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CRISPR-Cas9 genome editing in iPSCs for functional genetic screening

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Abstract

Induced pluripotent stem cells (iPSCs) are widely used for disease modeling, drug discovery, and cell therapy development. However, iPSCs are difficult to engineer with an efficient CRISPR/Cas9 system for functional genetic screens. We used WTC11 human iPS cells to generate functionally validated, lentiviral-transduced Cas9 lines, characterized by high gene-editing activity and sustained potential to differentiate. Flow cytometry was used to check for the expression of pluripotency markers Oct3/4, TRA1-60, and SSEA-4 in the iPS-Cas9 cells, and their actual pluripotency was then confirmed by testing the ability to differentiate into the three germ layers ectoderm, mesoderm, and endoderm. Furthermore, genome-wide transcriptome analysis was performed to confirm the expression of germ-layer-specific markers in the differentiated cells. Sustained Cas9 activity was confirmed in the differentiated cells. This study provides proof-of-principle that patient-derived iPSCs can be used to enable CRISPR/Cas9 functional genetic screening technology in reconstituted patient-specific tissues/disease models.

Introduction

- Human Induced pluripotent stem cells (iPSCs) are derived from adult somatic cells that have been genetically reprogrammed to co-express defined pluripotency factors associated with embryonic stem cells (ESCs).
- Due to their ability to proliferate and differentiate into derivatives of all three primary germ layers (i.e., ectoderm, mesoderm, and endoderm) iPSCs have significant potential for biological and therapeutic applications.
- Genetic screening applications of CRISPR/Cas9 based iPSCs may be used to
  (i) discover new drugs
  (ii) treat life-threatening diseases through cell therapy-based strategies
  (iii) monitor protein dosage in neurodevelopmental disorders
  (iv) investigate therapeutic strategies for viral infections

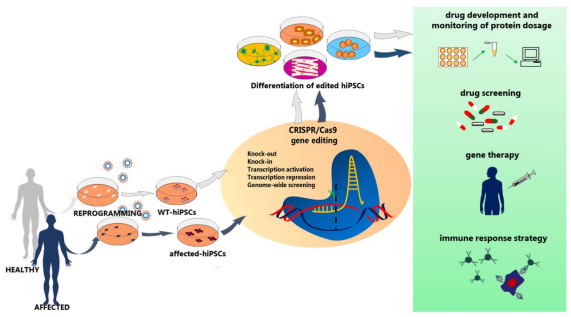


Fig 1: Workflow of the research involving hiPSCs and CRISPR/Cas9 gene editing for the investigation of new drugs and therapeutic alternatives (De Masi, Spitalieri, Murdocca, Novelli, & Sangiuolo, 2020)

Materials & Method

- WTC11 human iPS cells were obtained from Coriell Institute for Medical Research.
- WTC11 human iPS cells were used to generate lentiviral-transduced Cas9 lines.
- Cas9 activity was assayed and confirmed using Cellecta's CRISPRuTest™-EF1L kit.
- The optimal promoter for the sustained expression of Cas9 was found using Cellecta's PromoterTest™ Custom Assay Kit
- The pluripotency of WTC11-Cas9 cells was confirmed by flow cytometry using conjugated Ab for Oct3/4, TRA1-60, and SSEA-4.
- To validate the ability of the WTC11-Cas9 cell line to differentiate into the three germ layers—ectoderm, mesoderm, and endoderm—StemDiff Trilineage Differentiation Kit (StemCell Technologies) was used.
- Genome-wide transcriptome analysis was performed using Cellecta's DriverMap™ Human Genome-Wide Gene Expression Profiling Assay

Results

- To identify optimal promoter for the sustained expression of Cas9 in iPS cells, Cellecta's PromoterTest™ Custom Assay Kit was used.
- Human EF1a promoter was found to be the strongest promoter to sustain the highest expression in iPS cells.

