Inhibiting HDAC1–4 in DSRCT Cells via CRISPR-Cas9 Gene Editing

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Introduction

Desmoplastic Small Round Cell Tumor (DSRCT) is a rare and aggressive pediatric cancer with limited treatment options and poor survival rates. This cancer is linked to a genetic mutation that disrupts normal cell function. We are studying a group of proteins called histone deacetylases (HDAC1–4), which can turn off tumor suppressor genes that protect against cancer. Using CRISPR-Cas9 gene editing, we tested whether DSRCT cells rely on these HDACs to survive.

Objective

To determine whether HDAC1–4 are essential for DSRCT cell survival by using CRISPR-Cas9 to disrupt expression and assess changes.

Methods

Guide RNAs (gRNAs) targeting HDAC1–4 were cloned into GFP- or mCherry-tagged Cas9 plasmids, amplified in *E. coli*, and sequence-verified. Control plasmids expressing GFP or mCherry alone were prepared. DSRCT cells were co-transfected with HDAC and control constructs in replicates. Gene disruption and transfection efficiency were monitored via fluorescence microscopy. A custom ImageJ macro was used to merge red and green channels, producing yellow composite images to assess co-expression and HDAC dependencies.

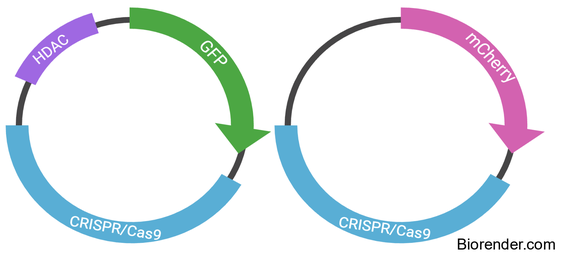

Figure 1: Vector maps for mCherry and GFP tagging

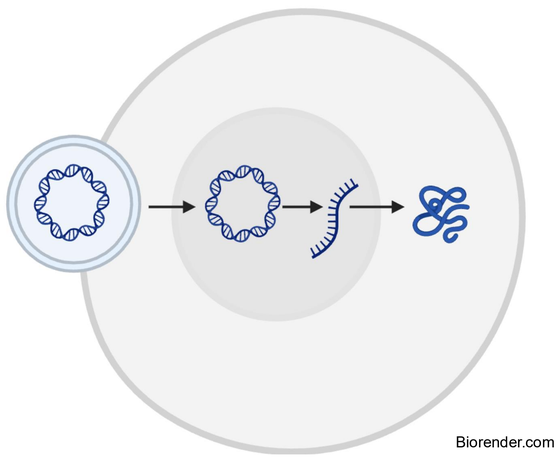

Figure 2: Plasmid uptake into cell

CRISPR Mechanism

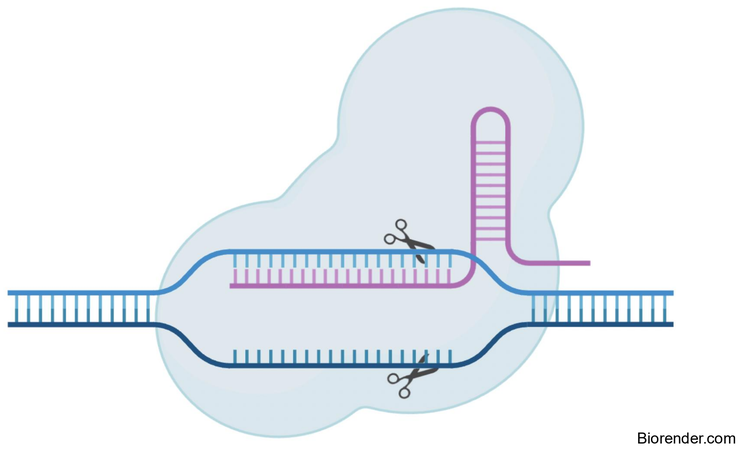

Figure 3: CRISPR-Cas9 complex cutting DNA

Fluorescence Microscopy

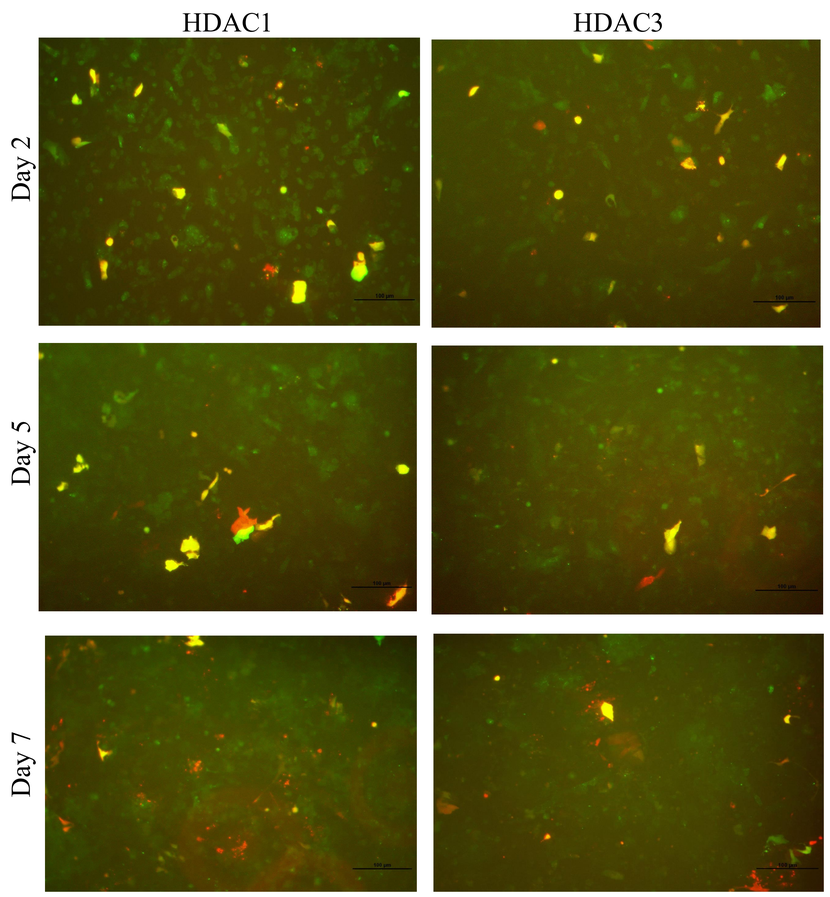

Figure 4: Merged green and red fluorescence microscopy images for HDAC 3 (right side) and HDAC 1 (left side) on all three days of photo collection.

The number of dual-positive (yellow) cells co-expressing both the HDAC-targeting plasmid and a control plasmid was quantified at on days 2,5, and 7. All HDACs showed decreased co-expressing cells after a week, as expected for normal plasmid uptake.

Results

