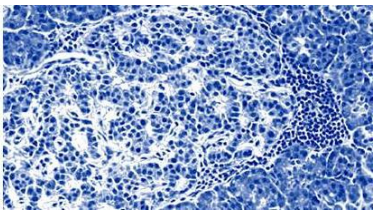
nPOD
Network for Pancreatic Organ Donors with Diabetes



Genome Scale *in vivo* CRISPR Screen Identifies *RNLS* as a Modifier of Beta Cell Vulnerability in Type 1 Diabetes

AUTHORS

Erica P. Cai[1†], Yuki Ishikawa[2†], Wei Zhang[2†], Nayara C. Leite[3], Jian Li[1], Shurong Hou[4], Badr Kiaf[2], Jennifer Hollister-Lock[1], Nese Kurt Yilmaz[4], Celia A. Schiffer[4], Doug A. Melton[3],Peng Yi[1*] & Stephan Kissler[2*]

[1]Section for Islet Cell and Regenerative Biology and [2]Section for Immunobiology, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA.
[3] Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138, USA.
[4] Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School Worcester, MA 01605, USA.

[†] These authors contributed equally.
[*] These authors jointly supervised the study.

PURPOSE

We performed a genome-scale CRISPR screen in beta cells to search for gene mutations that protect against autoimmune killing. Our goal was to identify novel gene targets that could be modified in stem cell-beta cells to enable a cell replacement therapy for type 1 diabetes.

METHODS

We transduced the mouse NIT-1 beta cell line with a lentiviral CRISPR-Cas9 library at a low multiplicity of infection to generate a pool of mutant cells each carrying one of 60,000 possible mutations covering more than 20,000 genes. We leveraged the selective pressure of autoimmunity in the NOD mouse model to identify mutant cells capable of resisting immune-mediated killing *in vivo*. We validated the protective capacity our lead candidate gene in both mouse models and in human stem cell-derived beta cells.

SUMMARY OF RESULTS

Our genome-wide CRISPR screen in the NOD model identified only 11 genes whose mutation appeared to protect beta cells against autoimmunity. Remarkably, one of these genes was Renalase (*Rnls)*, a gene previously associated with the overall risk and the age-of-onset of human T1D by GWAS. Extensive validation in mouse models confirmed that *Rnls* deletion confered protection against immune-mediated killing. Significantly, protection was associated with ER stress resistance, and this phenotype was replicated in *RNLS* knockout human SC-beta cells. Although the function of *RNLS* is unknown, its crystal structure has been solved. Using structure-based modeling, we identified an FDA-approved

drug that can be repurposed to inhibit *RNLS*. We show that oral drug treatment protected beta cells grafted into overtly diabetic NOD mice, leading to disease reversal.

CONCLUSIONS

We have identified the GWAS candidate gene *RNLS* as a modifier of beta cell vulnerability in T1D. We propose that *RNLS* could be targeted to protect stem cell-beta cells against autoimmunity and to enable a cell replacement therapy for T1D. Furthermore, we have identified an FDA-approved oral drug capable of protecting beta cells against autoimmunity. Based on its favorable safety profile in human and its efficacy in diabetic mice, we believe that this drug could be a promising candidate for clinical trial in patients with new onset T1D.