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Short Communication

Epigenome Editing using dCas9-DNMT3A Fusion Proteins for Therapeutic Silencing of Oncogenes in Glioblastoma

Carl Hancock*

Department of Biotechnology and Bioengineering, University of Sao Paulo, Sao Paulo, Brazil

DESCRIPTION

Glioblastoma Multiforme (GBM) represents the most aggressive primary brain malignancy, characterized by aberrant oncogene expression and resistance to conventional therapies. Epigenome editing technologies utilizing catalytically inactive Cas9 (dCas9) fused to epigenetic modifiers offer promising approaches for therapeutic gene silencing without permanent DNA sequence alterations. This investigation explores the application of dCas9-DNMT3A fusion proteins for targeted DNA methylation and subsequent silencing of the Epidermal Growth Factor Receptor (*EGFR*) oncogene in GBM cell lines and patient-derived xenografts [1-3].

The experimental design incorporated a dCas9-DNMT3A-3L fusion construct, where the DNMT3A catalytic domain was enhanced with the regulatory 3L domain to improve methyltransferase activity. Multiple gRNA sequences were designed to target CpG-rich regions within the *EGFR* promoter, with particular focus on the core promoter region spanning -200 to +50 base pairs relative to the transcription start site [4,5].

Glioblastoma Multiforme (GBM) is one of the most aggressive and lethal forms of brain cancer, characterized by rapid proliferation, high invasiveness, and resistance to conventional therapies. Despite advancements in surgical resection, radiation, and chemotherapy, the prognosis for GBM patients remains poor, with median survival rarely exceeding 15 months. A major contributing factor to this treatment resistance is the epigenetic plasticity of GBM cells, which allows them to dynamically regulate gene expression without altering the underlying DNA sequence. Among the many oncogenic pathways active in glioblastoma, several are driven by aberrant gene expression that could be therapeutically targeted through epigenetic reprogramming.

Epigenome editing offers a novel therapeutic approach by enabling precise, locus-specific modifications to the epigenetic landscape of cancer cells. One promising strategy involves the use of catalytically dead Cas9 (dCas9) fused to DNA methyltransferases such as DNMT3A. This fusion protein can be guided by sequence-specific single guide RNAs (sgRNAs) to the promoter regions of oncogenes, where it deposits methyl groups to silence transcription. Unlike conventional gene knockouts, this method does not disrupt the genomic sequence, reducing the risk of unintended mutations while allowing reversible and tunable gene repression [6].

In the context of glioblastoma, dCas9-DNMT3A-mediated epigenetic silencing presents a targeted, non-permanent therapeutic option for turning off key oncogenes that drive tumor growth and resistance. This introduction explores the principles, mechanisms, and therapeutic implications of using dCas9-DNMT3A fusion proteins for epigenome editing in glioblastoma, highlighting their potential to reshape cancer treatment through precision gene regulation [7,8].

Targeted methylation analysis revealed significant hypermethylation at *EGFR* promoter CpG sites, with average methylation levels increasing from 12% to 78% following dCas9-DNMT3A treatment. Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) analysis demonstrated corresponding 4.2-fold reduction in *EGFR* mRNA expression, with Western blot confirmation of decreased *EGFR* protein levels. Importantly, methylation changes were specific to targeted regions, with minimal off-target methylation detected through genome-wide RRBS analysis. ChIP-seq experiments revealed altered chromatin architecture at the EGFR locus, with decreased H3K4me3 and H3K27ac activating marks and increased H3K9me3 repressive marks [9].

Functional studies demonstrated significant reduction in cell proliferation, with colorimetric assays showing 58% decrease in viability compared to control cells [10]. Colony formation assays revealed 73% reduction in clonogenic potential, while invasion assays demonstrated 64% decrease in invasive capacity. *In vivo* experiments using patient-derived xenograft models showed significant tumor growth suppression, with treated tumors exhibiting 67% reduction in volume compared to controls.

CONCLUSION

Immunohistochemical analysis confirmed sustained *EGFR* silencing and reduced proliferation markers throughout the

Correspondence to: Carl Hancock, Department of Biotechnology and Bioengineering, University of Sao Paulo, Sao Paulo, Brazil, E-mail: carlhancock@ufrhs.br

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treatment period. DCas9-DNMT3A fusion proteins enable precise epigenetic silencing of oncogenes in GBM, offering a reversible therapeutic approach with significant anti-tumor efficacy. The specificity of methylation targeting, sustained gene silencing, and functional tumor suppression demonstrate the therapeutic potential of epigenome editing technologies. This work establishes a foundation for developing epigenetic therapies that complement existing treatment modalities for aggressive brain cancers.

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