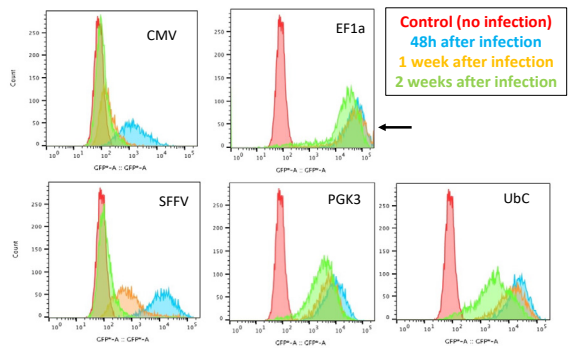
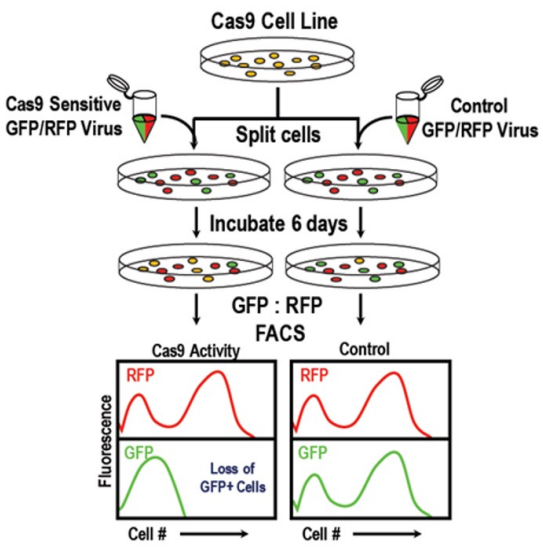


Fig 2: FACS readout shows that EF1a sustained the highest expression in iPSCs.

Results (cont.)

CRISPR-Test Assay



- Cellecta's CRISPRuTest™(EF1L Kit) was used to assess the nuclease activity of Cas9 in the cells of interest.
- CRISPRtest and CRISPRu-Test Kits provide quantitative FACS-based readouts of Cas9 activity.

Polyclonal WTC-11-EF1L Cas9-Cell-Line (48.5 % KO)

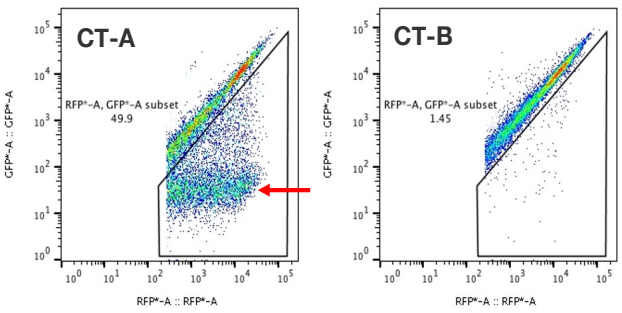


Fig 3: Polyclonal iPS cells expressing Cas9 from EF1a promoter demonstrated 48.5 % GFP KO efficiency.

a. WTC-11-EF1L-Cas9 clone #1 (90% KO)

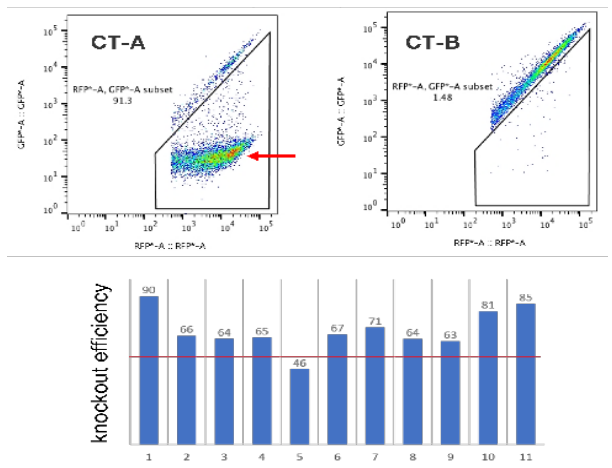


Fig 4: Clones from WTC11-Cas9 pool cells were isolated to obtain higher knockout efficiency
a. Clone 1 showed 90% GFP-KO efficiency
b. Clonal KO Efficiency

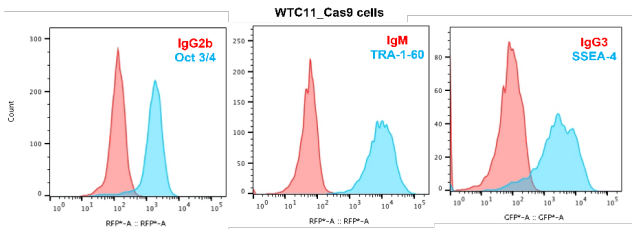


Fig 5: The expression of pluripotency markers in WTC11-Cas9 cells was confirmed by flow cytometry using conjugated Ab for Oct3/4, TRA1-60, and SSEA-4.

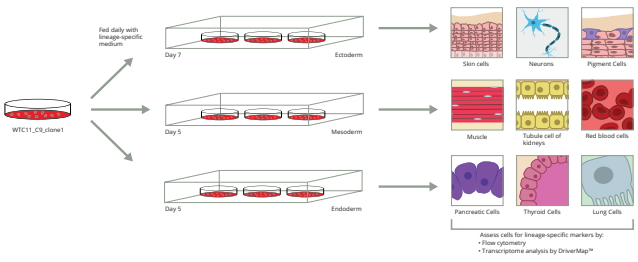


Fig 6: The differentiation capability of iPSC-Cas9 cells was confirmed using STEMdiff™ Trilineage Differentiation Kit.

