Name(s)

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Project Number

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Project Title

Targeting Susceptibility to Mutations in the Cell Cycle: Disruption of the Ade2 Gene in Yeast Using CRISPR/CAS9

Abstract

Objectives

If we arrest the cell cycle in S phase and M phase using inhibitors, we will see significant differences in DNA damage. In S-phase, we find DNA synthesizing, whereas in M-phase, DNA has condensed into their chromosomal structures. By doing so, we will be able to conclude in which stage of the cell cycle is the cell most vulnerable to mutations and translate it into CRISPR editing to see if the same results are shown.

Methods

The procedure involves dividing the yeast cells in two groups for each trial and, within those groups, three experimental procedures in which the cells will be treated with cell cycle inhibitors [DMSO (negative control), hydroxyurea (arrests the S-phase), and nocodazole (arrests the M-phase)]. One half will be the used to measure the DNA damage susceptibility of the yeast cells at a cell cycle phase by exposure to UV irradiation via comet assay. The other half will be used to measure the CRISPR editing efficiency by culturing the cells in galactose-containing media to induce expression of the Cas9 protein, affecting the edited ade2 gene to completely knock out both ADE2 genes, allowing for the growth of red yeast colonies.

Data will be collected by measuring the comet assay via application ImageJ and counting the number of red colonies from the plated yeast. ImageJ will allow us to precisely measure the density and lengths of each gel streak which should correlate with the DNA damage and translate into the yeast red colony count. For precise analyzation, we ran a total of 5 trials to find averages and standard deviation of our data.

Results

Cells targeted at the S-phase experienced an 11% higher mutation rate whereas cells in the M-phase saw a -1% rate less compared to our DMSO control, which is directly translated into our CRISPR efficiency with plated yeast whose cells showed proportional numbers to the comet assay cells treated under the same conditions.

Conclusions

We see that the cell is most susceptible to DNA alterations in the S-phase, while in the M-phase the cell experiences less. The trials treated with Hyd. showed longer and denser streaks, which means damage and mutations are occurring, and that directly translated into gene editing using CRISPR since they also produced a higher yield of red colonies compared to the rest of the trials with different treatments. The trials treated with Noc. experienced less genomic changes than our control (DMSO), which could indicate that the M-stage is less likely to experience DNA alterations.

Summary Statement

As more genetic mutations occurred in S-phase than in M-phase in yeast cells, it can be correlated that S-phase has higher susceptibility to mutations and CRISPR editing.

Help Received

Our advisor supervised us while we were doing our experimental trials. Certain materials were also donated by graduate students from Stanford, and our yeast strain donated by the Tech Museum.