Optimization of precise gene editing in human iPS cells

We are looking for R&D collaborators.

Background

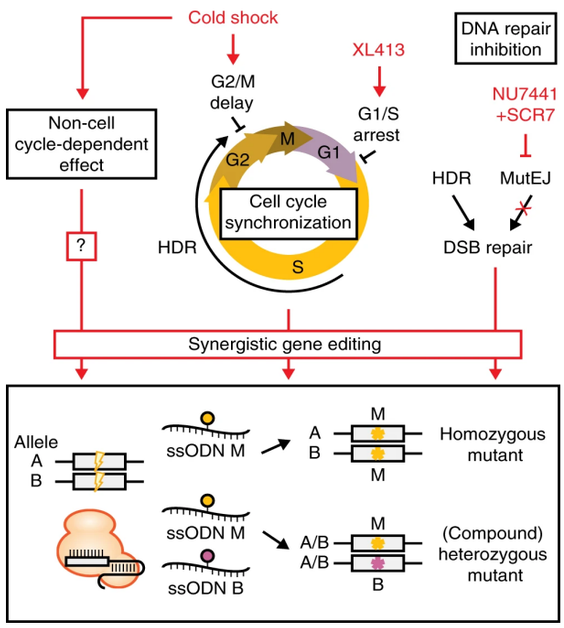
The discovery of CRISPR-Cas system for genome editing has revolutionized experimental biology. Research labs all over the world are using multiple variations of the system to answer fundamental questions in genetics, model diseases, and develop therapies. However, precise gene editing to generate single-nucleotide modifications needed for accurate disease modeling and therapeutic development has seen limited success. The Woltjen Lab has developed methods to optimize precise gene editing in iPS cells by combining a series of techniques using CRISPR-Cas9.

Technical Summary

To make single nucleotide modifications, CRISPR-Cas9 is usually used to generate targeted DNA double-strand breaks in the genome. These are subsequently resolved by the cell's endogenous DNA repair pathways. Human cells have several strategies to repair double-strand breaks such as non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ), referred to collectively as mutagenic end joining (MutEJ), and homology-directed repair (HDR). Researchers exploit the latter to generate designer, single nucleotide changes: they introduce CRISPR-Cas9 into the cell and provide a customized repair template, i.e. donor plasmid or single-stranded donor oligonucleotide (ssODN), to make the cells repair the breaks using HDR. However, the process is complicated by HDR being limited to specific phases of the cell cycle, while MutEJ is not. This often results in low HDR/MutEJ ratios, i.e. less precise editing.
The Woltjen Lab optimized HDR editing with custom ssODN templates by a combination of two techniques: 1) controlling cell-cycle progression with cold shock and 2) modulating DNA repair pathway choice with pharmacological inhibition. For the latter, the researchers treated the cells with various small molecules reported to inhibit particular DNA repair pathway components. As a result, the researchers confirmed improved HDR rates and HDR/MutEJ ratios and managed to successfully generate both homozygous and heterozygous mutants of human induced pluripotent stem cells (iPSCs) (Maurissen and Woltjen 2020, Fig.1).

Figure 1.

Schematic illustrating precise gene editing approach by releasing the cells into the HDR permissive S and G2 phases of the cell-cycle combined with DNA repair inhibition to improve HDR rates. Additional non-cell-cycle-dependent effects of cold shock may also play a role. Leveraging these mechanisms, homozygous mutations are generated at high efficiency, while heterozygous mutations may be generated without indels by using a combination of mutant and blocking ssODN templates.



The researchers confirmed that their method of gene editing via cell-cycle synchronization and DNA repair pathway modulation is transferable between electroporation instruments and to other iPS cell lines. They are looking for collaborators to apply these techniques to cell lines other than iPSCs and test various pharmaceutical cocktails to find optimal combinations for DNA repair pathway modulation.

Technology Readiness Level

3

Approach has been validated in iPS cell lines

Potential Applications

- Disease modeling
- Drug screening

Possible Collaboration Mode(s)

- R&D collaboration
- Licensing
- IP Acquisition
- Other

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Inventor(s)

Knut Woltjen, Ph.D.
Thomas Luc Maurissen, Ph.D.

SOCIETY ACADEMIA COLLABORATION FOR INNOVATION (SACI)
Kyoto University Division

215, 2/F, International Science Innovation Building
Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto
606-8501 JAPAN

Email: gtcunit@mail2.adm.kyoto-u.ac.jp
Phone: +81-75-753-5692