Results (cont.)

Endoderm Cell-Line (WTC11-Cas9 clone #1)

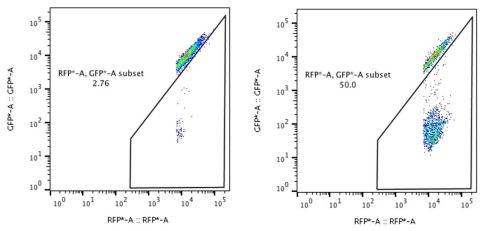


Fig 7: The heterogeneous population of endoderm cells derived from WTC11-Cas9 clone #1 showed 47% GFP-KO efficiency. Higher Cas9 activity is expected to be found in selected endoderm clones.

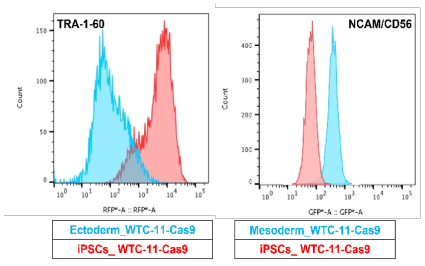
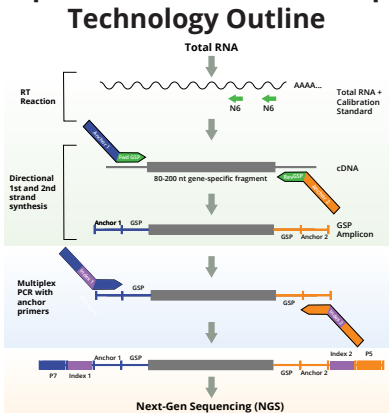


Fig 8: Pluripotency/differentiation markers in undifferentiated and differentiated WTC-11-Cas9-clone#1 cells:
a) Pluripotency marker TRA-1-60 present in iPSCs, absent in differentiated WTC-11-Cas9 clone#1 cells
b) Mesodermal differentiation marker NCAM/CD56 absent in iPSCs, present in differentiated WTC-11-Cas9 clone#1 cells

DriverMap™ Genome-wide Gene Expression Technology Outline



- Multiplex RT-PCR-NGS assay for expression profiling of up to 19,000 genes in a single test-tube assay
- RT-PCR enables use of total RNA from tissue, blood, cells, etc. (no mRNA enrichment, no globin depletion).
- PCR sensitivity down to 10pg—single-cell level-96-well format allows for cost-effective high-throughput application

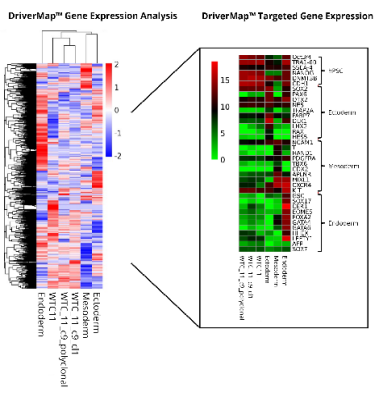


Fig 9: Genome-wide transcriptome analysis and targeted expression analysis confirm the key markers present in iPSCs and differentiated WTC-11-Cas9 clone#1 (ectoderm, mesoderm and endoderm cell lines).

Discussion

- We showed that iPS cells can be successfully engineered to express high levels of active Cas9.
- After screening multiple viral and mammalian promoters, we successfully identified EF1a as the optimal promoter for sustained Cas9 expression in iPSCs. Viral promoters like CMV and SFFV got inactivated a few days after transduction.
- We were able to generate both polyclonal and clonal validated iPSC-Cas9 lines with efficient Cas9 activity (~90% KO efficiency in selected clones) with sustained potential to differentiate.
- We were able to show that Cas9 activity is retained after differentiation of iPSC-Cas9 cells.
- iPSCs can be used to enable CRISPR/Cas9 functional genetic screening technology in reconstituted patient-specific tissues/disease models.

References:
- De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G., & Sangiuolo, F. (2020). Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery. Human Genomics, 14(1). https://doi.org/10.1186/s40246-020-00276-2
- Ye, L., Swingen, C., & Zhang, J. (2013). Induced Pluripotent Stem Cells and Their Potential for Basic and Clinical Sciences. Current Cardiology Reviews, 9(1), 63–72. https://doi.org/10.2174/157340313805076278