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OVEREXPRESSION OF MTDNA REPAIR GENE — *MUTYH* — USING CRISPRA TO ENHANCE RESISTANCE TO GENOTOXIC AND OXIDATIVE STRESS

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Abstract

Mitochondria is mostly susceptible to oxidative DNA damage mainly because of proximal ROS production, lesioning mtDNA and resulting in mitochondrial dysfunction and follow up cellular machinery failure. This work discusses the possibility of increasing mtDNA repair by inducing overexpression of mtDNA repair gene — *MUTYH* — using controlled CRISPRa technique.

Mitochondrial DNA is vulnerable to oxidative and genotoxic stress due to its proximity to reactive oxygen species (ROS) mainly generated by the electron transport chain, lack of protective histones, and limited DNA repair capacity, unlike nuclear DNA. Accumulating damage in the mitochondrial genome highly contributes to mitochondrial dysfunction, cellular energy failure, and pathogenesis [1].

MUTYH gene product — Adenine DNA glycosylase — detects and excises adenine opposite mispaired 8-oxoG (a common ROS-induced lesion), initiating BER to prevent G:C → T:A transversions, and after adenine removal, downstream BER enzymes (*OGG1*, *APE1*, *POLβ*, *LIG1/LIG3*, *PARP1*, *XRCC1*) restore the correct G:C pair. *MUTYH* has two splicing variants — nuclear & mitochondrial. Their fundamental difference is the presence or absence of the first exon, which encodes the mitochondrial localization signal (MLS) [2, 3].

There is evidence suggesting *MUTYH* influences apoptosis signaling if overexpressed, where it is believed that persistent lesions or repair intermediates (AP sites, single-strand breaks) can trigger cell death. Therefore, studies show contradicting results about the direct action of this gene. Survival or death? [4–6].

In this project, we investigate whether overexpressing a key mitochondrial DNA repair gene — *MUTYH* — via CRISPR activation (CRISPRa) system enhances cellular resistance to ionizing radiation and chemical oxidation and mitigates radiation-induced mitochondrial dysfunction. Although MUTYH gene knockdown/knockout or overexpression experiments were conducted before [7–9], there was no mention of using CRISPRa constructs to overexpress this particular gene in literature to our knowledge, therefore, this work will be performed for the first time in HEK293T and HeLa cell lines.

We designed sgRNAs targeting three distinct locations upstream of the transcription start site (−80, −165, −255 nt) to selectively guide dCas9-VPH protein complex towards mitochondrial variant of MUTYH. We aim to assess the grade of overexpression using our designs (singularly and in combinations) and the protective role of controlled elevated expression of *MUTYH* gene on the overall mitochondrial genome stability against chemical and radiation-induced damage. Outcomes will be evaluated through assays measuring mtDNA integrity and mitochondrial function, and cellular viability.

This work may provide insights into mtDNA repair genes and controlled mechanisms of action and novel strategies for enhancing mitochondrial resilience and improving therapeutic responses in oxidative stress-associated conditions.

References

1. Richter C. et al. Oxidants in mitochondria: from physiology to diseases // Biochimica et Biophysica Acta (BBA) — Molecular Basis of Disease. 1995. Vol. 1271, No. 1. P. 67–74.

2. Out A. A. et al. Leiden Open Variation Database of the MUTYH gene // Human Mutation. 2010. Vol. 31, No. 11. P. 1205–1215.

3. Arai T. et al. Up-regulation of hMUTYH, a DNA repair enzyme, in the mitochondria of substantia nigra in Parkinson's disease // Acta Neuropathologica. 2006. Vol. 112, No. 2. P. 139–145.

4. Oka S., Nakabeppu Y. DNA glycosylase encoded by MUTYH functions as a molecular switch for programmed cell death under oxidative stress to suppress tumorigenesis // Cancer Science. 2011. Vol. 102, No. 4. P. 677–682.

5. Oka S. et al. MUTYH, an adenine DNA glycosylase, mediates p53 tumor suppression via PARP-dependent cell death // Oncogenesis. 2014. Vol. 3, No. 10. P. e121.

6. Raetz A. G. et al. The DNA repair enzyme MUTYH potentiates cytotoxicity of the alkylating agent MNNG by interacting with abasic sites // Journal of Biological Chemistry. 2020. Vol. 295, No. 11. P. 3692–3707.

7. Hwang B.-J., Shi G., Lu A.-L. Mammalian MutY homolog (MYH or MUTYH) protects cells from oxidative DNA damage // DNA Repair. 2014. Vol. 13. P. 10–21.

8. Kremer T. M. et al. Protection of pulmonary epithelial cells from oxidative stress by hMYH adenine glycosylase // Respiratory Research. 2004. Vol. 5, No. 1. P. 16.

9. Gupta A. et al. Mammalian MutY Homolog (MYH or MUTYH) is critical for telomere integrity under oxidative stress // OBM Geriatrics. 2022. Vol. 6, No. 2. P. 196.