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PROMOTING CRISPR HOMOLOGY DIRECTED REPAIR IN INDUCED PLURIPOTENT STEM CELLS BY COMBINING SMALL MOLECULES

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CRISPR-Cas9 is a bacterial system that has been artificially synthesized so it can be harnessed to edit the human genome. In the synthesized version, a small piece of RNA with a "guide" sequence binds to a specific DNA sequence in the genome and the enzyme Cas9. The Cas9 enzyme can identify the targeted DNA sequence and cut it. After the DNA is cut, it is repaired in one of two ways: Non-homologous End Joining (NHEJ) or Homology Directed Repair (HDR). NHEJ, the more common pathway, typically results in an insertion or deletion (indel) that results in a truncated gene product leading to loss of function ("knock-out) of the gene of interest. HDR, the more technically challenging method, is used to knock-in a genetic modification into a cell line or animal model (often a mutation associated with a certain disease). Despite the research community's high interest in HDR, the process remains very inefficient.

To improve the efficiency of the HDR system, our lab ***hypothesized that treating the induced pluripotent stem cells (iPSCs) with small molecules would synergistically increase donor DNA insertion into the genome***. Various concentrations of SCR7, L755507, and Resveratrol dissolved in DMSO were tested, and the least cytotoxic mixture was chosen.

This project focused on using HDR to insert a point mutation into the transcription factor gene *NFATC1*, converting the wild-type ATG sequence to TTG, substituting a methionine for leucine at amino acid position 527. This gene was chosen because *NFATC1* has been identified as a novel candidate atrial fibrillation susceptibility gene. To test our hypothesis, a combination of Scr7 (6.3µM) and L755507 (3.6µM) dissolved in 1µl of DMSO was used to treat half of the cells that went through the CRISPR process.

Preliminary results

- iPSCs tolerated DMSO, but at increasing concentrations, entire colonies collapsed.
- Resveratrol was extremely cytotoxic to iPSCs, even at low concentrations.
- The least cytotoxic combination of molecules was Scr7 (6.3µM) and L755507 (3.6µM) dissolved in 1µl of DMSO, keeping more cells alive for expansion post-electroporation.
- 69% of iPSC clones that were Sanger sequenced exhibited a variety of indel mutations at target site.
- No HDR events have been identified in 70 iPSC clones screened.

Conclusion

We conclude that even though this approach efficiently generated a number of different indel mutations in the iPSC clones, the combination of small molecules Scr7 (6.3µM) and L755507 (3.6µM) does not increase HDR efficiency.

Work in progress

Based on statistics for 1% probability, our lab must isolate, expand, and sequence at least 230 iPSC clones in order to find a single HDR clone. We currently are expanding the next set of 67 clones. If we do not find an iPSC clone containing the desired HDR insertion, we may explore testing other small molecules or different combinations of small molecules.