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"Inhibiting HDAC1-4 in DSRCT cells via CRISPR-Cas9 Genomic Editing"

Introduction

Desmoplastic Small Round Cell Tumor (DSRCT) is a rare and aggressive soft tissue sarcoma caused by a chromosomal translocation resulting in the EWSR1::WT1 fusion gene. Patients face poor prognoses despite aggressive multifaceted treatment with surgery, chemotherapy, and radiation, underscoring the need for novel targeted approaches. Histone deacetylases (HDACs) regulate chromatin structure and gene expression by promoting chromatin condensation and transcriptional silencing. Aberrant HDAC activity can repress tumor suppressor genes, supporting oncogenic phenotypes. We aim to determine whether DSRCT cell survival is dependent on HDAC1, HDAC2, HDAC3, and HDAC4. To investigate this, we used a CRISPR-Cas9 genome editing tool to inhibit HDAC gene expression. The system uses a guide RNA (gRNA) to direct the Cas9 endonuclease to specific genomic loci, where it induces double-strand breaks, resulting in gene disruption.

Methods

CRISPR-Cas9 gene editing was used to inactivate HDAC1 through HDAC4 in DSRCT cells. Guide RNAs targeting HDAC1 through HDAC4 were cloned into Cas9-expressing plasmids tagged with GFP or mCherry fluorescent proteins. These constructs were transformed into *Escherichia coli* for amplification. Plasmids were purified using miniprep and sequence verified. Control plasmids expressing only GFP or mCherry without HDAC gRNAs were also prepared. DSRCT cells were co-transfected with paired combinations of HDAC gRNA expressing plasmids and controls. Transfection efficiency and gene disruption were monitored by fluorescence microscopy, and HDAC dependencies were assessed by tracking changes in the ratio of GFP- to mCherry-positive cells over time.

Expected Results

We hypothesize that activation of HDAC3 and HDAC4 will lead to significantly decreased cell proliferation, indicating their role as potential dependencies in DSRCT. HDAC1 and HDAC2 deletions may yield less pronounced effects due to possible functional redundancy. We anticipate activation of apoptotic pathways in HDAC3 and HDAC4-deficient cells.

Conclusion

Identifying HDAC3 and HDAC4 as essential genes for DSRCT cell survival would highlight vulnerabilities in this treatment-resistant tumor. These findings could support the development of selective HDAC inhibitors that restore tumor suppressor gene expression and inhibit tumor cell growth. This work contributes to the growing field of targeted epigenetic therapy and may advance precision medicine approaches for rare sarcomas.