifically, the have adapted the type III secretion system (T3SS) of Pseudomonas aeruginosa [1] to inject TALEN proteins into a cell using a complex, needle-like structure, instead of bacterially derived toxins. In their new study, published in *Stem Cells Translational Medicine*, they now outline how to use this system for gene editing in human pluripotent stem cells (hPSCs), to serve as a basis for ongoing research into creating disease models and human gene therapy [2].

This new study builds upon previous work by the same group in which they used TALENs delivered by T3SS to target a GFP transgene in modified HeLa cells [3]. This same strategy also functioned in mouse embryonic stem cells (mESCs) with a GFP transgene at a multiplicity of infection (MOI) of around 100 for 3 hours. This mediated an efficient delivery of proteins to the mESC nucleus where they remained stable until 8 hours after the end of bacterial infection. Bacterial delivery mediated a two-fold higher GFP-targeting efficiency than that found for TALEN-coding plasmid transfection and isolation of GFP-negative cells demonstrated the expected DNA double-strand breaks (DSBs) at the intended target site. From this starting point, the researchers then refined the process, discovering that the optimal conditions for bacterial delivery of TALEN into mESCs is a 3-hour infection at an overall MOI of 400 (200 for each of the TALEN pair). The next stage of the process involved using the bacterially delivered TALENs to induce a single base change, rather than creating DSBs. To do this, they introduced a single-stranded oligonucleotide DNA template for homologous recombination in the GFP gene in order to convert a GAG codon to a TAG stop codon, so switching off GFP. Excitingly, this method resulted in a three-fold higher efficiency of target gene modification than conventional transfection approaches.

Finally, the study turned to the application of this strategy in GFP-expressing human ESCs (hESCs) and human induced pluripotent stem cells (hiPSCs). Initial studies found human PSCs to be sensitive to the bacterial cytotoxicity of the strain used up until this point, and this required the application of another P. aeruginosa strain which lacked five additional toxicity-inducing genes but maintains a high type III secretion capacity. Three hours of infection at an overall MOI of 100 induced GFP silencing at a greater efficiency than plasmid transfection and also allowed, in a separate experiment, for the targeting of a region of the HPRT1 gene which contains a mutation which causes neurological and behavioral problems [4]. This efficiently mediated DSBs at the HPRT1 site, and the introduction of a single-stranded oligonucleotide (ssODN) designed for homologous recombination to introduce a single nucleotide change mediated high-efficiency modification of the target gene.

This exciting study demonstrates a novel and highly efficient method of effecting targeted gene changes and is summarised in the adjoining figure. The bacterial nature of the process makes it simple to scale up, easy to manipulate, and safe, as the bacteria can be simply eradicated using simple antibiotic treatments. The authors also note that recent developments such as simpler single-chain TALEN strategies [5] and improved CRISPR/Cas9 mediate gene targeting strategies [6, 7] may also be bacterially delivered, in the hope that this may lead to the generation of clinically relevant systems for the treatment of human genetic diseases.



**Discussion Points**

* Are engineered bacteria the key to improving your gene editing work?
* Are there any off-target effects?
* How can we reduce bacterial toxicity?
* Can they also be used to express reprogramming factors in order to generate iPSCs?
* Can CRISPR/Cas9 technology be adapted to function via bacterial infection?

**References**

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