CRISPR/Cas9-Mediated Targeting of Glycolytic Enzymes in Cancer Metabolism as a Therapeutic Intervention

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Theme: Good Health and Wellbeing (SDG 3)

Research Problem

GLOBALLY

1 OUT OF 6 DEATHS is due to cancer → Conventional Cancer Therapies → Lack of specificity

SUSTAINABLE DEVELOPMENT GOALS → 3 GOOD HEALTH AND WELL-BEING → Prevention of mortality due to noncommunicable disease

Main Objective

To design and construct a CRISPR/Cas9 mediated toolkit to target the metabolic reprogramming of cancer cells via two independent approaches by,

1) Knocking out the Allosteric domain of liver type PFK-1
2) Knocking out the PFKFB3 isoform of PFK-2 to reduce the PFK-1 activity

to treat cancers.

Warburg effect

- Cancer cells consume high glucose level compared to normal cells through "Aerobic glycolysis" even in the presence of high oxygen level and functioning Mitochondria.
- Downregulation of glycolytic enzymes will reduce the proliferation rate of cancer cells by slowing down the glycolysis.

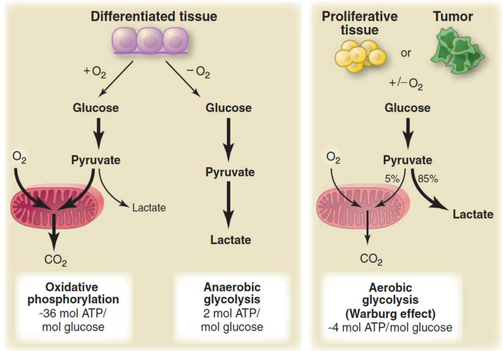

Figure 1: The difference in glucose metabolism between a normal differentiated tissue and a cancer proliferative tissue

Glycolysis – Rate limiting step

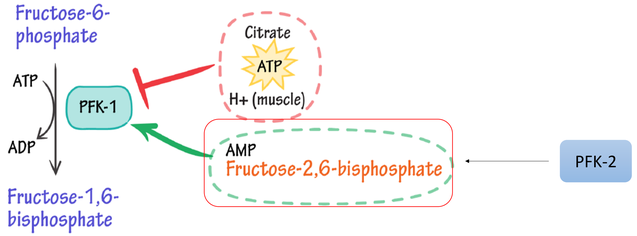

Figure 2: Illustration of the rate limiting step of glycolysis

SgRNA designs to target the two glycolytic enzymes; PFK-1 and PFK-2

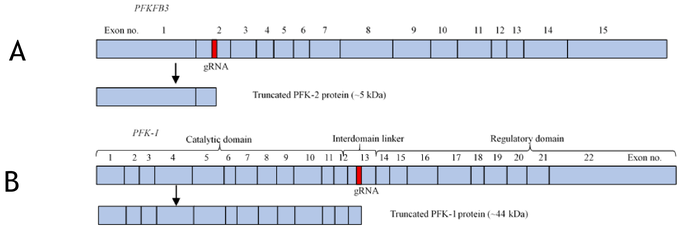

Figure 3: Location of designed gRNAs targeting (A) PFKFB3 (exon 2) and (B) PFK-1 (exon 13)

Confirmation of the cloning of sgRNA by Colony PCR

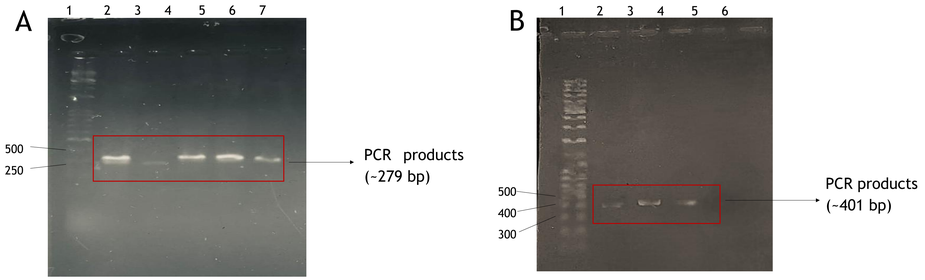

Figure 4: UV visualized agarose gel electrophoresis images of Colony PCR products, of (A) PFK-1 (B)PFK-2

Conclusion

This study aimed to downregulate the elevated glycolysis of cancer cells by knocking out the glycolytic enzymes in the rate limiting step using two independent approaches; disruption of the *PFKFB3* gene by targeting exon 2 and disruption of regulatory domain of the PFK-1 enzyme by targeting exon 13 via specifically designed sgRNAs. This allows to maintain the basal level of glycolysis in healthy cells while significantly affecting the metabolic reprogramming in cancer cells.

Future directions

- In vitro validation using HepG2 cells.
- Detection of truncated protein.
- Sequencing to verify the precise genome modification.
- Proliferation assay (MTT assay) to investigate the effect of modified gene.

Acknowledgement

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