Genetic Interactions with Transcription-Coupled Nucleotide Excision Repair

Travis Dowd[1,2], Rui Wang[3], Junjie Chen[3]

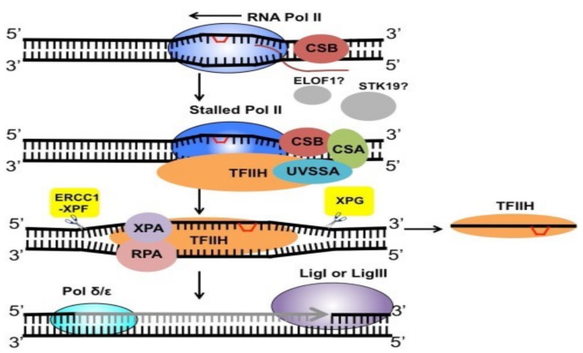
1. Rice University, George R. Brown School of Engineering, Houston, TX
2. MD Anderson, Student Undergraduate Research Program, Houston, TX.
3. MD Anderson, Department of Experimental Radiation Oncology, Houston, TX

Email: tjd13@rice.edu

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Introduction

- Transcription-Coupled Nucleotide Excision Repair (TC-NER) is a subpathway of nucleotide excision repair that repairs DNA lesions on actively transcribed genes.
- When TC-NER fails as occurs in Cockayne Syndrome (CS), it is unknown what repair mechanisms assume the responsibility of DNA damage repair (DDR).
- CS patients experience UV sensitivity, premature aging, neurodegeneration, and dwarfism.[1]
- CS patients have deficiencies in TC-NER-specific proteins Cockayne Syndrome type A (CSA) and Cockayne Syndrome Type B (CSB).[1,2]



- A CRISPR/Cas9 full genome synthetic lethality screen on CSA$^{-/-}$ and CSB$^{-/-}$ cells can be used to identify DDR pathways that replace deficient TC-NER in humans.
- We plan to use the chemotherapeutic drug Illudin-S, whose clinical use is limited due to high toxicity, in synthetic lethality screening to cause DNA lesions repaired almost exclusively by TC-NER.[3]
- To use Illudin-S, we must determine the drug doses ideal for maintaining 400 times coverage of the CRISPR gRNA library through the screen while displaying sufficient cellular toxicity to cause a 20% cell reduction (LD20) at each timepoint.[4]

Materials & Methods

293A cell transient transfection using Mirus LT1 transfection reagent. Puromycin selection 2 days later.

Single cell clone formation using a 96 well plate.

Western blot analysis to test gRNA knockout efficiency.

2 Week Incubation

Plasmid DNA ligation and Sanger sequencing to confirm single clone knockout.

Western blot analysis to confirm single clone knockout.

Culture single cell clone knockouts until there are an adequate number for LD20 screen seeding at 5 million cells per replicate per treatment.

Control | 0.5 µg/mL | 1.0 µg/mL | 1.5 µg/mL | 2.0 µg/mL

Validated Knockout Cells
LD20 Screen

- 5 million cells were seeded to ten 150 mm plates. Two technical replicates were used, each with a 0, 0.5, 1.0, 1.5, and 2.0 µg/mL Illudin-S concentration.[2]
- Cells were cultured in 25 mL DMEM with bovine serum.
- Cells were passaged, counted, and reseeded at 5 million cells per dish every three days for 18 days.
- Cells of each dose had the assigned dose readministered every reseeding.
- When a cell count dropped below 5 million, data was cut off for that treatment since the next passage could not be adequately seeded to maintain an equal number of cells between treatment groups.

Results & Discussion

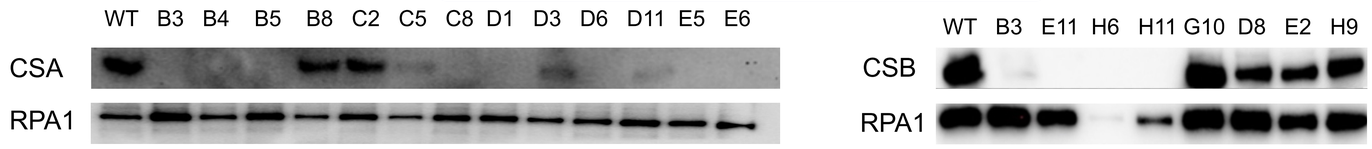


Fig. 1 Western blot tests the CSA and CSB knockout cell candidates. We achieved a 33% and 38% knockout efficiency for CSA and CSB, respectively. We used RPA1 as a loading control for both protein blots, with a weight of 70 kDa. CSA appeared at 46 kDa, while CSB appeared at 168 kDa. The clone number of each single cell clone tested for knockout validation is shown above the blots. The first lane in each blot is a wild type (WT) positive control.