The highest co-expression of GFP and mCherry cells was observed on Day 2 across all HDAC-targeted conditions, indicating successful transfection. A notable decline in double-positive cells occurred by Day 5, consistent with expected plasmid dilution over time. Average cell counts from duplicate samples were calculated to compare remaining cell populations at each timepoint. On Day 2, HDAC2 exhibited the highest average cell count (118), followed by HDAC1 (108.75), HDAC4 (85), and HDAC3 (82.5). By Day 5, all groups declined, with HDAC2 again showing the highest average (62), while HDAC4 had the lowest (53). By Day 7, HDAC4 showed a notable increase (80.0), while HDAC1 (59.75), HDAC2 (63.75), and HDAC3 (58.5) remained steady. Despite these fluctuations, no clear differences in cell viability or growth were observed among the four HDAC gRNA groups. This is likely due to the short duration of the experiment, which limited the ability to detect downstream effects of HDAC inactivation. Future studies should extend the observation period to at least 21 days and increase the number of transfections and replicates to improve statistical power. These adjustments will be essential to determine whether HDAC1–4 are functionally required for DSRCT cell survival and to clarify their potential as therapeutic targets.

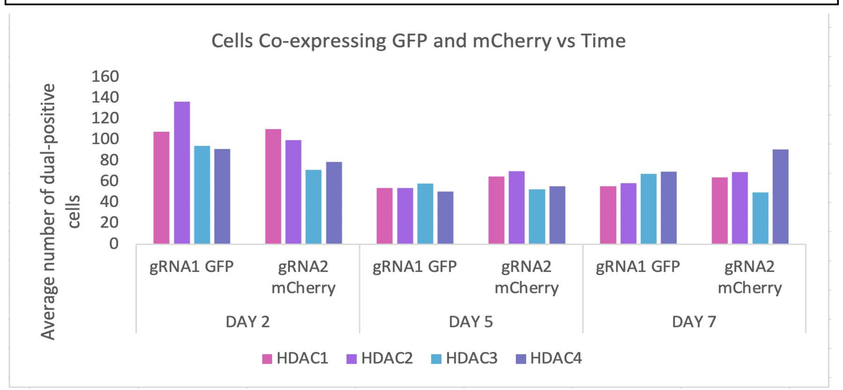

Figure 5: A bar graph with number of cells co-expressing GFP and mCherry over time

Conclusion

These preliminary results suggest that HDAC inactivation did not produce distinct effects on DSRCT cell growth within the observed timeframe, as no noticeable differences were detected among the four HDAC gRNA conditions. The decline in double-positive GFP and mCherry cells by Day 5 is consistent with normal plasmid dilution and degradation, indicating transient transfection. However, the short experimental duration likely limited the ability to detect changes resulting from gene disruption. Future studies should extend the observation period to at least 21 days to allow sufficient time for downstream effects of HDAC loss to manifest. Additionally, increasing the number of biological replicates and performing multiple independent transfections will enhance the statistical power and reproducibility of the results. These improvements will be critical for accurately evaluating the role of HDAC1–4 in DSRCT cell survival and for validating their potential as therapeutic targets.

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