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).\(^1,2\)

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

Results & Discussion

Fig. 1 Western blot tests the CSA and CSB knockout cell candidates. We achieved a 33% and 38% knockdown 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 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.

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.

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References