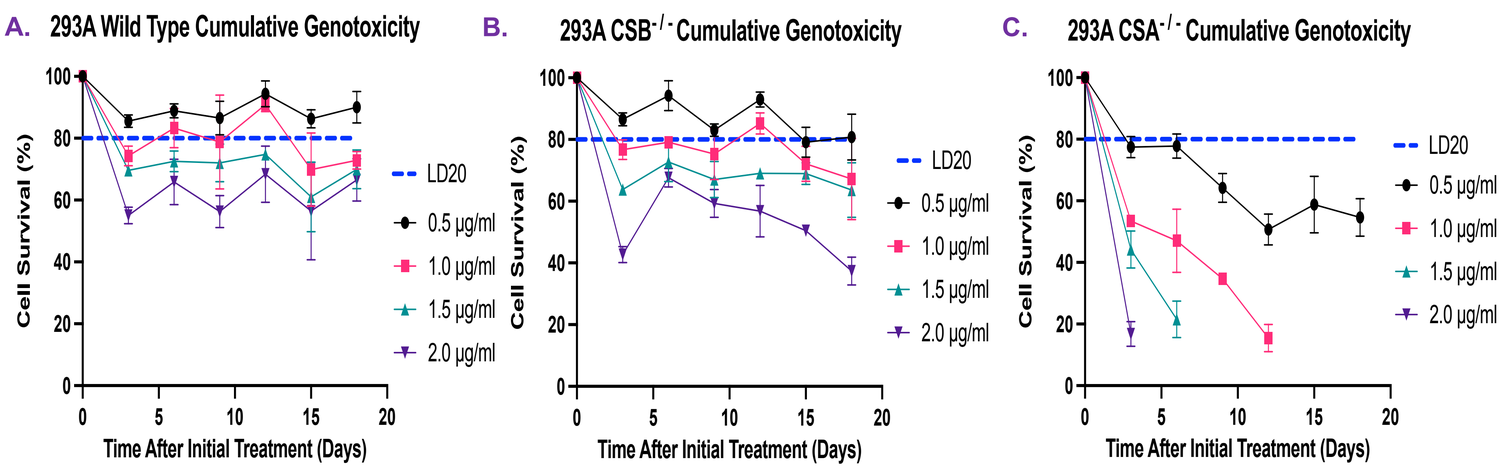


Fig. 2A-C To calculate percent survival, we divided the cell count for each Illudin-S concentration by the 0 µg/mL control cell count at every passage. We conducted this calculation separately for both technical replicates and averaged them. The error bars show variation in replicate measurements. The targeted 80% cell survival is denoted by the blue dotted line for the LD20 screen data within each knockout cell type. **A)** proves that 20% lethality for WT cells was consistent at each passage for the 1.0 µg/mL concentration within cell survival fluctuations. This concentration is thus a possible candidate for future 293A WT screens. WT cells did not suffer extensive cumulative genotoxic effects as expected since they possess an efficient TC-NER pathway. **B**) shows that by the screen's end, the 0.5 µg/mL dose achieves 20% lethality for CSB$^{-/-}$ cells, making it an LD20 candidate in future screens. Because they are TC-NER deficient, CSB$^{-/-}$ cells experience heightened cumulative genotoxic effects at higher Illudin-S doses in comparison to WT cells. **C**) exhibits the considerably higher sensitivity to Illudin-S that CSA$^{-/-}$ cells demonstrate in comparison to WT or CSB$^{-/-}$. All concentrations caused excessive cell death, making none LD20 candidates. All concentrations other than the lowest 0.5 µg/mL dose resulted in less than 5 million cells prior to the screen's conclusion.

- The heightened sensitivity of CSA$^{-/-}$ cells to Illudin-S is unexpected due to the more central role of CSB in the TC-NER pathway. We double checked the validity of the cell knockouts with Western blotting, which confirmed our results. Further drug screens are necessary to validate the heightened sensitivity of CSA$^{-/-}$ to Illudin-S as observed in this study.

Conclusions & Future Directions

- Lower Illudin-S doses are most suitable to maintain adequate gRNA library coverage in 293A full genome CRISPR synthetic lethality screens. Especially for TC-NER deficient cells, we will execute further Illudin-S drug screening at lower drug concentrations to identify LD20 concentrations for 293A CRISPR screens, using this screen's lowest 0.5 µg/mL concentration as the highest concentration tested.

Following confirmation of proper Illudin-S doses for the 293A cell line, we will conduct LD20 screens on other cell lines such as the HCT116 colon cancer cell line to prepare for CRISPR synthetic lethality screens using other cell types.

Acknowledgements

- Special thanks to the Chen Lab and its members for their guidance on this project, especially Samuel Swisher.
- Thank you to the MD Anderson postdoctoral association for helping with the deign and presentation process.
- Thank you to Dr. Marites Melancon and Dr. Chandra Bartholomeusz for their support of the Student Undergraduate Research Program (SURP).
- Funding for this project was provided by MD Anderson and SURP.

References

1.) Lainé J-P, Egly J-M. When transcription and repair meet: a complex system. Trends Genet. 2006;22(8):430–6.
2) Geijer ME, Zhou D, Selvam K, Steurer B, Mukherjee C, Evers B, et al. Elongation factor ELOF1 drives transcription-coupled repair and prevents genome instability. Nat Cell Biol. 2021;23(6):608–19.
3) Jaspers NGJ, Raams A, Kelner MJ, Ng JMY, Yamashita YM, Takeda S, et al. Anti-tumour compounds illudin S and Irofulven induce DNA lesions ignored by global repair and exclusively processed by transcription- and replication-coupled repair pathways. DNA Repair (Amst). 2002;1(12):1027–38.
4) Olivieri M, Durocher D. Genome-scale chemogenomic CRISPR screens in human cells using the TKOv3 library. STAR Protoc. 2021;2(1):100321.